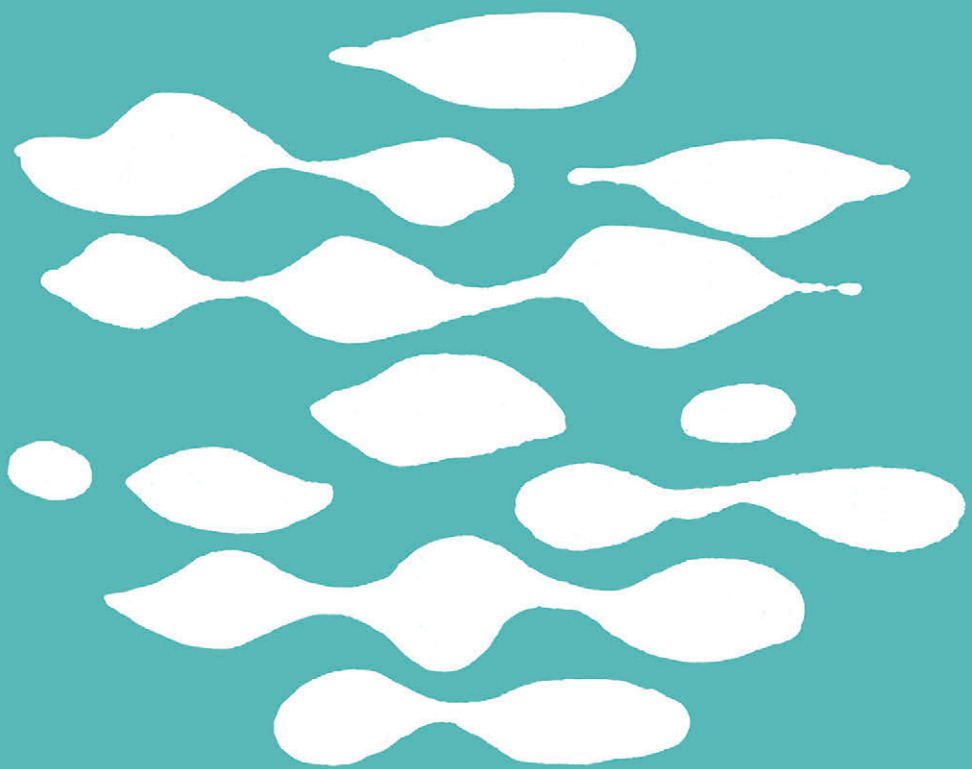
DEVELOPMENTS IN HYDROBIOLOGY

Advances in Decapod Crustacean Research

edited by
José P.M. Paula, Augusto A.V. Flores and
Charles H.J.M. Fransen



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Advances in Decapod Crustacean Research

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Advances in Decapod Crustacean Research

Proceedings of the 7th Colloquium Crustacea Decapoda Mediterranea, held at the Faculty of Sciences of the University of Lisbon, Portugal, 6–9 September 1999

Edited by

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Preface

The Colloquia Crustacea Decapoda Mediterranea are devoted to decapod crustacean research, and organized on a 3-yearly basis by institutions of the Mediterranean geographical area. The scope of these meetings has progressively widened throughout the sequence of events, and presently welcomes contributions from crustacean research worldwide. The seventh Colloquium was held in Lisbon, Portugal, from 6 to 9 September 1999, at the Faculty of Sciences of the University of Lisbon.

The Scientific Committee of 7CCDM was composed of C. Almaça (Portugal), K. Anger (Germany), D. Calazans (Brazil), G. Charmantier (France), P. Clark (UK), P. Dworschak (Austria), W. Emmerson (South Africa), D. Felder (USA), R. Forward Jr (USA), C. Fransen (Netherlands), C. Froglia (Italy), R. Hartnoll (UK), R. Ingle (UK), D. Jones (UK), A. Koukouras (Greece), R. Manning (USA), P. Ng (UK), P. Noël (France), J. Paula (Portugal), H. Queiroga (Portugal), F. Sardà (Spain), F. Schram (Netherlands), R. Seridji (Algeria), E. Spanier (Israel), Z. Stevcic (Croatia), M. Turkay (Germany) and M. Vannini (Italy).

The Organizing Committee was based at the Marine Lab of Guia, University of Lisbon, Portugal, and was composed of C. Almaça, C. Bartilotti, R. Calado, A. Cartaxana, J. Cruz, M. Dornelas, T. Dray, A. Flores, O. Luis, A. Marçal, S. Morais, L. Narciso, R. Nogueira Mendes, A. M. Passos, J. Paula, O. Santos, J. Saraiva, A. Sousa Dias and Z. Stevcic.

During the event 12 plenary talks, 82 regular oral presentations, 101 poster presentations and 1 seminar were presented. The meeting hosted the 2nd Crustacean Larval Conference, and had a number of dedicated sessions, as the Mangrove Crustacea and the Thalassinid sessions. Within this publication papers from all sessions were invited, except for those presented for the larval conference which are published elsewhere.

We thank the Portuguese sponsors who generously provided financial support for the Colloquium organization and publication of these proceedings: Instituto do Mar – Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Fundação para a Ciência e Tecnologia, Fundação Luso-Americana para o Desenvolvimento and the Secretaria de Estado do Ambiente. A debt of gratitude is owed to *Hydrobiologia* (Kluwer Academic Publishers) and the Editor-in-Chief, Professor H. Dumont, for publishing the Proceedings as a special volume in the series *Developments in Hydrobiology*. We would like to thank all members of the Organizing Committee and the many students and technicians involved in the organization of the meeting. In particular we thank the facilities provided in the University of Lisbon by Prof. C. Almaça at the Department of Zoology and Anthropology, Prof. F. Catarino at the Botanical Garden, and Prof. J. Pinto Paixão at the Faculty of Sciences.

JOSÉ PAULA
AUGUSTO FLORES
CHARLES FRANSEN
(guest editors, on behalf of the Organizing and Scientific Committees of the 7CCDM)


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Phylogeny of decapods: moving towards a consensus

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Key words: cladistic analysis, Decapoda, developmental genes, fossils, morphology, phylogeny, sequence data

Abstract

Although the recognition of four broad groups within Decapoda – natantians, macrurans, anomurans and brachyurans – has long been a staple of textbooks and even the primary taxonomic literature, a precise resolution of phylogenetic relationships within the order has proved more difficult. Indeed, there have been as many schemes of decapod taxonomy and phylogeny as there were experts who wished to offer an opinion. In this decade, utilization of explicit cladistic methods of analysis and the application of molecular techniques have produced a series of clear hypotheses concerning the relationships within many of the groups of Decapoda. It is apparent that earlier conflicts of opinion can be related in part to the implicit problems of dealing with paraphyletic groups near the base of the tree that are too broadly defined by only general or plesiomorphic features. Comprehensive morphological analyses of both fossil and living forms, with attention being paid to defining synapomorphies, can lead to resolution of old controversies. Molecular techniques hold great promise towards providing further resolution, but currently suffer from insufficiencies of sampling. Nevertheless, where once there was chaos and vexation, there is now some enlightenment. The situation can only improve, but the broad outlines of decapod deep history are already emerging.

Introduction

There have been as many taxonomies and schemes of phylogeny for the Decapoda as there have been experts willing to offer an opinion. Sometimes, experts have been willing to offer more than one opinion. Burkenroad (1963, 1981) held different views at different times, erecting the Pleocyemata in 1963 to contain all abdominal egg-brooders (Table 1) to general acclaim but then abandoning use of the term in 1981 (Table 2), though the clade clearly remained on his cladogram (Fig. 1). Textbooks typically often still employ terms like Natantia and Macrura in classifications, whereas among specialists these terms have fairly well passed out of formal taxonomic use (Tables 1 and 2). Natantians and macrurans are now perceived as stages in the evolution of decapod body plans, and even anomurans are coming to be interpreted in this same light (cf. Burkenroad, 1981; Scholtz & Richter, 1995). Nevertheless, one can still find Anomura employed as a taxon (Table 1), even as a consensus is now emerging that this group is paraphyletic. Only the Brachyura among the old classic suborders is now perceived as a real monophyletic group.

There are many reasons for these disagreements. First, they arise from the differences in perception about the basic nature of taxa that have their roots in phylistic *versus* cladistic approaches to classification and tree building. The old phylistic approaches of evolutionary systematics (Rasnitsyn, 1996) treat primitive groups as a monophylum by uniting them on the basis of plesiomorphic features alone. Macrurous natantians do not form a true monophylum in the cladistic sense since their long-tailed, swimming habitus is essentially a primitive one.

Second, differences can arise from whether or not fossils are included within an analysis. As an example, Schram & Hof (1998) clearly demonstrate what can happen when fossils are included or deleted from an analysis; major shifts of clades can occur. The lesson to be drawn from that exercise is that, while fossils may be frustrating to deal with, often lacking

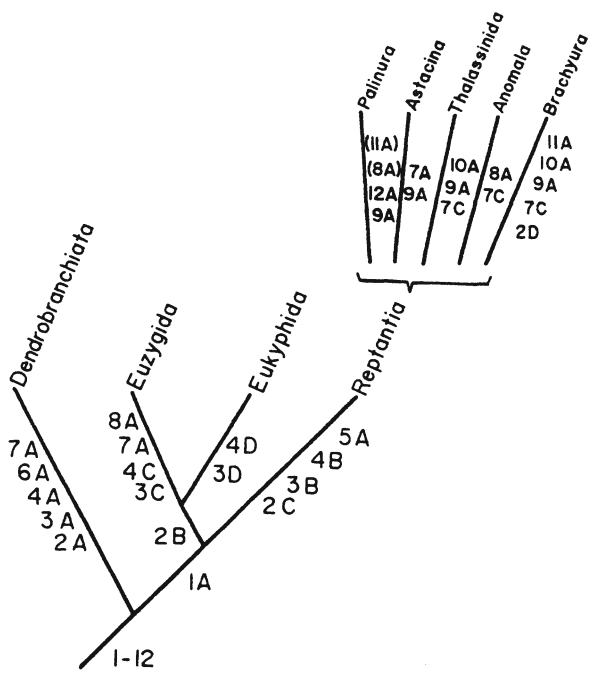

Figure 1. Cladogram of decapod relationships from Burkenroad (1981). Although the clade itself is characterized by a good apomorphy (1A = pleopod brooding of eggs) and is widely accepted among decapod workers, Burkenroad deliberately chose not to recognize the Pleocyemata in this paper. In addition, while Burkenroad believed there were five clearly defined Supersections of Reptantia, his characters could not further define relationships within that Suborder. For details concerning characters, consult Burkenroad (1981).

information we may wish we had, they nonetheless often contain enough information that in fact helps determine the basic structure of phylogenetic trees.

Third, we need to be very careful about how we use characters. This is especially crucial in terms of the use of soft-anatomy features observable only in living forms. For example, information from molecular sequences, developmental genetics, and/or neuroanatomy might seem to indicate apparently robust sister groups (Fig. 2a). However, more inclusive and comprehensive analyses, including larger arrays of characters and/or taxa (Fig. 2b), might actually argue against such groups (see Jenner, 1999; Jenner & Schram, 1999; Schram & Jenner, 2001).

Because of limitations of space, what follows is only a very general overview of some of the issues currently at play in discerning the phylogeny of the Decapoda; and it remains a very personal one at that since it focuses on such matters as have drawn my attention for one reason or another or struck my fancy.

Table 1. Classification of Decapoda from Glaessner (1969)

Order Decapoda Latreille, 1803
- Suborder Dendrobranchiata Bate, 1888
  - Superfamily Penaeoidea de Haan, 1849
  - Superfamily Sergestoidea Dana, 1852
- Suborder Pleocyemata Burkenroad, 1863
  - Infraorder Caridea Dana, 1852
- Infraorder Stenopodidea Huxley, 1879
  - Infraorder Uncinidea Beurlen, 1930
  - Infraorder Astacidea Latreille, 1803
- Infraorder Palinura Latreille, 1803
  - Superfamily Glypheoidea Winckler, 1883
  - Superfamily Eryonoidea de Haan, 1841
  - Superfamily Palinuroidea Latreille, 1803
- Infraorder Anomura H. Milne-Edwards, 1832
  - Superfamily Thalassinoidea Latreille, 1831
  - Superfamily Paguroidea Latreille, 1803
  - Superfamily Galatheoidea Samouelle, 1819
  - Superfamily Hippoidea Latreille, 1825
- Infraorder Brachyura Latreille, 1803
  - Section Dromiacea de Haan, 1833
    - Superfamily Dromioidea de Haan, 1847
    - Superfamily Homoloidea White, 1847
    - Superfamily Dakoticancroidea Rathbun, 1917
  - Section Oxystomata H. Milne-Edwards, 1834
    - Superfamily Dorripoidea de Haan, 1841
    - Superfamily Calappoidea de Haan, 1833
    - Superfamily Raninoidea de Haan, 1833
  - Section Oxyrhycha Latreille, 1803
  - Section Cancridea Latreille, 1803
  - Section Brachyrhycha Borradaille, 1907
    - Superfamily Portunoidea Rafinesque, 1815
    - Superfamily Xanthoidea Dana, 1851
    - Superfamily Ocypodoidea Rafinesque, 1815

A more inclusive treatment will have to be presented elsewhere.

Morphology and a natural taxonomy

Of course the 'Holy Grail' of all our work is to arrive at a system of classification that reflects the phylogeny of the Decapoda, and vice-versa. When I accepted the invitation to prepare a contribution of this subject, I naïvely thought that the effort would be a straightforward one and that I could report a complete and acceptable phylogeny of the Decapoda. The issue, naturally, is a lot more complicated than I thought. While

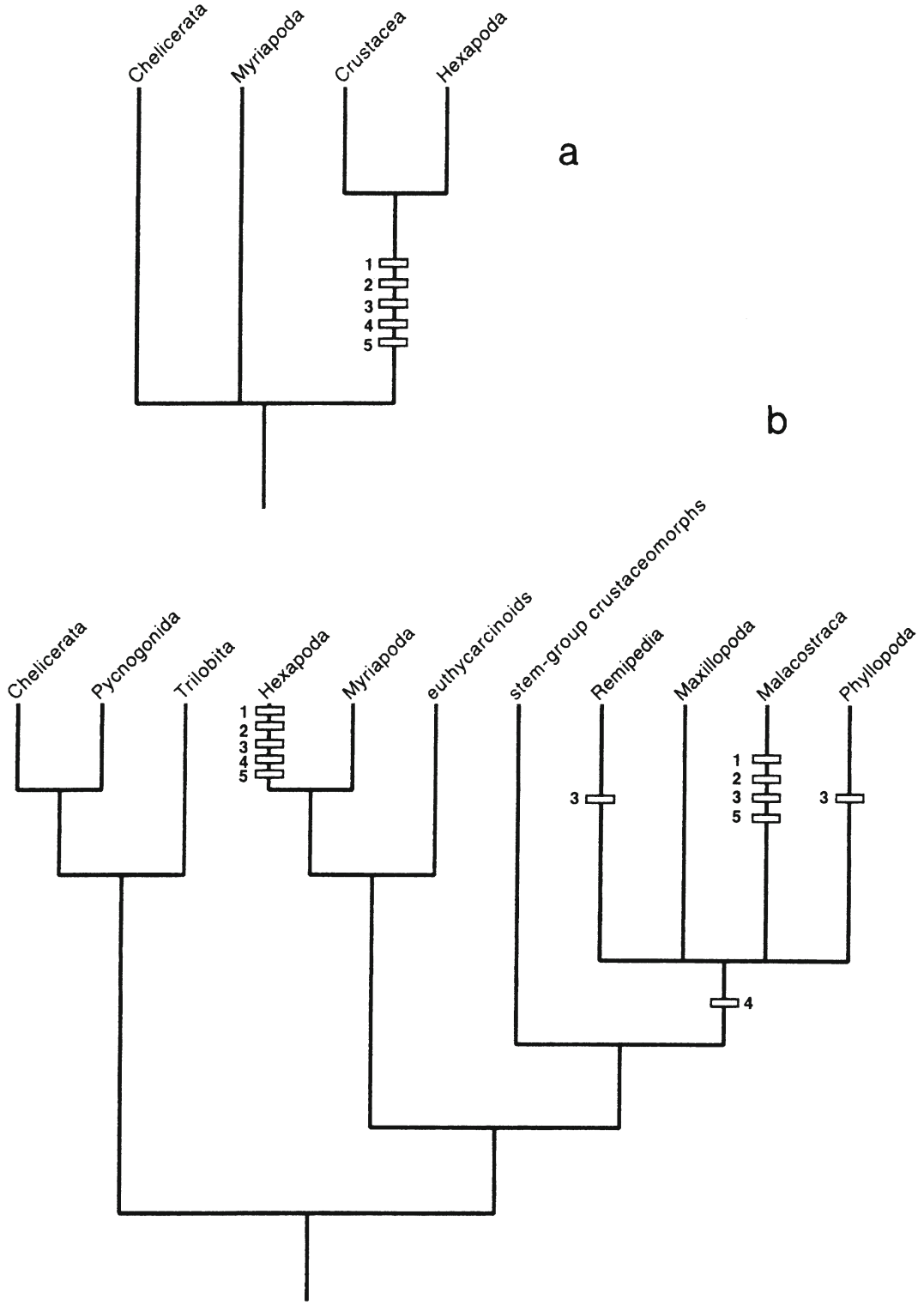

Figure 2. Hypothetical cladograms from Schram & Jenner (2001). (a) A very restricted (pruned) phylogeny of arthropods, and (b) a more comprehensive phylogeny including different crustacean types, pycnogonids and fossil arthropods. Although a particular set of characters may indicate an apparently well-supported clade, addition of other taxa, especially fossil groups, can in fact suggest a distinctly different alternative hypothesis. 1: complex neural chiasmata; 2: pattern of axon growth; 3: distinctive mitochondrial gene order; 4: ommatidia composition in compound eye; 5: neuroblast form. (For details, consult Jenner & Schram, 1999.)

Table 2. Classification of Decapoda modified from Schram (1986)

Order Decapoda Latreille, 1803
Suborder Dendrobranchiata Bate, 1888
Superfamily Penaeoidea de Haan, 1849
Superfamily Sergestoidea Dana, 1852
Suborder Eukyphida Boas, 1880
Infraorder Procaridídea Felgenhauer & Abele, 1983
Infraorder Caridea Dana, 1852
Suborder Euzygida Burkenroad, 1981
Infraorder Stenopodidea Huxley, 1879
Infraorder Uncinidea Beurlen, 1930
Suborder Reptantia Boas, 1880
Infraorder Astacidea Latreille, 1803
Infraorder Thalassinidea Latreille, 1831
Infraorder Palinura Latreille, 1803
Infraorder Anomala Boas, 1880
Infraorder Brachyura Latreille, 1803
Section Dromiacea de Haan, 1833
Section Archeobrachyura Guinot, 1877
Section Eubrachyura de St. Laurent, 1980
Subsection Heterotremata Guinot, 1977
Subsection Thoracotremata Guinot, 1977

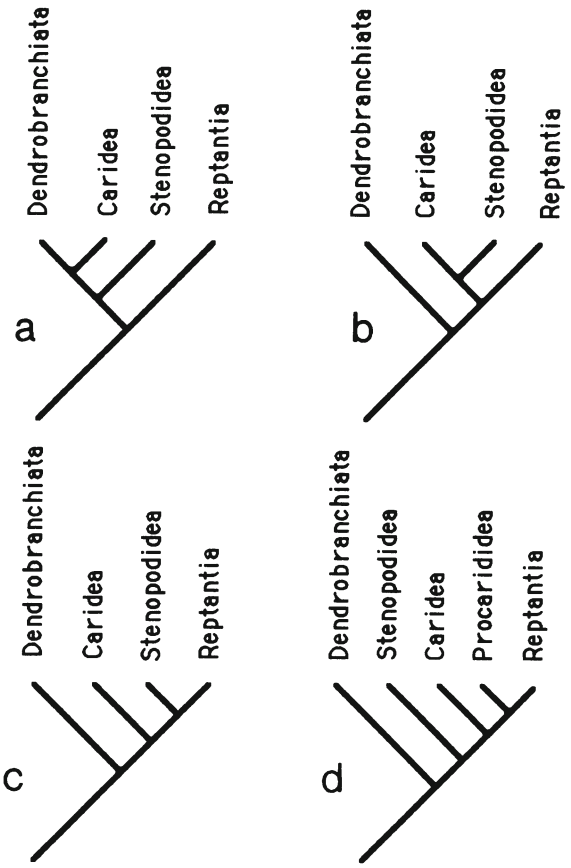

Figure 3. Various hypotheses of relationship among natant decapods. (a) From Borradaile (1907); (b) Burkenroad (1963, 1981); (c) De St. Laurent (1979), Abele & Felgenhauer (1986), Abele (1991); (d) Felgenhauer & Abele (1983). The current consensus favors the tree in (c).

there is a growing consensus about some parts of the decapod family tree, other sectors will take much more work to resolve. However, we are not there yet. For instance, a few years ago, there were several alternative schemes for the relationships of the natantian groups to each other (Fig. 3). Today, one of these is gaining the upper hand (Fig. 3c). Nevertheless, within natant groups, such as the Caridea, work on elucidating phylogenetic relationships is only proceeding very slowly (e.g. see Christoffersen, 1987, 1988, 1989, 1990).

The central core for all this right now remains morphology. There are other important sources of information to be sure, as will be seen below. However, at this time, morphology still forms the only comprehensive database. In this regard, a major step forward occurred with the publication of the overview of Scholtz & Richter (1995). While their treatment focused on the phylogeny of the Reptantia, their inclusion of a wide array of out-group taxa ensured that the basis existed for a more comprehensive analysis. The investigation of Scholtz & Richter (1995) employed the 'Method of Hennig,' essentially a paper and pencil approach that relies on the *a priori* recognition of ground patterns. They employed some 63 binary characters to sort 44 in-group taxa and polarised their data set employing 6 out-group species. This resulted in the recognition of 7 monophyletic clades [Polychelida (Achelata (Homarida (Astacida (Thalassinida (Anomala, Brachyura)))))] in an essentially asymmetrical cladogram (Fig. 4). The relationships seemed well supported, except for the position of the Astacida, for which Scholtz & Richter (1995) could not choose between it being a separate clade positioned between the Homarida and the Thalassinida, or a sister group to the latter.

Examination of the character set of Scholtz & Richter (1995) uncovered some duplication of features: e.g. their characters D3 and J3, which both deal with a lack of chelae on pereiopods; or G1 and L5, which both involve the mobility of the last thoracic sternite, the so-called 'fractostern,' a most important feature in their matrix. In addition, some binary fea-

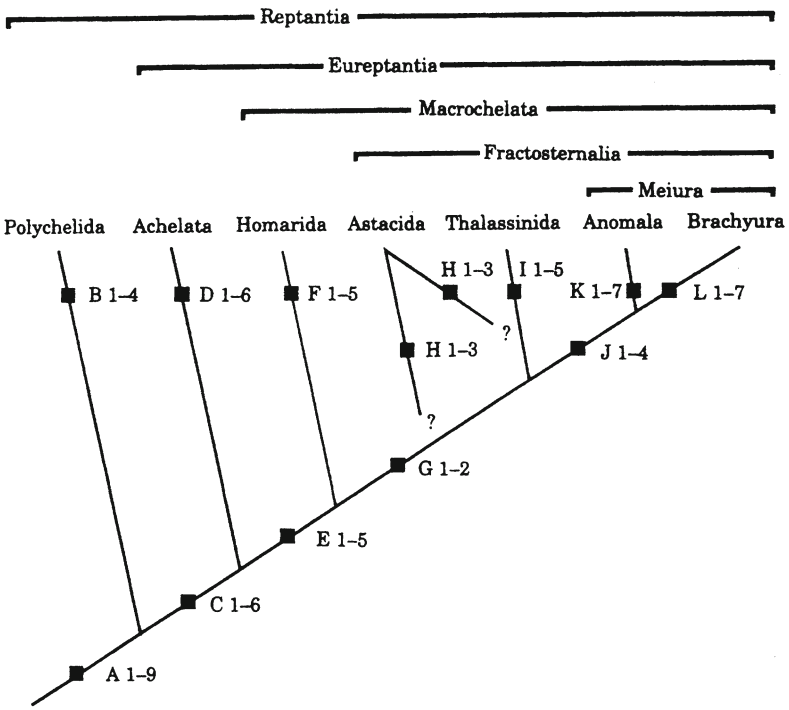

Figure 4. Cladogram of relationships of the Reptantia with suggested names of inclusive clades. For details of apomorphic features consult Scholtz & Richter (1995).

tures that deal with larval types (B4, D6, F4 and H3) result in inappropriate character scorings when employed separately and require a multi-state approach to establish consistency. Even so, a conversion of their raw data into a numerical matrix suitable for a parsimony analysis by PAUP* 4.0 resulted in a duplication of their original result (Fig. 5), with some exceptions. Astacida definitely emerged as a separate clade, sister to all the other Fractosternalia. However, relationships within the Homarida are far from absolutely clear. *Enoplometopus debelius* emerges as a separate clade in a strict consensus tree, something already suspected as a possibility by Scholtz & Richter (1995: 319), while the rest of the Homarida remained unresolved. Only in a 50% majority rule tree (not shown), in which *Thaumastocheles zaleucus* appeared in a separate clade between the Homarida and the Fractosternalia, do the rest of the Homarida occur as a resolved clade. However, a problem arose at this stage in my analysis in that because of the great redundancy in the taxon list, some 32 700 trees resulted before a memory overload occurred. So, while the main clades of Scholtz & Richter (1995) appeared for the most part in the final result, no resolution was possible of course within clades.

To facilitate the use of the database of Scholtz & Richter (1995) with additional taxa, and to allow incorporation of new features, I recast the 63 original characters to eliminate redundancies and inappropriate scorings to yield a base list of 59 features. I then took the features from Burkenroad (1981) and added them to the character list where appropriate to arrive at 65 characters. This allowed the natant out-group taxa of Scholtz & Richter (1995) to be taken into the analysis, with *Euphausia* sp. then serving as a new out-group. The resulting 14 400 trees duplicated the results earlier for the Reptantia alone and also arranged the natantians into a transition series near the base of the tree (Fig. 6).

The next step was to remove the redundancy of the taxon list by removing taxonomic equivalents (Wilkinson, 1995). Representative species were selected for the clades that had consistently appeared up until this point. After that was done, some 18 trees resulted, although the resolution among the basal natantians evident in the previous analysis disappeared (Fig. 7). *Enoplometopus debelius* continued to appear in a separate clade. At this point, though the character set certainly can be refined further, I believe that we have a basic data set that can begin to be employed 'exper-

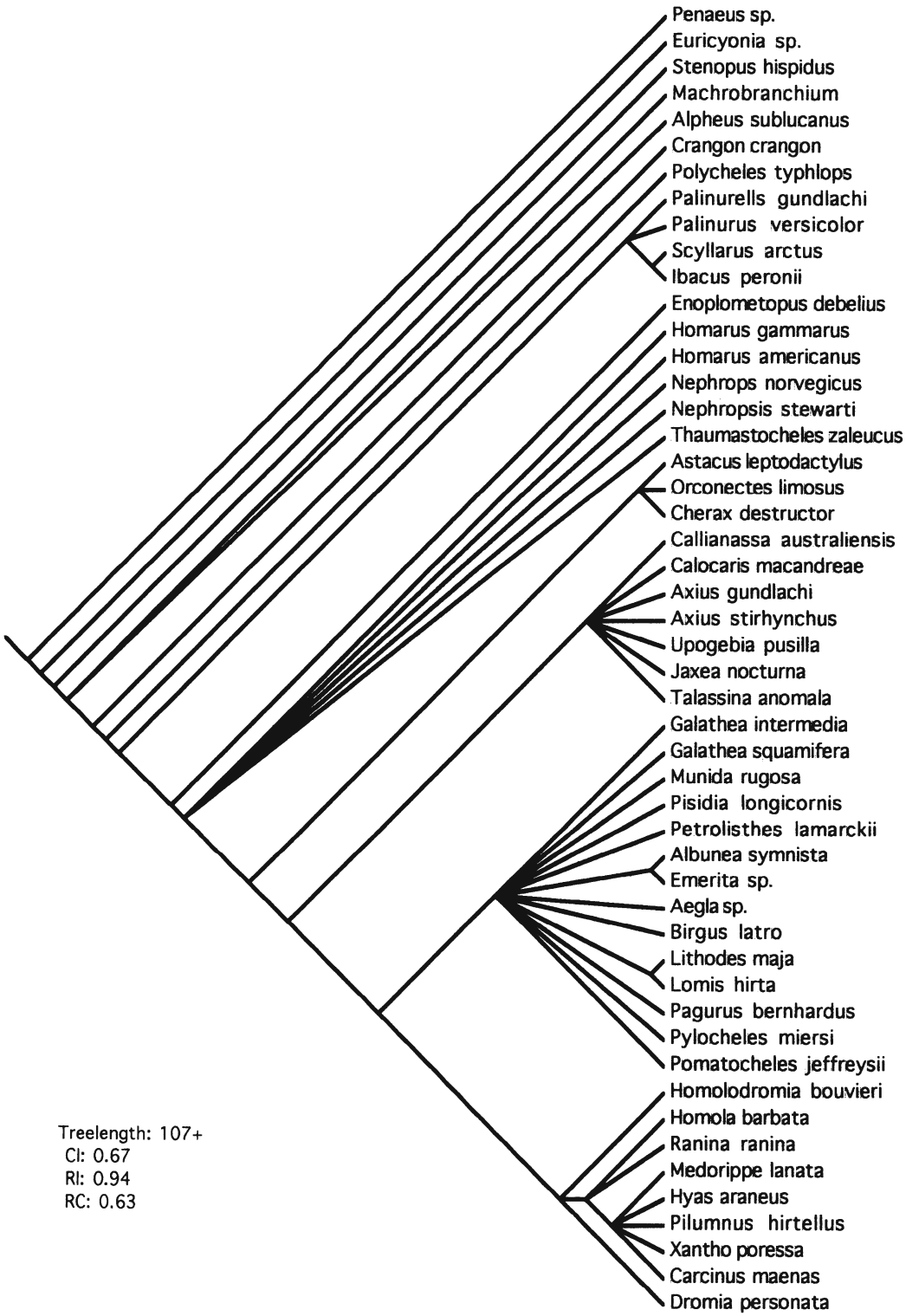

Figure 5. The strict consensus of 32 700 trees of Reptantia that resulted from the analysis with PAUP* 4.0 of a data matrix derived directly from that of Scholtz & Richter (1995). Diagonal format employed to emphasize polychotomies.

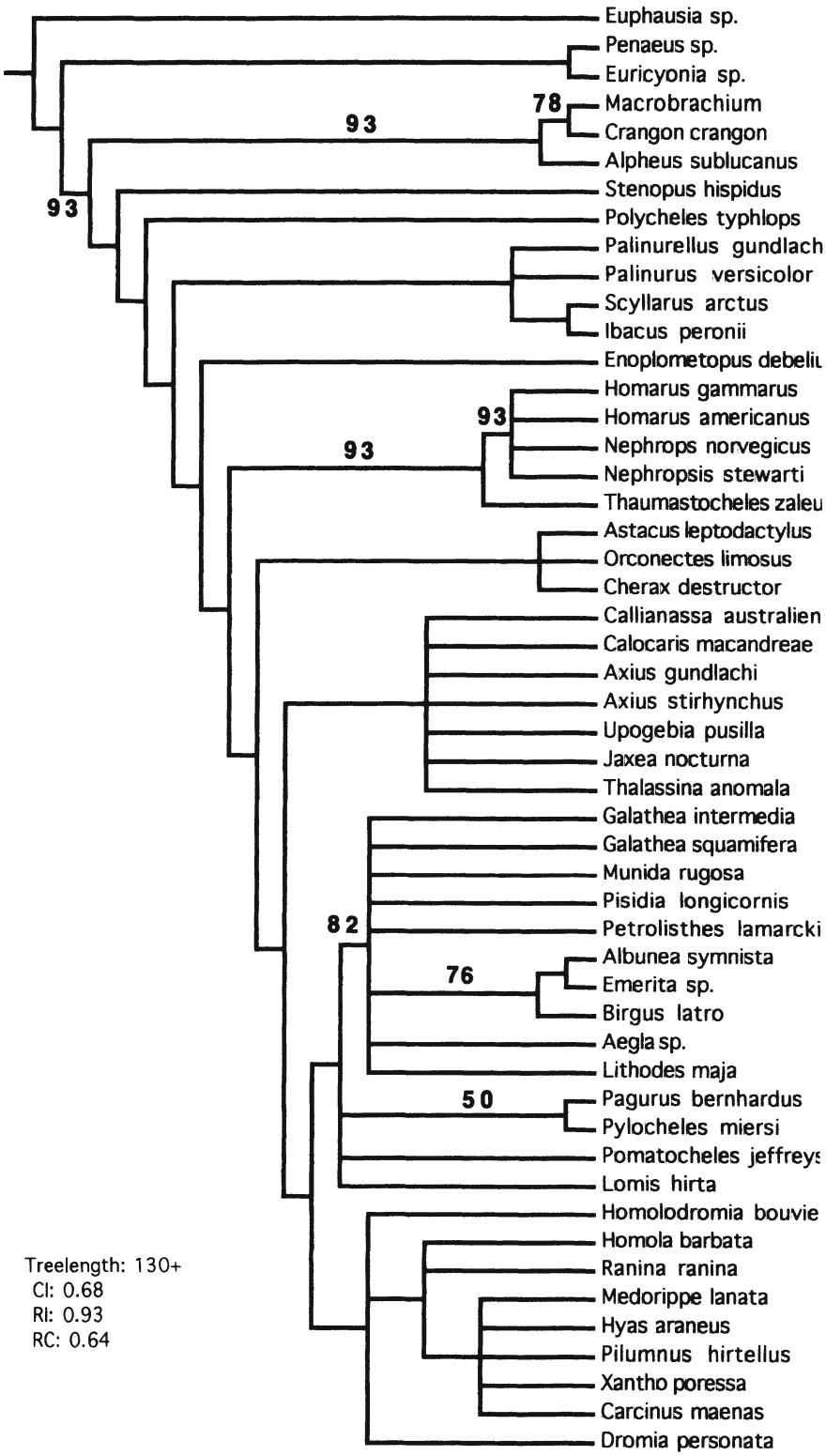

Figure 6. The 50% majority rule tree of 14 400 trees of Decapoda resulting from a reconfigured character set from that used in Figure 5 (see text for details) employing the features derived from Scholtz & Richter (1995) with the addition of characters from Burkenroad (1981). All branches 100% unless otherwise noted.

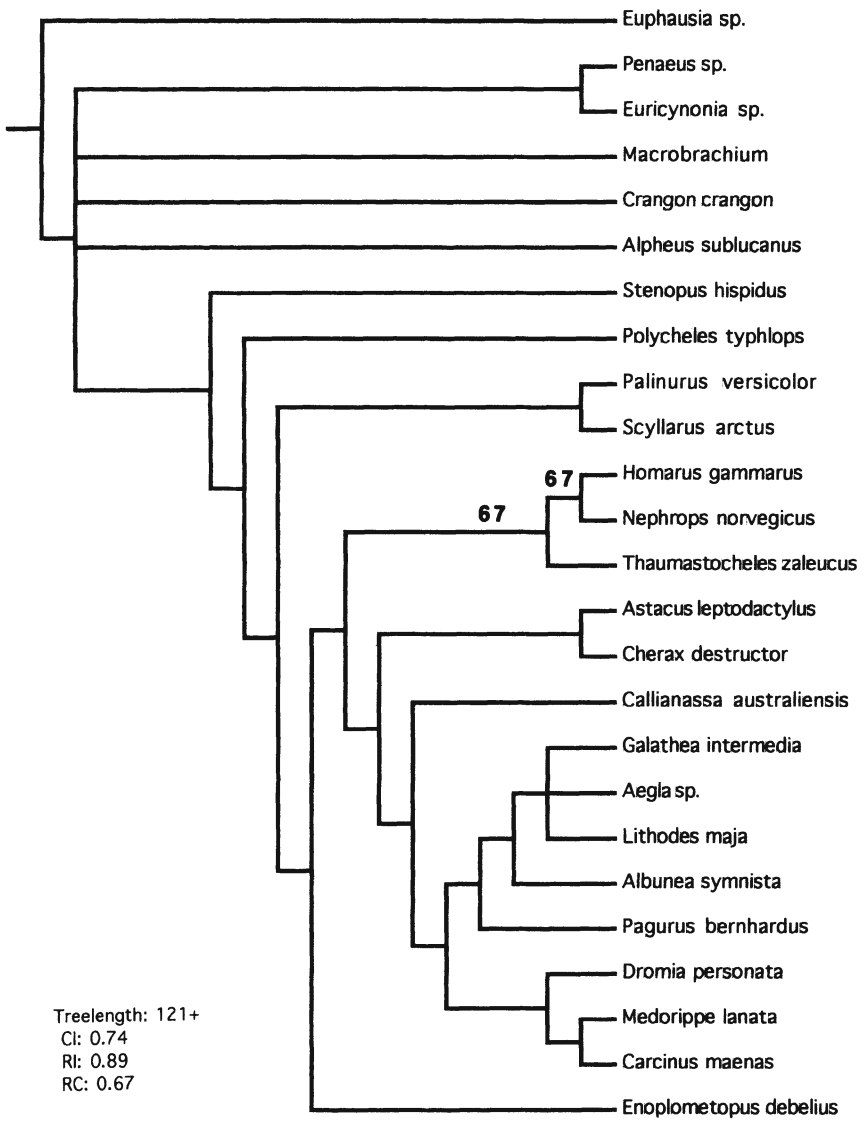

Figure 7. The 50% majority rule tree of 18 trees of Decapoda resulting from a reduced taxon list (same characters used in Fig. 6). All branches 100% unless otherwise noted.

imentally.' Toward that end, I decided to assess the position of taxa, both fossil and living, not included in the original set. As a test, I scored *Neoglyphea inopinata* for the features in my character list. In most classic schemes (Table 1), the Glypheoidea are included within the Palinura, and thus I expected to see *Neoglyphea* emerge fairly low in the tree. However, in this case (Fig. 8), *Neoglyphea* appeared in a polychotomy with higher fractosterns! Scholtz & Richter (1995: 304) suggested as much. Admittedly, my initial scoring of characters was based only on my reading of the excellent description and illustrations of Forest & De Saint Laurent (1981). However, study of the type specimens and related skeletal preparations made by De Saint Laurent in the collections of the Paris Museum confirmed that *Neoglyphea inopinata* in fact possesses the two diagnostic apomorphies of the Fractosternalia, an articulated eighth thoracic sternite or fractostern, and a secula with three sclerites.

Nevertheless, the results of the analysis so far indicate two things. We may agree about the sequence of clades among natantians and that there is a clade Meiura high in the tree. However, the evolutionary events and relationships among the 'macrurans' in the middle of the tree will require a great deal more investigation. The answers may not be easily forthcoming

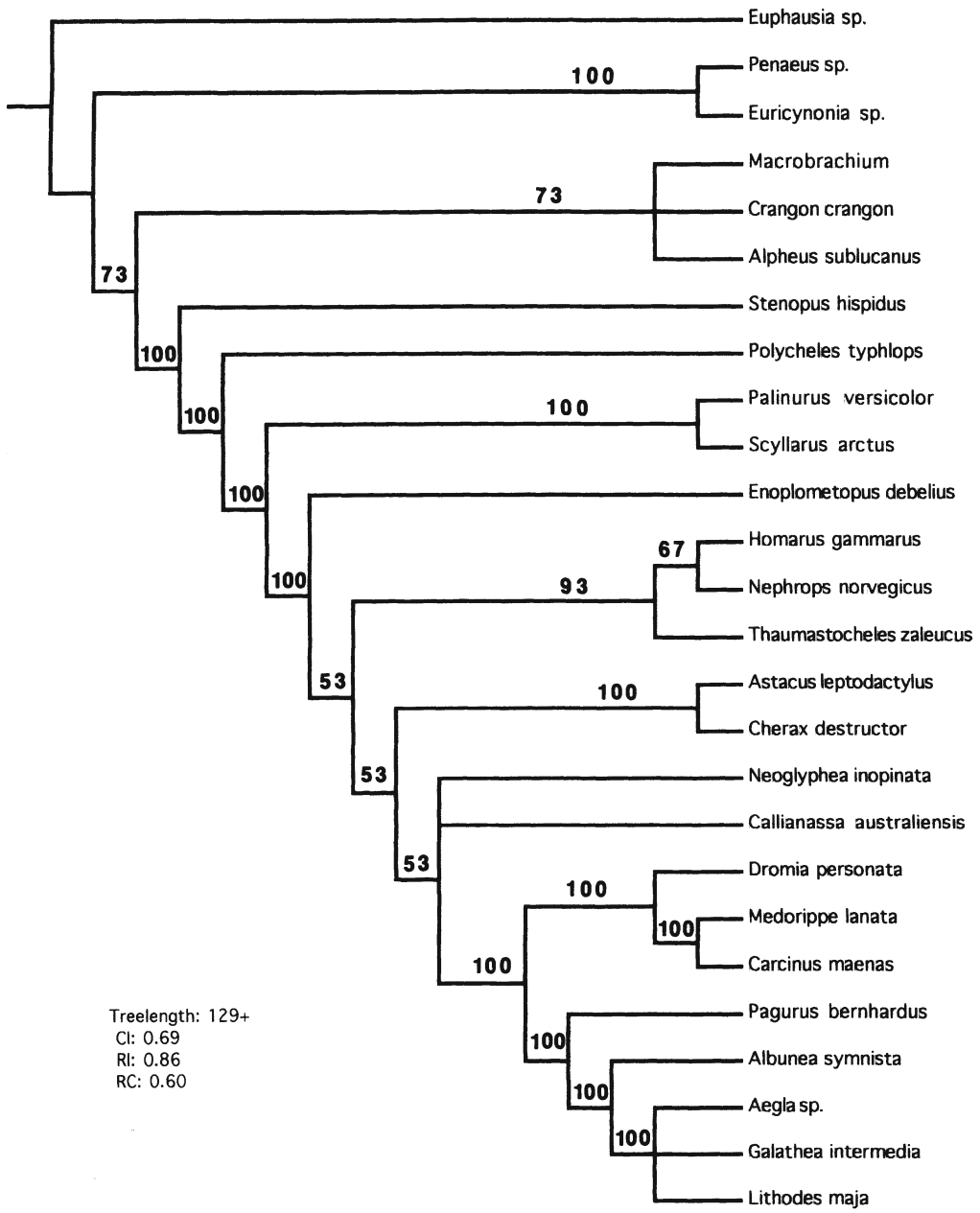

Figure 8. The 50% majority rule tree of 45 trees of Decapoda resulting from the same data set as Fig. 7 except for the addition of *Neoglyphea inopinata*.

either, since an important source of information about biodiversity in this part of the tree will have to be based on fossils. The fossil taxa could be difficult to compare directly with the wealth of information available from examination of living forms. Nevertheless, if we recast the tree of Figure 8 into a stratigraphic context (Fig. 9), we can see that a tremendous number of discoveries in the fossil record of decapods await us.

Anomala: the use of different sources of evidence

The issue of Anomura and Anomala have vexed carcinologists almost since the word 'Go' (for a summary, see McLaughlin & Holthuis, 1985). Nevertheless, a fine example of the wide range of studies that are going on relevant to decapod phylogeny is provided by study of the Anomala. McLaughlin (1983a, b) began to deal with the issue of relationships from a morpho-

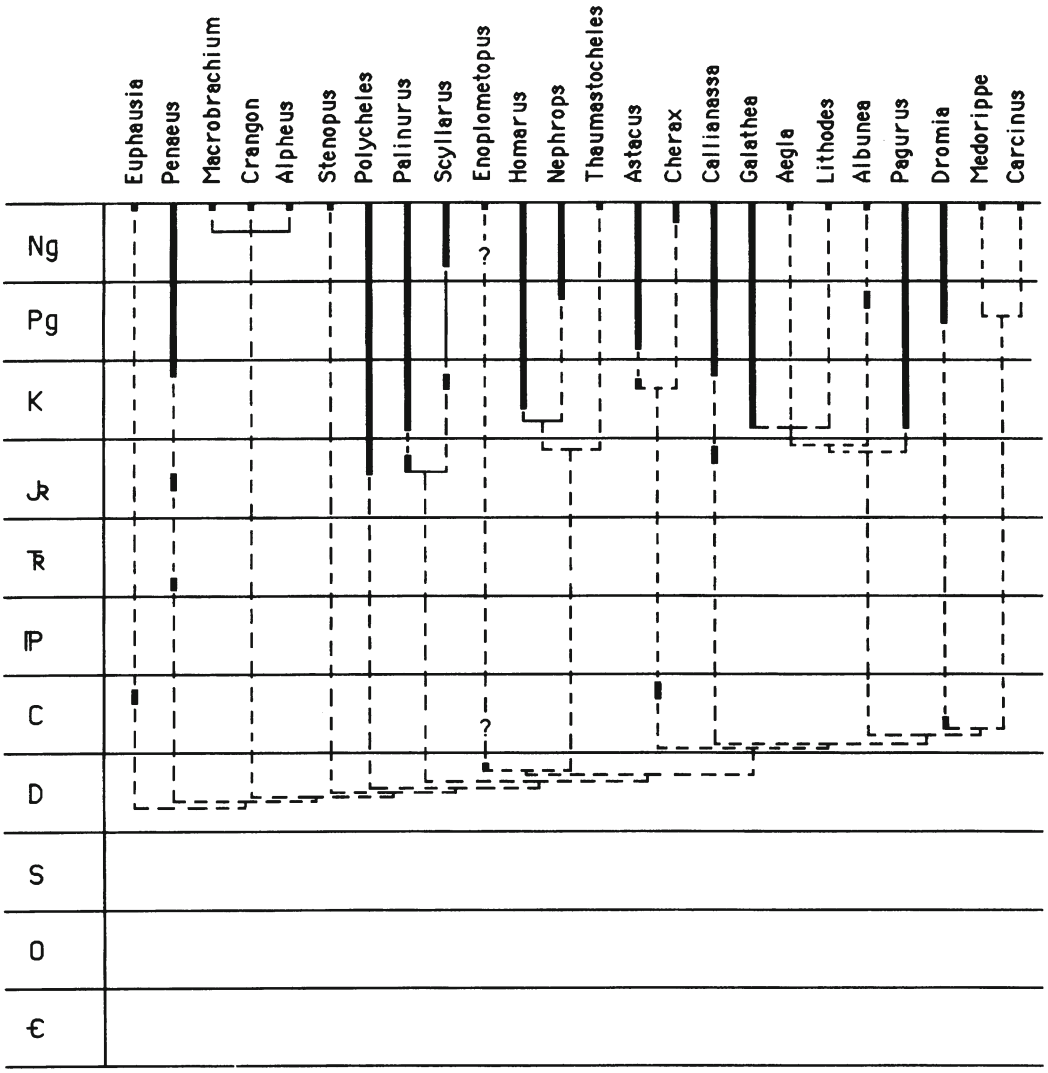

Figure 9. The tree of Fig. 7 rendered into a stratigraphic context. The Carboniferous euphausiacean is linked to certain possible such fossils known from various Coal Age Lagerstatten (see Schram, 1986), and the Carboniferous astacidan is suggested from probable burrows of such (see Hasiotis, 1999). For the sake of convenience, the Devonian 'lobster' genus *Palaeopalaemon* is linked to *Enoplometopus*, although the basis for this must be further explored. The carboniferous dromiacean based on the genus *Imocaris* (see Schram & Mapes, 1984). Note the considerable array of ghost ranges and phantom lineages (dashed lines). Question mark indicates uncertainty about linking *Palaeopalaemon* with the living enoplometopids.

logic perspective in examining the position of *Lomis* and exploring the question of 'what is a hermit crab?' Along these same lines, i.e. focusing on individually important taxa to extrapolate to larger scale issues of phylogeny, Martin & Abele (1986) proposed a family level phylogeny for Anomura that grew out of their study of the genus *Aegla*.

The analysis of Martin & Abele (1986) recognized separate thalassinidean and anomalan clades. However, their study illustrates quite effectively several very important issues of concern. First, they produced a well-resolved phylogeny of 'anomuran' families. However, in doing so, they got out exactly what they put into it. The data were analysed at a family level, and what they achieved was a phylogeny of families. As we will see, analyses by other authors at a genus and species level (e.g. see Richter & Scholtz, 1994) have called into doubt some of the families and superfamilies within the Anomala. One needs to be careful how data are entered into any computer-driven phylogenetic analysis, since it is on the basis of those data that the patterns will be analysed.

Second, Martin & Abele (1986) provide trees derived from both a phenetic UPGMA clustering program and a cladistic parsimony analysis. In doing so, they nicely illustrate the care that needs to be taken with programs that group on the basis of strict similarity, i.e. phenetic analyses, since such approaches fail to sort out relationships among 'primitive' groups, often lumping them into clades near the bases of trees. Thus false signals of monophyly may be indicated, when paraphyly may in fact more accurately describe the relationships.

Third, a phylogenetic analysis can only work with the taxa that are put into the programs. While the Martin & Abele (1986) hypothesis for anomuran phylogeny would appear to emerge as indeed very robust, with lots of congruent characters supporting branches, it is essentially a phylogeny of *only* anomurans rooted to a phylogenetically distant genus *Penaeus*. In these analyses, clades are drawn based on either shared derived features, or degrees of similarity of the taxa given. If more proximal out-groups were utilized, or if additional taxa, in this case brachyurans, were used to effectively sort relationships among an entire potential monophylum, what we could call in the terminology of Scholtz & Richter (1995) the Fractosternalia, it is possible that other hypotheses of relationships could have emerged. Martin & Abele (1986) is a fine study, and I have no argument at all with their results, which are explicitly presented as hypotheses only. However, we all need to keep in mind the nature of the data we put into these analyses, both in terms of the characters as well as the taxa (Jenner & Schram, 1999).

Unless we perform comprehensive cladistic analyses, we cannot be sure that we are in fact dealing with monophyla. Tudge (1997) employed an entirely different source of data towards elucidating relationships of 'anomurans' when he examined ultrastructure of sperm and spermatophore morphology. Although the principal focus was directed at 'anomurans,' a wide array of decapods including astacids, homarids and brachyurans were also analysed. Even though the character set was narrowly cast towards sperm only, the resulting tree structure is interesting (Fig. 10). Thalassinideans emerge as polyphyletic and, while Anomala itself is monophyletic, most families of anomalans are either para- or polyphyletic. The wide range of taxa used, grounded in a rather comprehensive database of characters yields *a* phylogeny and certainly indicates that spermatozoan ultrastructure will be an important source of data in more comprehensive, total evidence approaches to the issues of anomuran phylogeny.

Finally, there are times in which restricted analyses can be useful. Cladistic analyses need not always be directed at producing a phylogeny *per se*. McLaughlin & Lemaitre (1997) were actually only interested in assessing old ideas about the processes and occurrences of carcinization. Their data were collected and analysed at a generic level and might appear to 'demolish' many well-established family and superfamily taxa. However, the authors caution that what they focused on in the analysis were only features directed at assessing degrees of carcinization and not the total array of hard morphological features that might have been employed in a more comprehensive analysis. McLaughlin & Lemaitre (1997) arrived at a fresh understanding of what carcinization actually represented, and in the process they clarified the supposed relationship between lithodids and pagurids.

Brachyura and the use of molecules and sperm

No treatment of decapod phylogeny can escape consideration of molecular issues. However, up until this point, there have been relatively restricted uses of molecular sequence data, although the number of research groups generating and using sequence data is growing. For example, Kim & Abele (1990) and Abele (1991), as part of a larger program to address crustacean phylogeny with 18S rRNA and 18S rDNA data (e.g. see Spears & Abele, 1997), examined the relationships of natant taxa to each other using a limited data set and largely confirmed the results derived from morphology (Abele & Felgenhauer, 1986).

However, one area of study where I believe molecule sequences will be of immense help will be in elucidating the phylogenetic relationships of Brachyura. Ever since the benchmark work of Guinot (1978, 1979), which recognized three groups of brachyurans based on location of male gonopores, the phylogeny of the Brachyura has attracted strong interest. Very quickly after Guinot, De Saint Laurent (1980a, b) elucidated the essentially paraphyletic nature of Guinot's Podotremata while offering a caution against relying too heavily on gonopore locations alone. Subsequently, Spears et al. (1992) using 18S rRNA confirmed the paraphyly of the podotremes (Fig. 11). However, Guinot et al. (1994) in examining sperm structure in Homolidae concluded that a podotreme type sperm could be characterized and thus used

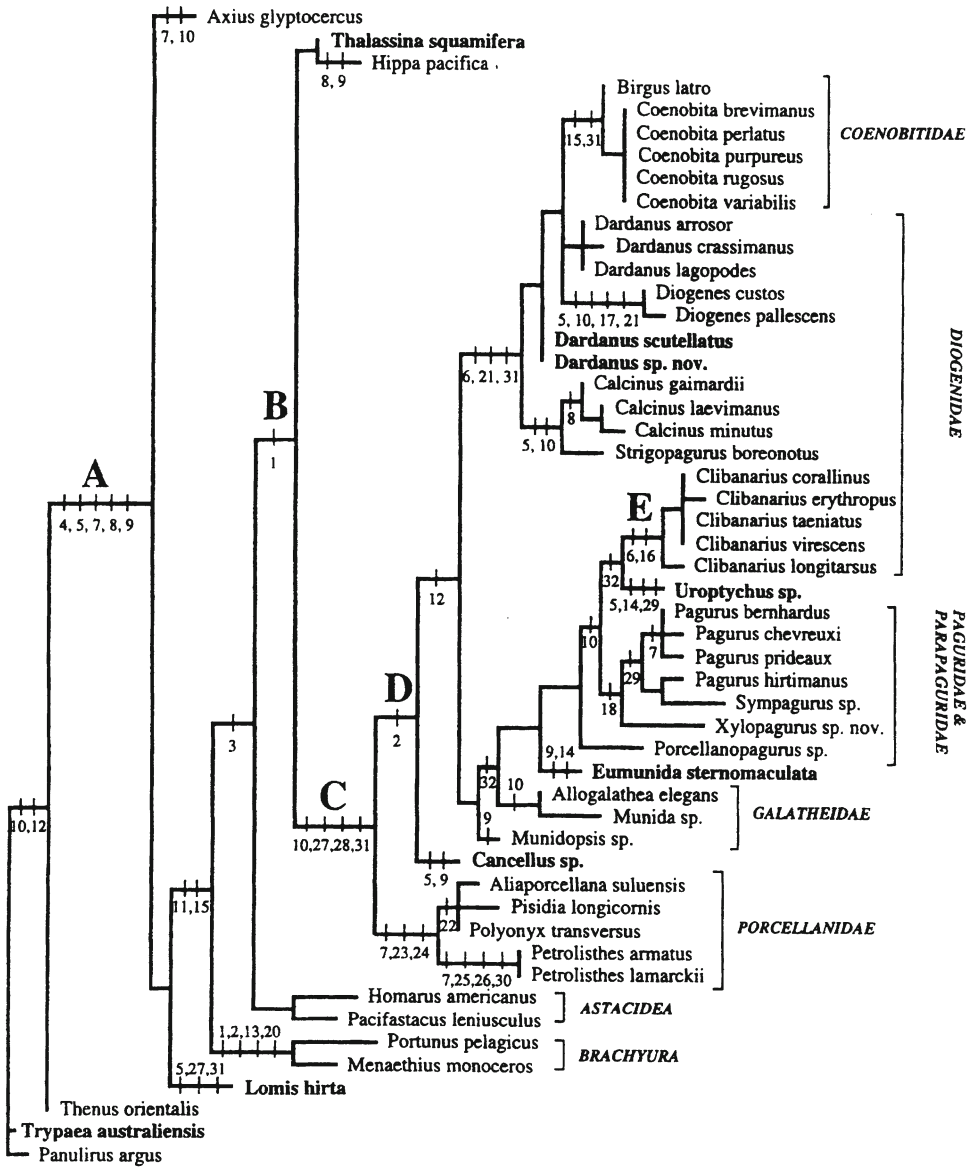

Figure 10. A phylogram for anomurans of a 50% majority rule tree derived from 26 equally parsimonious trees based on analysis of only spermatologic features (see Tudge, 1997 for details). Note the polyphyletic Thalassinida with this data set. Also, while Anomala is monophyletic, the constituent families are mostly para- or polyphyletic.

to justify a monophylum Podotremata. Nevertheless, Guinot et al. (1998) pointed out that, while a dromiacean sperm type could be defined, neither Dromiidae nor Dynomenidae would appear to be monophyletic based on sperm characters alone. Clearly, more comprehensive studies of sperm and molecular sequences of rDNA are needed.

Moreover, within the Heterotremata and Thoracotremata, the situation is far from resolved. The old, classic Sections of the Brachyura from Borradaile (1907) no longer seem very effective. Most authorities these days settle for grouping families within more inclusive superfamilies. However, nested sets of relationships remain obscure. Recently, Schubart et al. (2000) have begun to build a database of 16S rDNA for Eubrachyura with some intriguing results (Fig. 12). While the thoracotremes cluster in a monophyletic clade (with a problematic inclusion of pinnotherids), the heterotremes as a whole would appear to be characterized as more-or-less paraphyletic. This is not a

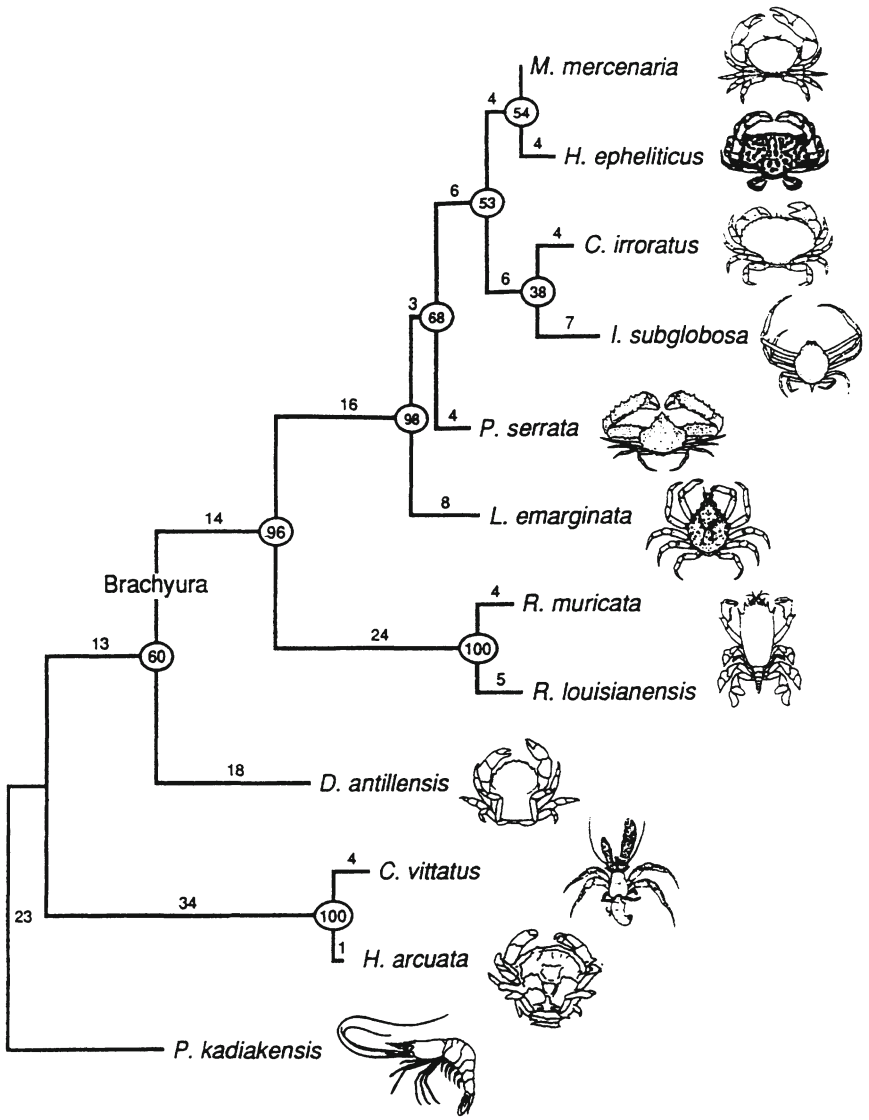

Figure 11. The inferred relationships of Meiura, with the number of steps indicated for each branch, based on analysis of 18S rRNA and confirming the para- or possibly even polyphyletic nature of the podotrematous brachyurans (from Spears et al., 1992). Circled numbers indicate bootstrap values.

complete analysis of all families, although the authors have done additional work (Schubart, pers. com.), and the authors need to include additional relevant outgroups. However, current sequence banks for even 18S rDNA do not contain a full array of brachyurans. Such comprehensive analyses from several molecules will be necessary before we can seek a solution to this problem. In addition, there is no reason to doubt that a more broadly based examination of brachyuran comparative anatomy (cf. Von Sternberg et al., 1997) and larvalogy (in the manner of Rice, 1980, 1983) could make contributions as well towards a final synthesis.

Astacida: a focal point of many problems

Let us return to that array of macrurans in the middle of the decapod tree that will probably continue to give us trouble for some time to come. In particular, I want to focus on the Astacida, the crayfish. An intriguing group, they seem to encapsulate in one taxon a great many problems we will have to come to grips with in our quest for consensus over decapod phylogeny.

First of all, there is a problem with their apparent age (see Fig. 9). The earliest body fossil crayfish are Mesozoic and include the extinct families

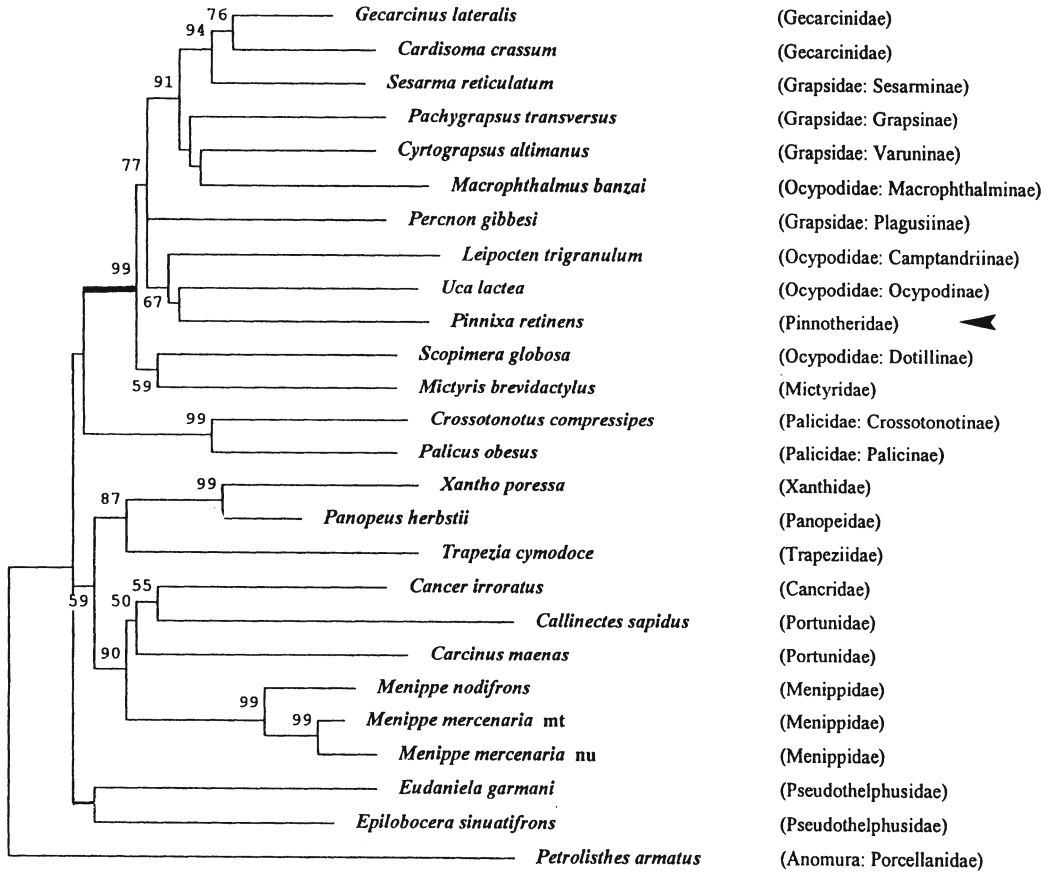

Figure 12. The pattern of relationships among several brachyuran families based on analysis of 16S rDNA (from Schubart et al., 2000). Numbers represent confidence levels from an internal node test. The clade of Thoracotremata marked with heavy black line. While the analysis is not a comprehensive one for all families of Eubrachyura, it is interesting to note the possibility of the paraphyletic nature of Heterotremata (if not polyphyletic, note arrow for heterotrematous pinnotherids).

Protastacidae Albrecht, 1983 from the Jurassic and Cretaceous of Germany, and the Cricoidoscelosidae Taylor et al., 1999, from the Cretaceous of China, both families whose status needs to be critically evaluated. However, the group seems much older than this. Kowalewski et al. (1998) report trace fossils of crayfish burrows from the Triassic, and Hasiotis (1999) even records similar burrows from the Late Pennsylvanian indicating an origin for Astacida probably sometime in the Early Carboniferous. Thus, it would appear that we lack body fossils for more than half of crayfish history, missing information that undoubtedly would lend some insights into the origins and early anatomical evolution of the crayfish.

We might have guessed this was so from consideration of crayfish biogeography alone. The distribution of modern forms (Fig. 13) has always been cited as a classic example of 'disjunct distributions.' Indeed, examination of the pattern based solely on the present day arrangement of the continents makes it difficult to develop logical scenarios to explain the evolution of the group. However, if that same modern distribution is plotted on a paleogeographic map of the Triassic (Fig. 14), the anomalies from the modern geography begin to disappear. One could postulate that the Astacidae were a subtropical to north-temperate family, extending from what is the present northwestern United States across Canada and Greenland into what is today Europe. The Cambaridae appear to have been a tropical to subtropical group in waters across the paleo-equator of Pangaea, connecting perhaps in habitats along the northern coast of the Paleo-Tethys Ocean to what is today eastern Asia. This confirms that the Superfamily Astacoidea is certainly Laurasian in origin (Scholtz, 1995a, 1998, 1999). The Parastacidae are clearly a south-temperate family, occupying freshwater habitats of Gondwanaland. One could in fact use the inferred paleogeographic distri-

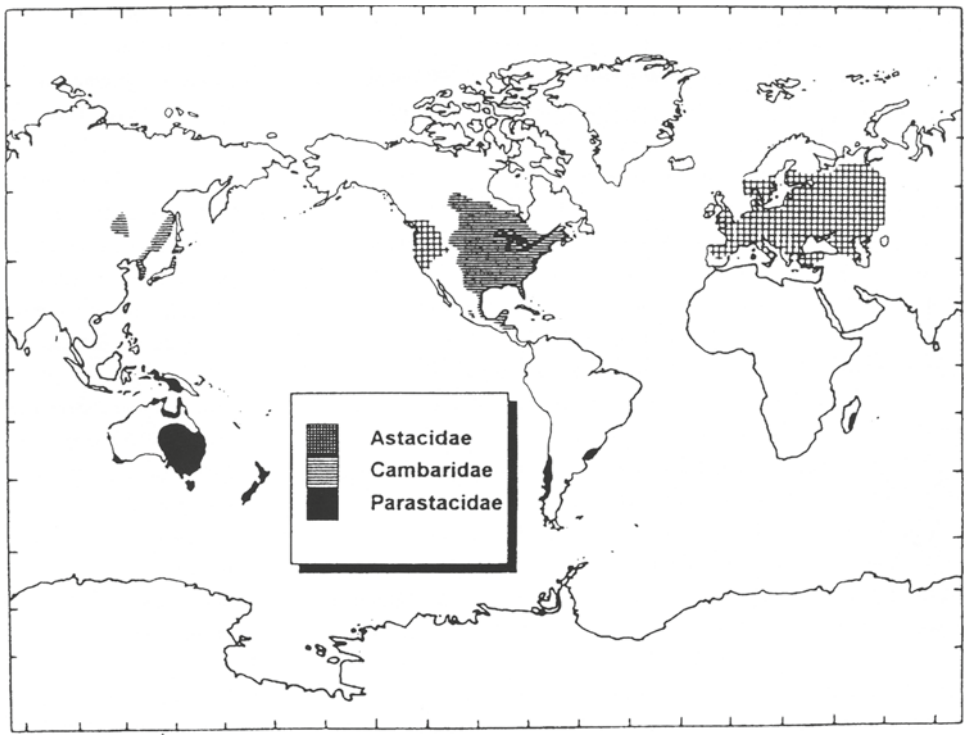

Figure 13. Modern distribution of crayfish families (from Holdich, 1999).

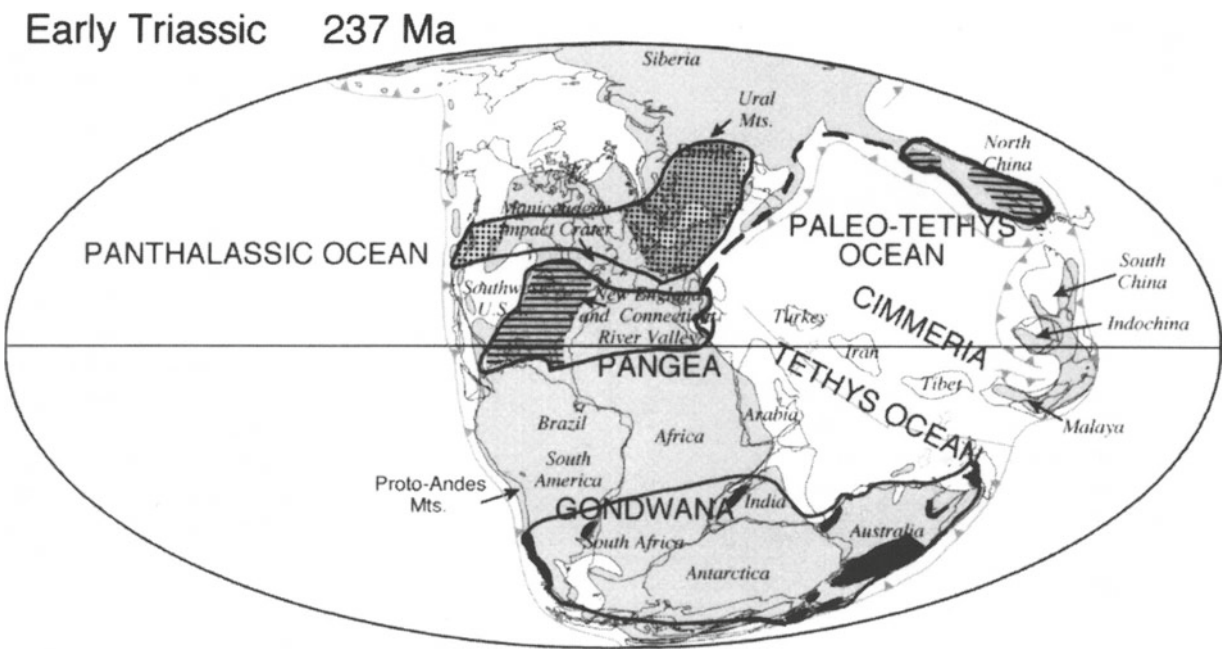

Figure 14. Modern distribution of crayfish families plotted on a paleogeographic map of the Triassic (from Scotese, 1997). Three tracks can be discerned: (1) a subtropical/north-temperate track of Astacidae; (2) a tropical subtropical Cambaridae; (3) south-temperate Parastacidae. Fossil representatives of these families might be expected in the intermediate areas included within the heavy black lines in any time periods since the Early Triassic.

butional tracks to predict areas where explorations for crayfish fossils should be carried out. Given the inferred Triassic distribution, it seems obvious that the origins of the group would have to be sought in pre-Triassic time, as already suggested by Scholtz (1999), giving credence to the claim of Hasiotis (1999) for Carboniferous crayfish burrows. Obviously, we have a great deal more to discover about the history of crayfish.

Despite the work of Scholtz & Richter (1995), the elucidation of relationships within Astacida is still tied to the old idea of Astacidea [= erymids + nephropids + astacids + cambarids + parastacids]. As an example, Tshudy & Babcock (1997) performed a phylogenetic analysis of 'clawed lobsters.' They rooted their tree to *Eryma* as an out-group and recognized two families: the Nephropidae Dana, 1852, which includes the fossil and living marine, clawed lobsters, and a new family, the Chilenophoberidae, an amalgam of Mesozoic 'proto-lobsters.' However, within the Chilenophoberidae they included *Pseudastacus*, a Jurassic genus from Germany. As mentioned above, Albrecht (1983) placed the Protastacidae within the true crayfish. The status of the Protastacidae presents problems. One could question whether these are crayfish. First of all, they are marine taxa. In addition, their carapace groove pattern is really erymid, or clytiopsid, in pattern, and what little can be discerned of the tail fan is not particularly crayfish like. A close reading of Albrecht (1983) reveals that he is an evolutionary systematist and still writes of trends and grades. A rigorous cladistic analysis of his information would more than likely not give the pattern he envisioned. The Tshudy & Babcock (1997) database is more inclusive than the features employed by Albrecht (1983), who focused almost exclusively on a selection of the carapace grooves. Nevertheless, at the very least it is clear that despite our best efforts to produce careful analyses of relationships we still often lack any certain knowledge of what taxa constitute monophyletic groups.

A computer, or a person, given any array of taxa and a selection of characters, can produce on command *a* phylogenetic tree. The question is, does the tree mean anything? One must be very careful. A tree is a tree – a pictorial representation of a matrix of information. It is only as good as the information that goes into the matrix. One must focus on identifying monophyletic groups because not to do so is to run the risk of getting paraphyletic or even polyphyletic groups out of a cladistic analysis conducted without due regard for fundamentals (Jenner & Schram, 1999). Without attention to this crucial issue, we will never be able to sort out the relationships among the macrurous Reptantia.

The origin of Decapoda

The issue of paleogeography emerges again in connection with the origin of Decapoda. That event undoubtedly lies in the deep recesses of the Palaeozoic. The earliest known decapod is *Palaeopalaemon newberryi* in the Upper Devonian of North America (Schram et al., 1978), a macrurous 'lobster' of some kind (Fig. 15). That species is not too far away in time from the fossil species *Imocaris tuberculata* from the Mississippian (Lower Carboniferous) of North America (Schram & Mapes, 1984), which appears to be a dromiacean. The appearance of the Eumalacostraca in the fossil record is abrupt (Schram, 1981a, 1983) – a classic punctuated event. However, a hint as to what could have happened is to be gotten from the paleogeography of contemporaneous trilobites.

When Eldredge was developing his allopatric model of speciation in the Middle Devonian phacopid trilobites (Eldredge 1971, 1972, 1973), he charted the paleogeographic and paleohabitat preferences for his species and subspecies of *Phacops*. His conclusions about allopatric population shifts across the Devonian seas of North America of course ultimately lead to the well-known concept of Punctuated Equilibrium. Eldredge (1974) postulated an allopatric model where changes in anatomy occurred quite rapidly in isolated peripheral populations of his trilobites. The main source of the lineage centered on the shallow marginal seas, whose deposits today stretch across the Middle Atlantic States of America. The peripheral isolates can be collected from the contemporaneous deposits further west, located in the Midwestern States extending from Ohio across to Iowa. These latter deposits represent the deeper water epeiric seas further offshore from that of the shallow water marginal seas to the east (Eldredge & Eldredge, 1972).

Why is this interesting for decapods? *Palaeopalaemon newberryi*, our first decapod, is to be found in these deeper, offshore, epeiric sea deposits of the American Midwest, albeit of the slightly younger Upper Devonian. The obvious working hypothesis is that decapods may be scarce in the latter half of the Palaeozoic because their natural habitat up until that point may have been even deeper water. The few decapod

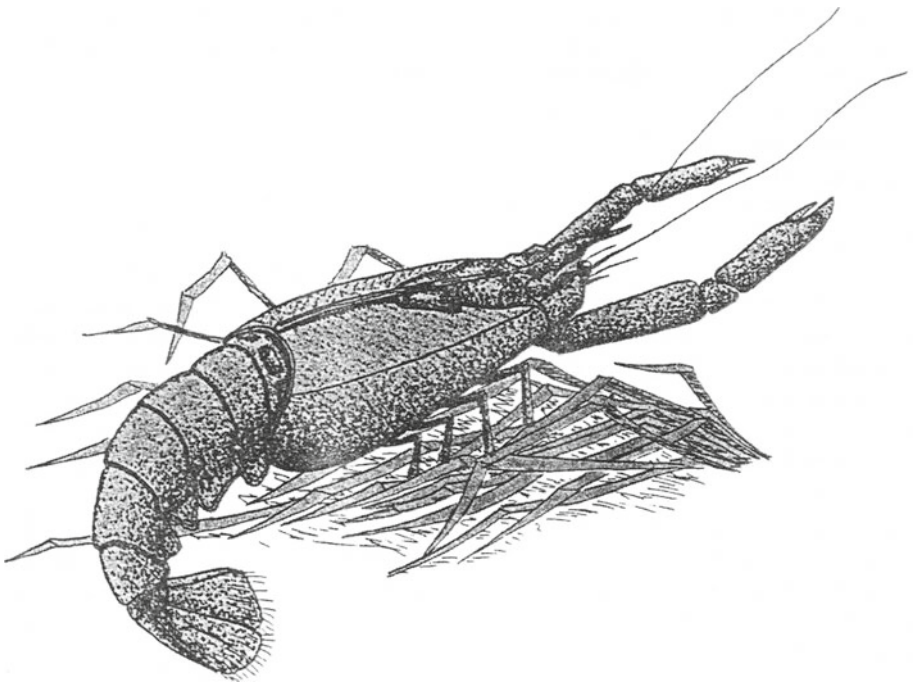

Figure 15. Reconstruction of *Palaeopalaemon newberryi*, the earliest known decapod from the Upper Devonian of North America (from Hannibal & Feldmann, 1985).

species we have in the Palaeozoic – reptantians at that – perhaps are there only because they represent a few pioneer types that ventured up out of the continental shelf and/or slope waters onto the margins of the offshore, epeiric seas. It is a pattern the reverse of that of the trilobites, which seem to have evolved into the epeiric seas from shallower water.

Probably this model is too simple. Would it also apply to the natant precursors to the Reptantia? What about the origins of other Eumalacostraca? Many of these non-decapod eumalacostracans have a predominantly shallow near-shore, or even fresh water, component (Schram, 1981b) in Carboniferous time. Did these syncaridan, peracaridan and hoplocaridan types also come out of the deep sea? Or did these non-decapod groups have an independent trajectory in shallow, near-shore seas? Again, much needs to be discovered in the Paleozoic fossil record before any consensus can emerge.

Developmental genetics, evolution and phylogeny

Finally, something must be said about the discoveries coming to light from the work of developmental geneticists. This research in regards to crustaceans is just in its infancy. Only a few species relative to the wide morphological diversity of Crustacea as a whole have been studied. Certainly, a great deal more will need to be done in order to get some good insights into the evolutionary history of Decapoda, let alone have any direct significance for consideration of phylogeny. We can summarize a few things here.

Some work has been done on mapping *Hox* gene expression in Malacostraca and relating this to degrees of maxilliped development (Averof & Patel, 1997). However, there have only been limited investigations to date and these studies concern only two of the *Hox* genes, *Ubx* and *abdA*. Nevertheless, what has been seen so far indicates that a concerted effort towards a comprehensive survey and mapping of all *Hox* genes in crustaceans will undoubtedly prove effective towards increasing our understanding of the genetic forces that shaped the evolution of the decapod Bauplan.

More extensive work has been done to elucidate the patterns of expression of *engrailed* (*en*). Aside from basic similarities of *en* expression in the head of crustaceans to the expression seen in insects (Scholtz, 1995b), a peculiar pattern is manifest in decapods. The crayfish *Cherax destructor* displays a total of 9 *en-*

grailed stripes appearing in the course of development in the pleon (Scholtz, 1995c). Whether this represents an autapomorphy for *Cherax* (or even the crayfish), or is the revelation of some underlying primitive pattern for malacostracans is not clear. Furthermore, it appears that the Malacostraca possess a pattern of repeated cell divisions in the ectoderm and mesoderm of the post-naupliar germ band that is unique for arthropods (Scholtz & Dohle, 1996). In connection with this, the malacostracan ground plan seems to include the possession of 19 ectoteloblasts arranged in a ring. Two derived conditions from this ground pattern are recognized. Amphipods have apparently lost the ectoteloblasts altogether, and all crayfish families share the possession of 40 ectoteloblasts (Scholtz, 1993) as a synapomorphy.

These are only tantalizing titbits, but we can only look forward to a considerable amount of undoubtedly important phylogenetic information coming to light in the next several years.

Conclusion

We are nowhere near to approaching a complete consensus on the phylogeny of Decapoda and consequently a universally accepted natural taxonomy of the group. Our understanding of the phylogenetic relationships among the Decapoda has improved in the last 15 years, and at least everyone agrees that we are dealing with a monophyletic group. However, we still are not entirely clear where all the monophyletic groups within the Decapoda sit. While we can have as a working goal the production of a phylogeny for the group as a whole, it would seem efficacious towards this end to concentrate for now on trying to identify the monophyletic groups within the decapods. This can have some immediate benefits in terms of providing a framework for the practical applications of phylogenetic studies in the fields of nature conservation and resource management. The long-term objective will in time emerge of its own accord: a robust, well-supported phylogenetic tree for the order tied to a natural taxonomy of the group.

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Notes on the position of the true freshwater crabs within the brachyrhynchan Eubrachyura (Crustacea: Decapoda: Brachyura)

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Abstract

Cladistic and phenetic relationships of 51 eubrachyuran crab genera, comprising 36 genera of marine crabs and 18 genera of true freshwater crabs from 7 families, were investigated using 121 parsimony-informative adult morphological characters. The data matrix was subjected to four different treatments: (1) a cladistic analysis with a combination of unordered and ordered characters, (2) a cladistic analysis with all characters unordered, (3) neighbour-joining, and (4) UPGMA phenetic analyses. The parsimony analysis conducted with a combination of ordered and unordered characters produced a set of hypotheses which supported monophyly of a Pseudothelphusidae+Potamoidea clade. Furthermore, exemplar genera of the Bythograeidae and Pinnotheridae formed an unresolved polytomy with the Pseudothelphusidae+Potamoidea group, the Thoracotremata. The trichodactylid freshwater crabs were positioned as the sister taxon of the basal portunoid *Carcinus*, but were unresolved relative to other portunoids and geryonids. Second, the parsimony analysis conducted with all characters unordered resulted in a [bythograeid, pseudothelphusid+potamoid, pinnotherid, thoracotreme] group with no hierarchical resolution, which in turn formed a polytomy with a goneplacid+portunoid clade and a polyphyletic Xanthoidea. And third, phenetic groupings of the eubrachyuran genera invariably placed the pseudothelphusids with the potamoids, and this clustered with a group containing the thoracotremes (either in whole or part). Support was thus found for morphological connections among the nontrichodactylid freshwater crabs, thoracotremes, bythograeids, and pinnotherids, and for the placement of the trichodactylids within the Portunoidea. These two latter findings (that used a range of genera from each family) are broadly congruent with a previous cladistic analysis of selected eubrachyuran familial groundpatterns that used a basal exemplar of each marine and freshwater crab family (Sternberg et al., 1999). However, it is clear that the large scale homoplasy identified here may nullify any reliable hypothesis of brachyrhynchan groupings at this stage.

Introduction

Freshwater crabs have historically received relatively less attention than their marine relatives, and until recently there have been few serious attempts to identify the relationships between the freshwater crabs to other brachyurans found in marine environments. This situation has gradually improved over the past 30 years and there is currently a wave if interest in freshwater crab biology. This has taken the form of an explosion of alpha-taxonomy and an increase in the number of described species from 600 to more than 950 (e.g. Bott, 1955, 1970; Rodríguez, 1982, 1992; Ng & Naiyanetr, 1993; Cumberlidge, 1999; Cumberlidge & Sternberg, unpublished), and an increase in the number of families from three (Bott, 1955) to 12 (Bott, 1970; Cumberlidge 1999). At present, most authors (Ng, 1988; Cumberlidge, 1999) recognise seven or eight families (Trichodactylidae, Deckeniidae, Gecarcinucidae, Parathelphusidae, Potamidae, Potamonautidae, Pseudothelphusidae, and Platythelphusidae). The recent literature on freshwater crabs includes a number of important monographic revisions of the faunas of the Neotropics, Africa and Asia (Rodríguez,

1982, 1992; Ng, 1988; Ng & Naiyanetr, 1993; Magalhães & Türkay, 1996a,b,c; Cumberlidge, 1999). This dramatically improved database, together with the availability of techniques such as cladistics, have laid the foundations for much-needed phylogenetic and biogeographic analyses of the group. The worldwide distribution of freshwater crabs throughout the inland waters of the continents and islands of the tropics and subtropics means that these decapods hold great potential as indicators of past geological events. However, in order for this potential to be realized, it must first be determined whether the freshwater crabs are a monophyletic group, and whether they originated relatively recently, or whether they are of more ancient origin.

A key attribute of all true freshwater crabs is direct development whereby larval stages are lacking and the eggs produce young crabs. The broad, shallow female sternoabdominal cavity and the equally-broad abdomen together form a brood pouch for the relatively small number (~25–200) of large eggs and the hatchling crabs. The biogeographic importance of the freshwater crabs arises from their restriction to inland fresh water habitats of the continents, and their relatively poor powers of dispersal. This is because freshwater crabs lack the dispersive planktonic larval stage seen in most marine crabs. This means the geographic range of freshwater crab species is in part limited by their low dispersal capabilities, their low fecundity, their restriction to ecosystem microhabitats, their intolerance to desiccation, and to saline habitats (Rodríguez, 1986). As a consequence, freshwater crabs become isolated relatively easily and they tend to exhibit high rates of endemism. It is common for a relatively small geographic area to support a high species diversity of freshwater crabs (e.g. Ng, 1988; Ng & Naiyanetr, 1993; Cumberlidge, 1999).

Freshwater crabs are distributed pantropically along the lowland watersheds of South America, the Andean and Central American cloud forests, and some islands in the Caribbean; throughout sub-Saharan Africa; in southern Europe and parts of the Middle and Near East; Madagascar; the Seychelles; India and Southeast Asia; China, the Philippines, Indonesia, New Guinea, and Australia (Bott, 1970; Rodriquez, 1982, 1992; Ng, 1988; Ng & Naiyanetr, 1993; Magalhães & Türkay, 1996a,b,c; Cumberlidge, 1999). If the group should prove to be monophyletic, and if the group can be demonstrated to have an ancient origin, then this circumtropical distribution pattern could be interpreted in terms of plate tectonic movements and continental fragment migration; if not, then other explanations must be sought. However, the age and origin of the freshwater crabs is far from certain, and this is due in part to a poor fossil record for the group, with the oldest fossils dating back to the Miocene, 25–30 million years ago (Bott, 1955; Glaessner, 1969). On the other hand, all freshwater crabs are highly derived heterotremes, and this latter group has a more complete fossil record. It is likely that the heterotremes underwent a post-Cretaceous radiation (Glaessner, 1969) and it is, therefore, reasonable to assume that the freshwater crabs may have first appeared at some time in the early to mid-Cenozoic era (65–30 mya). Other attempts to establish the time of origin of the freshwater crabs include evidence from dated tectonic movements. For example, if the South American, African, Madagascan, and Indian freshwater crabs constitute a monophyletic group, then the stem group must have been present in or near the inland waters of the ancient southern continent of Gondwana. Because the breakup of Gondwana is believed to have taken place around 120–100 mya, authors have postulated an origin of freshwater crabs in excess of 120 mya (Ng & Rodríguez, 1995; Ng, Stevcic & Pretzmann, 1995). However, this reasoning has been questioned (Sternberg et al., 1999) because such an early origin would require (1) that the freshwater crabs significantly predate the eubrachyuran radiation, and (2) that the Brachyura as a whole is a great deal older than current data allow. On the other hand, if the freshwater crabs are an unnatural (polyphyletic) group, their distribution would reveal little about past geological events and if each of the freshwater crab families on different continents had a separate marine crab ancestry and a more recent (post-Cretaceous) origin (Pretzmann, 1973; Rodríguez, 1986; Ng & Rodríguez, 1995; Guinot et al., 1997).

The aim of the present study is to test the monophyly of the freshwater crabs, and to identify a possible marine sister group (or groups) of the Old World and New World freshwater crab families. Testing the monophyly of the freshwater crabs is intertwined with the identification of the marine sister taxon (or taxa) of the freshwater crabs, because knowledge of this sister group is a necessary prerequisite for the correct polarization of characters for cladistic analysis. The problem is that the freshwater crabs (alone of all brachyurans found in freshwater habitats) have no easily identifiable extant marine crab relatives.

The cladistic analysis of selected eubrachyuran familial groundpatterns by Sternberg et al. (1999) found

that the freshwater crabs fell into two broad lineages: (1) a clade that included the Neotropical Trichodactylidae within the Portunoidea, and (2) a clade that included all of the remaining freshwater crab families (Deckeniidae, Gecarcinucidae, Parathelphusidae, Platythelphusidae, Potamidae, Potamonautidae and Pseudothelphusidae). In addition, parsimony analysis of the eubrachyuran familial groundpatterns (Sternberg et al., 1999) positioned the Thoracotremata (*sensu* Guinot, 1977, 1979; Guinot & Richer de Forges, 1997) as the sister group of the Neotropical Pseudothelphusidae+Palaeotropical freshwater crabs, and placed the Trichodactylidae within the Portunoidea. The latter finding supports the hypothesis first presented by Rodríguez (1992), and at the same time falsifies the monophyly of the freshwater crabs. These findings have weakend the previous hypotheses that the freshwater crabs are either strictly monophyletic (Rathbun, 1904), extremely polyphyletic (Bott, 1970; Pretzmann, 1973), or that they are positioned within or near the Xanthoidea (Pretzmann, 1973; Rodríguez, 1986; Guinot et al., 1997).

The need for the present study arises out of the fact that the monophyletic status of many of the eubrachyuran marine crab families is itself uncertain. This uncertainty raises questions about the wisdom of relying on published accounts of familial groundpatterns of groups of marine crabs that may eventually prove to be unnatural entities. In order to overcome this problem, the present test of freshwater crab monophyly and sister taxon (or taxa) identification compares character states among genera that each represent a particular family, rather than comparing a familial groundpattern that relied on the correct selection of a representative of a putative family. In the present, study we have restricted our analysis to the brachyrhynchan eubrachyurans, which is the group of heterotreme crabs that includes both the freshwater crabs and their likely marine sister taxon (or taxa). In order to compare the taxic relationships resulting from the different treatments of these data, we have subjected our extensive dataset of adult morphological characters to both cladistic and phenetic analyses. As far as we are aware, this study constitutes the largest selection of taxa and the largest number of characters of any published cladistic analysis of the Eubrachyura to date.

Bott (1970) recognised eight Palaeotropical freshwater crab families and placed these in two superfamilies: the Gecarcinucoidea (for the Parathelphusidae, Gecarcinucidae and Sundathelphusidae) and the Potamoidea (for the Potamidae, Potamonautidae, Deckeniidae, Isolapotamidae and Sinopotamidae), presumably to reflect two distinct evolutionary lineages. Bott (1970) recognised a third superfamily (the Pseudothelphusoidea, for the Pseudothelphusidae and Potamocarcinidae) and placed the Neotropical Trichodactylidae in a separate family. However, there is no cladistic support for such an elaborate polyphyletic ancestry for the freshwater crab families (Sternberg & Cumberlidge, 1999; Sternberg et al., 1999).

Furthermore, the morphological characters traditionally used to separate the members of Bott's (1970) two Old World superfamilies (i.e. gecarcinucoids and potamoids) are of dubious significance (Cumberlidge, 1999). Available cladistic studies of freshwater crab relationships (Sternberg & Cumberlidge, 1999; Sternberg et al., 1999) support the grouping of all the Palaeotropical freshwater crab families into a single superfamily, the Potamoidea. For this reason, the term 'potamoid' in the present context refers to the clade comprised of all the Old World families.

Methods

A data matrix of fifty-one taxa (Appendix 1) and 121 characters (Table 2) was compiled using MacClade 3.06 (Maddison & Maddison, 1996). The taxonomic authorities for all of the taxa are given in full in Appendix 1, and the details of the characters used are given in Table 1. All the characters in Table 1 pertain to aspects of adult morphology. We have included genera from 22 families of marine eubrachyurans, but we have not included genera from highly derived families such as the Mictyridae and Palicidae, because such forms can bias investigations of taxic relationships (Danser, 1950).

Two different cladistic analyses (one using all unordered characters, the other using a combination of unordered and ordered characters) and two different phenetic analyses ['neighbor joining' (NJ) and UPGMA] were performed using PAUP 4.0 (Swofford, 2000, unpublished). In view of the complexity of the database, the two cladistic analyses were carried out using the 'general heuristic search' option for 100 bootstrap replicates. No outgroup was specified during the searches for the shortest trees. Phenetic relationships among the brachyrhynchan genera were determined in order to compare the resulting phenograms in which taxic groupings are based on total character state distances, with the consensus cladograms (batches 1 and 2) based on shared derived charac-

Table 1. Adult morphological character states used in the cladistic and phenetic analyses of brachyrhynchan relationships

1. Carapace frontal margin: with median incision (0); entire, without notch (1).
2. Degree of carapace frontal margin downward deflexion: none (0); moderate (1): vertical (2); 'pseudothelphusid' (3).
3. Carapace front: distinct and moderately broad (0); narrow, subtriangular to spatulate (1).
4. Carapace frontal margin: cut into distinct teeth or lobes (0); low, blunt lobes present (1); straight, no trace of lobes (2).
5. Lateral margin of carapace front: separated from medial-inferior orbital angle (0); associated with medial-inferior orbital angle (1).
6. Carapace frontal margin: singular (0); horizontally split into inferior and superior margins (1).
7. Supraorbital margin: distinct or faint notch(es) present (0); complete (1).
8. Supraorbital margin shape: semi-circular (0); sigmoidal and elongate (1).
9. Eyestalks and eyes: well-formed and functional (0); vestigial (1).
10. Number of supraorbital notches, if present: one (0); two (1).
11. Medial-inferior occlusive orbital tooth (see Rodríguez, 1992): absent (0); present (1).
12. Medial-inferior infra-orbital ridge: absent (0); present (1).
13. Epigastric crest: absent (0); present (1).
14. Position of the epigastric crest: posterior to the imaginary line linking the supraorbital margins (0); anterior to the imaginary line linking the supraorbital margins (1); located at point of frontal margin downward deflexion (2).
15. Epigastric lobes: well-formed (0); reduced to scars (1); barely discernable to absent (2).
16. Postorbital crest: absent (0); present (1).
17. Branchial groove: weak to absent (0); marginally developed (1); distinct (2).
18. Epibranchial crest: absent (0); present and tuberculated (1).
19. Crest associated with posterior-most carapace lateral tooth: absent (0); present (1).
20. Posterior-most carapace lateral tooth prominent: absent (0); present (1).
21. Carapace lateral margin (separating surface from the sidewall): weakly defined (0); defined by a '"cancroid' ridge (1).
22. Anterior half of carapace lateral margin: not distinctly convex (0); distinctly convex and delimited by a low line of tubercles (1); distinctly convex and delimited by a raised lateral margin (2).
23. Carapace lateral margin: indistinct (0); distinct for the entire length and sharply projecting (1).
24. Carapace posterolateral region: smooth or weakly tuberculated (0); with posterolateral carinae and/or rugosities (1).
25. 'Potamoid' posterolateral carapace carina: absent (0); present (1).
26. Carapace posterior border: defined by distinct but low carina (0); defined by sharp, high carina (1).
27. Carina defining posterior extremity of carapace: distinct but low (0); reduced in length and height (1); very reduced to absent (2).
28. Longitudinal orientation of epimeral sulcus: merging with carapace lateral margin approximately halfway along length (0); remaining subparallel to carapace lateral margin throughout length (1).
29. Carapace sidewall vertical groove: absent (0); vaguely defined (1); distinct (2).
30. Outline of carapace sidewall vertical groove: straight (0); semi-circular in outline (1).
31. Carapace sidewall vertical sulcus: distant from lateral-inferior orbital margin (0); flanking lateral-inferior orbital margin (1).
32. Carapace sidewall: smooth or weakly tuberculate (0); with carinae or rugosities (1).
33. Pterygostomial region: not projecting relative to suborbital region (0); dorsal region produced and shelf-like relative to suborbital region (1).
34. Antennular septum: distinct (0); very reduced in width, forming a thin bridge (1).
35. Basal antennal article: having a (sub)rectangular outline in frontal view (0); distinct distolateral tooth present (1).
36. Buccal frame vertical margins: parallel (0); detectable to moderate ventral widening (1); pronounced ventral widening (2).
37. Buccal frame vertical margins: parallel (0); detectable to moderate dorsal widening (1); pronounced dorsal widening (2).
38. Vertical margin of buccal frame: visible (0); covered by 3rd maxilliped exopods and/or ischia (1).
39. Carapace weak, flexible, bulbous in conformation: absent (0); present (1).
40. Carapace outline pseudothelphusid-like in dorsal view: absent (0); present (1).
41. Lateral regions of the epistome posterior margin everted to form roofs of efferent 'tubes': absent (0); moderately developed (1); very pronounced (2).
42. 'Deckeniid' conformation of the epistome: absent (0); present (1).

Continued on p.25

Table 1. Continued

43. Median projection on epistomial posterior margin (projecting into space between the 3rd maxilliped palps): slight to absent (0); moderately developed (1); pronounced, tongue-like (2); block-like (3).
44. Median projection on epistomial posterior margin: projecting ventrally (0); projecting outward (1).
45. Median projection on epistomial posterior margin flanked laterally by longitudinal incisions: absent (0); present (1).
46. Epistome with longitudinal notches present near the lateral regions, lateral to the endostomial ridges if present: absent (0); present (1).
47. Orientation of the epistome: facing ventrally (0); facing anteriorly (i.e. vertical when crab is upright) (1); posterior margin visible from the dorsal perspective (2).
48. Endostomial gutter: distinct (0); reduced to highly reduced (1); absent (2).
49. Endostomial gutter deep, defined by sharp margins: absent (0); present (1).
50. Endostomial ridges (defining median sides of the efferent channels): distinct (0); reduced (1); absent (2).
51. Posterior margin of the epistome with three low, ventral projections, one median and two near the lateral margins: absent (0); present (1).
52. Mandibular palp: 3-segmented (0); proximal and penultimate segments intermediately fused (1); 2-segmented (2).
53. Mandibular palp terminal segment: 'simple' (0); small anterior lobe present (1); large anterior lobe present (2).
54. Mandibular palp terminal segment: flat and laminar (0); somewhat enrolled (1).
55. Endopod of first maxilliped: flat and laminar (0); rolled, tube-like (1).
56. Length of first maxilliped endopod: not reaching anterior margin of the endostome (0); reaching the anterior margin of the endostome (1).
57. 'Portunoid-lobe' on first maxilliped endopod: absent (0); slightly developed (1); distinct (2).
58. Exopod of third maxilliped: robust, almost 0.5–0.3X the width of the ischium (0); moderately thin, equal to or slightly less than 0.25X the width of the ischium (1); thin, less than 0.1X the width of the ischium.
59. Exopod of third maxilliped: medial part of the base curving under the ischium (0); medial part of the base only slightly curving under the ischium (1); base not curving under the ischium (2).
60. Ischia, meri and palps of third maxillipeds: leaving a medial space (0); completely enclosing the buccal cavity (1).
61. Articulation junction of the third maxilliped ischia-meri: not constricted (0); constricted (1).
62. Teeth located along medial margin of third maxilliped ischium: distinct (0); reduced to absent (1).
63. Anterolateral border of third maxilliped merus: rounded (0); flared, moderately projecting (1); distinctly flared (2).
64. Anterior margin of third maxilliped merus adjacent to proximal segment of the palp: not projecting (0); forming a distinct lobe or spine-like (1).
65. Anterior margin of third maxilliped merus: slanted, nearly straight (0); with medial depression (1).
66. Outline of third maxilliped merus *Nectocarcinus*-like: absent (0); present (1).
67. Palp of third maxilliped: articulating at disto-medial angle (0); articulating at disto-lateral angle (1).
68. Terminus of third maxilliped palp distal segment: extending to ischium (0); extending to ischium-merus junction (1).
69. Male abdomen outline narrowly triangular: absent (0); intermediate (1); distinct (2).
70. Male abdomen an equilateral triangle in outline: absent (0); intermediate (1); distinct (2).
71. Male abdominal segments a3–a4: freely articulating (0); fused (1).
72. Suture between male abdominal segments a3–a4: visible (0); erased (1).
73. Male abdominal segments a4–a5: freely articulating (0); fused (1).
74. Suture between male abdominal segments a4–a5: visible (0); erased (1).
75. Male abdominal locking facets on a6: distinct (0); absent (1).
76. Male abdominal segment 6 widened along the posterior region: absent (0); present (1).
77. Male telson tongue-shaped: absent (0); intermediate (1); distinct (2).
78. Male telson triangular: absent (0); intermediate (1); distinct (2).
79. Male abdominal segments a5 and a6 laterally constricted: absent (0); present (1).
80. Male abdominal segments a2–a3 dorsoventrally curved: absent (0); present (1).

Continued on p. 26

Table 1. Continued

81.	Outline of female abdomen: thinly oval (0); oval, longer than broad (1); round (2); broadly oval (3).
82.	Female abdomen: all segments free (0); segments a3–a5 ankylosed (1); segments a3–a6 ankylosed (2).
83.	Female abdominal segment 1 covered by carapace: absent (0); present (1).
84.	Female telson: subtriangular in outline (0); semi-circular in outline (1).
85.	Female telson: subtriangular in outline (0); tongue-shaped in outline (1).
86.	Female pleopodal exopods: long, narrow and pediform (0); slightly broad and flattened (1); very broad and flattened (2).
87.	Female pleopodal endopods: long, narrow, and pediform (0); distal end slightly paddle-like (1); distal end paddle-like (2).
88.	Female pleopodal endopods lacking hinge: absent (0); present (1).
89.	*Sella turcica* reduced to rim-like structure: absent (0); present (1).
90.	Endosternites 3–4: incomplete and sheet-like (0); reduced to apophyse (1).
91.	Endophragmal apophyse 3–4: ends juxtaposed (0); ends well separated (1).
92.	Endosternites 4–5, 5–6: medially confluent (0); medially interrupted (1).
93.	Endosternites 6–7: medially confluent (0); medially interrupted (1).
94.	Anterior terminus of male sternal cavity: middle of sternite 4 (0); at, or anterior, to s3–4 boundary (1).
95.	Longitudinal, median line on sternite 4: absent (0); present (1).
96.	Posterior margin of sternite 3: merges smoothly with sternite 4 (0); laterally expanded relative to sternite 4 (1).
97.	Female sternum distinctly excavated to form a bowl-like egg-chamber: absent (0); present (1).
98.	Position of male penial openings: pereiopod 5 coxae (0); via paired apertures on sternite 8 or near the s7–8 border (1).
99.	Male first pleopod (gonopod) with terminal article: absent (0); present (1).
100.	Articulating joint of first gonopod terminal article: poorly developed (0); prominent (1).
101.	'Panopeid' ornamentation on first gonopod distal end: absent (0); present (1).
102.	First gonopod 6-shaped or geryonid-like in outline: absent (0); present (1).
103.	First gonopod stout with apical spine field (pseudothelphusid-like): absent (0); present (1).
104.	First gonopod subtriangular in cross-section, grapsid-like: absent (0); present (1).
105.	First gonopod thin, xanthid-like: absent (0); present (1).
106.	Second gonopod terminal segment and flagellum: at least equal in length to first gonopod (0); length approximately half that of first gonopod (1): short (2).
107.	Second gonopod flagellum with a whip-like end: absent (0); intermediate (1): present (2).
108.	Second gonopod terminal segment-flagellum articulation point: distinct (0); lacking (1).
109.	Second gonopod apex: styliform (0); spoon-shaped (1).
110.	Pereiopod 2–5 meri: margins rounded in outline (0); subtriangular in outline (1); sharply triangular in outline (2).
111.	Dorsal surface of pereiopod 2–5 meri: smooth or weakly tuberculate (0); rugose or with carinae (1).
112.	Pereiopod 2–5 dactyl spines: absent (0); present (1).
113.	Pereiopod 2–5 dactyl articulation knob: absent (0); present (1).
114.	Pereiopod 5 dactylus: styliform (0); spatulate (1).
115.	Pereiopod 5 dactylus-propodus lined with silk-like setae: absent (0); present (1).
116.	Ventral margin of the pereiopod 1 merus: rounded (0); sharp and demarcated with tubercles (1).
117.	Dorso-interior margin of the pereiopod 1 merus: straight (0); lined with low, irregular tubercles (1); lined with sharp, irregular teeth (2); line with a few sharp, curved teeth (3).
118.	Dorso-external surface of the pereiopod 1 merus: smooth (0); rugose or with carinae (1).
119.	Pereiopod 1 merus short, slightly longer than the carpus and squat: absent (0); present (1).
120.	Dorsal margin of the pereiopod 1 merus: inconspicuous or well-defined (0); with a curved, sharp tooth (1).
121.	Outer surface of pereiopod 1 propodus: smooth or weakly tuberculate (0); with one or more distinct longitudinal ridges (1).

Table 2. Data matrix of the 121 adult morphological characters used in the cladistic and phenetic analyses. Character state codes are: 0 = plesiomorphies; 1, 2, & 3 = apomorphies; p = 0 & 1; q = 1 & 2; r = 0 & 1 & 2; s = 2 & 3; and ? = undetermined

Combined Outgroup:						
0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	
0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0
Carpilius:						
0q01001000	0000200010	000000000?	?000000000	000?010001	0000001000	
0110000000	1100010000	0000000000	0000110000	0000002000	0010000010	0
Platyxanthus:						
0001000001	0000000110	000000000?	?000000000	000?010001	0000000000	
0010000000	0000000000	0001000001	0100110000	0000000000	0000000010	0
Eriphia:						
0102100001	0000000100	0000000021	0000000000	000?011000	1000000000	
0010000000	0000010000	0001000001	0100110000	0000001000	0010000010	0
Ozius:						
0101000001	0000000010	000000000?	?000001000	000?011000	1100000000	
0010100000	0000010000	0001000001	0100110000	0000002000	0010000010	0
Menippe:						
010p000001	0000000010	000000000?	?000p01000	000?010000	0100000000	
0010100000	0000000000	0001000001	0100110000	0000002000	0010000010	0
Panopeus:						
0002000001	0000000110	000000001?	?000101000	000?011001	0000000000	
0020100000	1111000000	0001000001	0100110000	1000120000	0000000010	0
Rhithropanopeus:						
0002000001	0000000110	000000000?	?000101000	000?011001	0000000000	
0020100000	1111000000	0001000001	0100110000	1000120000	0000000010	0
Beuroisia:						
0002010101	0010011000	000000000?	?000000000	000?010000	0000000q00	
0010000020	0000000000	1001000001	0110000000	0000000001	0110000000	0
Pilumnus:						
0102000001	0000000000	000000000?	?000001000	000?011000	0000000000	
0010100010	0000000000	0001000001	0100100000	0000120000	0010000010	0
Leptodius:						
0002000001	0000000010	000000000?	?000001000	000?010002	0000000000	
0020100000	1111000000	0001000001	0100100000	0000120000	0010000010	0
Actaea:						
0002000001	0000000010	000000000?	?000001000	000?010002	0000000000	
0010100000	1111000000	0001000001	0100100000	0000120000	0010000010	0

Continued on p.28

Table 2. Continued

Trapezia:						
0000101100	000?200010	000000200?	?000?01000	001p011100	0100010010	
0110000020	1111000000	0001000001	0000100000	0000120000	0020002010	0
Geryon:						
0000000101	0000000101	100001010?	?000101000	000?010000	0000001000	
0110010001	1010000201	1001000001	1110000000	0100000000	0000000001	1
Carcinus:						
0000000101	1000000100	100001010?	?000000000	0010011002	0100000000	
0021010001	1111000201	2100000001	1110000000	0100010000	0011100010	1
Nectocarcinus:						
000r000101	0000000110	100001000?	?000101000	000?010000	0000000000	
0001010020	1010000201	1000000001	1100000000	0000001001	0001101001	1
Benthochascon:						
0000000101	1000000101	100001010?	?000101000	000?011002	0100000000	
0020000010	0000000201	1000000001	1110000000	0100000000	0011100001	1
Bathynectes:						
0000000101	1000000101	100001010?	?000101000	000?011000	0100002000	
0020000010	0101000201	1000000001	1110000000	0100000000	0011103001	1
Trichodactylus:						
1102000100	1000200000	100000210?	?000100000	0030001012	0000012200	
0101010001	1p1p000200	2011010011	1110000000	0100001000	0000000000	0
Valdivia:						
1102000100	1000000000	100001010?	?000100000	0030002012	0000002210	
0101010001	1111000201	2110020111	1110000000	010000q000	0001103001	0
Sylviocarcinus:						
1002000100	1000000000	100001010?	?000100000	0030002012	0100002210	
0101010001	1111000201	2110020111	1110000000	0100002000	0001103001	0
Goneplax:						
1002011100	0000000000	000000000?	?010001000	000?011000	0000000000	
0020100020	0000000201	3001000001	1110000000	0000000000	0001100001	0
Carcinoplax:						
1002011100	1000200000	000000000?	?010101000	000?011000	0000000000	
0020p00020	0000000201	10p0000001	1110000000	0000000000	0001100001	p
Coenophthalmus:						
0000100101	0000000100	000001000?	?000001000	000?011000	0200001000	
01101p0011	0000002000	2000000001	1111000000	0000000000	0101103001	1
Cyanograea:						
1202001?10	0010000000	010000200?	?001?00101	0010001002	0000000100	
0100000020	0000000200	1001000001	1110000000	0000001001	1000001010	0

Continued on p.29

Table 2. Continued

Austinograea:						
1102001?10	0000000000	0100000200?	?001001101	0021002202	0000001110	
0100000020	0000000200	1001000001	1110000000	0000000001	0000002000	0
Bythograea:						
1102011?10	0010010000	0100000200?	?001001101	0021012102	0000002110	
0100000020	0000000200	1001000001	1110000000	0000000002	1000002100	0
Cancer:						
0000000001	0000000000	100001010?	?000000000	000?010001	0000001000	
0010000000	1111000000	1000000000	0000000000	0000000000	000000000	0p
Pinnixia:						
1202001000	000?000000	0000000200?	?000020010	0010011202	0000000?20	
0100001?00	0000002000	3001000001	1111001000	0000020001	1000000000	0
Pinnotheres:						
1102001000	000?000000	0000000200?	?000020010	0010011202	0000000?20	
0100001?00	0000002000	3001000001	1111001000	0000020000	0000000000	0
Epilobocera:						
1302001100	0100100000	0100002020	0000010101	0011101200	1220010111	
0?p0100120	0000000200	1001022101	1110000000	0010000101	1100012100	0
Fredius:						
1302001100	0100100000	0100002000	0000010101	0011101200	1220010?21	
0?00000120	0000000200	1001022101	1110000000	0010000111	1100012100	0
Kingsleya:						
1302001100	0100100000	0100002000	0000010101	0011101200	1220010?21	
0?00000120	0000000200	1001022101	1110000000	0010000111	1100012100	0
Potamon:						
1202001100	0110010000	0201102021	01000p0000	001p101200	1000010211	
0110100120	0000000200	q001021001	1110000011	0000000002	1100012100	0
Potamonautes:						
1102001100	0110012000	0201102021	0100010000	0011101200	0200010211	
0110100120	0000000200	1001021001	1110000011	0000000002	1100012100	0
Sudanonautes:						
1102001100	0110012000	0201102021	0100010000	0011101200	02p0010211	
0110100120	0000000200	1001021001	1110000011	0000000002	1100012100	0
Platythelphusa:						
1002001100	0110011000	0001102011	0100000000	0011101200	p000000211	
0110100020	0000000200	2001021001	1110000011	0000000002	1100012100	0
Globonautes:						
1102001100	0110011000	0101102021	0100020000	0021101200	1220010221	
01p0000120	0000000200	1001021001	1110000011	0000000002	1100012100	0

Continued on p. 30

Table 2. Continued

Socotra:						
1202001100	0110010000	0101101021	00000q0000	1021101200	0000110111	
0110000120	0000000200	q001021001	1111000011	0000000002	1100012100	0
Hydrothelphusa:						
1002001100	0110011000	0201102021	0100000000	0021101200	0210010221	
01p0100120	0000000200	q001021001	1110000011	0000000002	1100012100	0
Deckenia:						
1q02001100	0100000000	0201102021	0100020000	2121101200	0200210221	
0100000120	0000000200	2001021001	1110000011	0000000002	1100012100	0
Gecarcinucus:						
1202001100	0110010000	0100101011	0000000000	1021101200	0220210221	
0110100120	0000002000	0001011001	11?1000000	0000000002	1100011100	0
Holthuisana:						
1002001100	0110010000	0101102021	01000p0000	1010101201	0220110221	
0110000120	0000001010	1001021001	1111000010	0000000002	1100011100	0
Sayamia:						
1002001100	0110010000	0201102021	0100000000	101p101201	0220110221	
0110p00120	0000002010	q001021001	1111000000	0000000002	1100011100	0
Seychellum:						
1202001100	0110011000	0201100021	0100020000	2121101201	0220210221	
0100000120	0000000000	2001021001	1111000011	0000000002	110001?100	0
Cardisoma:						
1202001100	0101200000	0201101010	1100020000	000?001202	0001000220	
1100001120	0000102000	s0 00100001	1111000100	0001020002	1100012100	0
Grapsus:						
1202001100	0102000000	0011102020	11000q0000	000?001200	0000000220	
1100001120	0000001100	s0 00000001	1111000100	0001020002	1100012100	0
Euchirograpsus:						
0002000100	0100000000	001000202?	?000001000	000?001200	0000000220	
j 0110000020	0000000200	2000000001	1110000100	0001020002	0110012100	0
Varuna:						
0002000100	0100000000	001010000?	?0000p0000	000?000200	0000000010	
0120001120	0000102000	3000100001	1111000100	0001020002	1001112100	0
Sesarma:						
1202001100	0102000000	0011102000	0000000000	000?001200	0000000220	
1100001120	0000102000	3000100001	1111000100	0001020002	1100011100	0
Uca:						
1212001100	0101200000	0011?0000?	?000020000	000?001200	0000000220	
0100001110	0000102000	3000100001	1111000100	0001020002	1000012100	0
Ucides:						
1212001100	0101200000	0000?00010	1000020000	002000120?	0001000220	
0100001110	0000102000	s000100001	1111000100	0001020002	?0000111000	

ters. This approach identifies significant tree topology discrepancies when taxa are grouped based on their overall similarities, rather than grouped according to synapomorphies.

Results

Parsimony analysis with a combination of unordered and ordered characters (batch-1)

Figure 1 shows the 50% majority-rule consensus tree of the 100 bootstrap replicates (batch-1) that was obtained when characters **1**, **3**, **7**, **9**, **11–14**, **16**, **18–19**, **21**, **23–26**, **28–35**, **39**, **41–42**, **45–46**, **48–49**, **53**, **55–57**, **62**, **67**, **71–75**, **77–79**, **84–89**, **97–101**, **103–105**, **109–110**, **112–116**, and **119–121** were coded as ordered transformation series. Almost none of the apomorphies included in the analysis can be viewed as being uniquely derived. Instead, the majority of derived states have incongruent distributions when mapped onto the consensus tree (or any shortest length tree). No attempt was made to identify synapomorphies for each node, because of the general absence of hierarchical groupings (Fig. 1).

Batch-1 trees support a clade consisting of the Pseudothelphusidae and the potamoids, with the Thoracotremata, Bythograeidae and Pinnotheridae placed as the (unresolved) sister groups of the pseudothelphusid+potamoid clade (Fig. 1). These findings are partially consistent with the cladistic study of brachyrhynchan groundpatterns conducted by Sternberg et al. (1999) and Sternberg & Cumberlidge (1999). Also consistent with the groundpattern groupings obtained in Sternberg et al. (1999) is that *Carcinus* was placed as the sister taxon of the Trichodactylidae (see Rodríguez, 1992), with this clade forming a polytomy with the remaining portunoids and the Geryonidae. A weakly supported group consisting of the portunoids (inclusive of the geryonids and trichodactylids) and Goneplacidae s.s. (i.e. subfamilies Carcinoplacinae and Goneplacinae), and the *incertae sedis* genus *Beuroisia*, formed a trichotomy with the [bythograeid, pinnotherid, pseudothelphusid+potamoid clade, Thoracotremata] set. Interestingly, both the hypothesis presented in Sternberg et al. (1999) and the cladistic analysis presented here revealed no evidence for a monophyletic Xanthoidea.

Among the batch-1 trees (Fig. 1), the African potamonautids were found to have a polytomous arrangement relative to *Potamon*, *Platythelphusa*, *Globonautes*, [*Deckenia*+ *Seychellum*], *Hydrothelphusa*, and a [*Gecarcinucus* [*Holthuisana*+*Sayamia*]] group. A new freshwater crab genus from Socotra (*Socotra* Cumberlidge & Wranik, 2000) was likewise grouped in the above-mentioned polytomy (Fig. 1).

Parsimony analysis with all characters unordered (batch-2)

General heuristic search analyses of 100 bootstrap replicates were performed with all characters coded as unordered transformation series (batch-2). The 50% majority-rule consensus tree of the batch-2 hypotheses (Fig. 2) generated a pattern largely congruent with the batch-1 hypothesis (Fig. 1). The only trenchant difference between the two hypotheses is that the Portunoidea+Goneplacidae s.s. clade formed a polytomy with the [bythograeid, pinnotherid, pseudothelphusid+potamoid clade, Thoracotremata] set, Cancridae, Platyxanthidae, Carpiliidae, two eriphiid groups, a [[Panopeidae+Xanthidae] Pilumnidae] line, and the *incertae sedis* genus *Beuroisia*.

Phenetic analyses

Both the neighbour-joining (NJ) and UPGMA analyses of morphological distances generated a pattern of groupings somewhat consistent with the parsimony-generated results: the pseudothelphusids and potamoids exhibit a greater amount of overall morphological similarity with the thoracotremes than with any other heterotreme group (Figs 3 and 4). The pseudothelphusids as a group are placed next to the potamoids in both phenograms (Figs 3 and 4) indicating that they are sister taxa, regardless of the criteria used for grouping. The position of the trichodactylids, on the other hand, differs greatly according to the algorithm used. In the UPGMA analysis, the trichodactylids are shown to be part of the goneplacid, portunoid, and xanthoid cluster, although somewhat distant from each of these. Largely consistent with the batch-1 and batch-2 hypotheses, a UPGMA-based {{Pseudothelphusidae+Potamoidea} + {Thoracotremata {Bythograeidae +Pinnotheridae}}} grouping was apparent (Fig. 3). Also congruent with the parsimony-based results is the juxtapositioning of the goneplacids (+ *Beuroisia*) and portunoids by UPGMA. The xanthoids form a cluster with the Cancridae and hypothetical outgroup on the grounds of total morphological distance.

The results of the NJ analysis (Fig. 4) positioned the trichodactylids within a portunoid set, and the Goneplacidae s.s. is again placed as the nearest

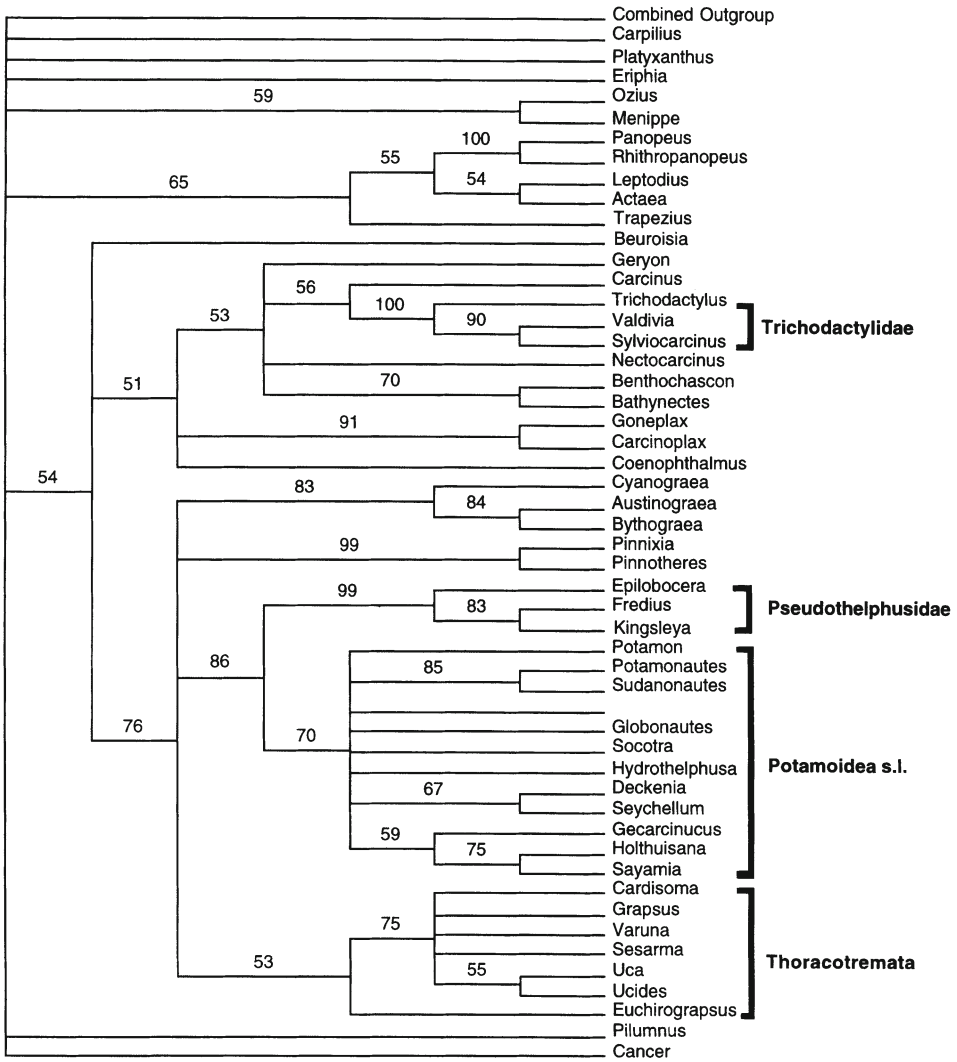

Figure 1. Bootstrap 50% majority rule consensus tree of Eubrachyuran relationships supported by bootstrapped parsimony using a combination of ordered and unordered characters. Numbers indicate bootstrap proportions >50 obtained from a heuristic search of 100 bootstrap replicates of 121 parsimony-informative characters for 51 ingroup taxa and a combined outgroup (Table 2), using the heuristic search option of PAUP 4.0 (Swofford, 2000).

morphological relation of the Portunoidea. In addition, the portunoid+goneplacid NJ 'line' is placed basal to a {pinnotherid {bythograeid {Thoracotremata {pseudothelphusid+potamoid}}}} pattern as is seen in the batch-1 hypothesis (Fig. 1). And the xanthoids form a cohesive set in the NJ tree (Fig. 4).

It should also be noted that the UPGMA and NJ calculated relationships among the potamoid genera are (at least in part) consistent with some recent taxonomic arrangements (e.g., Cumberlidge, 1999).

Conclusion

Parsimony searches for nested hierarchical relationships among 51 representative brachyrhynchan genera, using 121 morphological characters, resulted in a small set of hypotheses concerning the position of the freshwater crab families in the Eubrachyura (Figs. 1 and 2). The majority of the bootstrap replicate cladograms support a sister group relationship between the Neotropical Pseudothelphusidae and a Palaeotropical potamoid clade. This finding supports the results of the groundpattern analysis of Sternberg et al. (1999) and Sternberg & Cumberlidge (1999). Four almost invariant apomorphies are found among the pseudothelphusids and Old World freshwater crabs. These are: (i) a distinct semicircular vertical groove on the carapace sidewall, extending from the epibranchial tooth to the

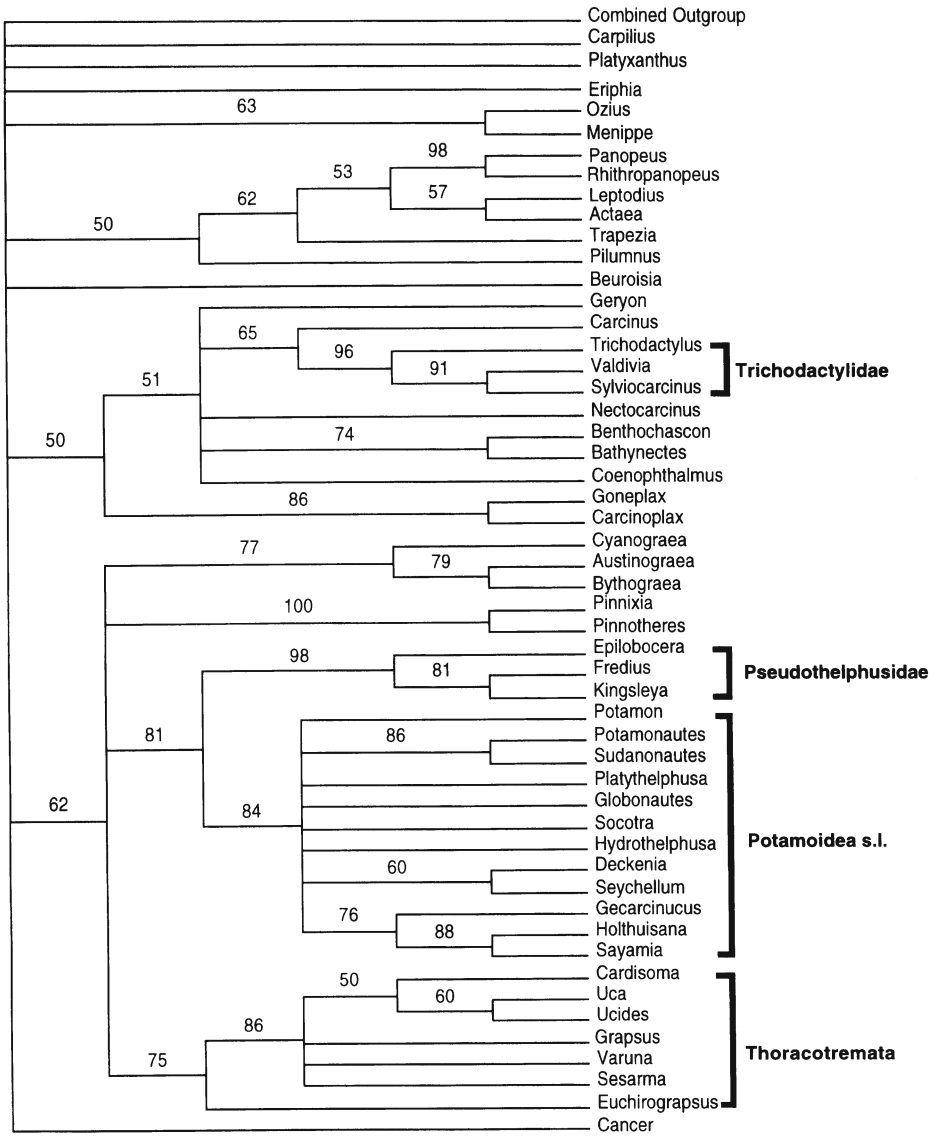

Figure 2. Bootstrap 50% majority rule consensus tree of Eubrachyuran relationships supported by bootstrapped parsimony using all unordered characters. Numbers indicate bootstrap proportions >50 obtained from a heuristic search of 100 bootstrap replicates of 121 parsimony-informative characters for 51 ingroup taxa and a combined outgroup (Table 2), using the heuristic search option of PAUP 4.0 (Swofford, 2000).

epimeral sulcus (**29-1**); (ii) a sharp and prominent median projection on the epistome (**43-1**), which is (iii) flanked by distinct incisions (**45-1**); and (iv) third maxillipeds which completely enclose the buccal chamber (**60-1**). These four apomorphies support a node linking the pseudothelphusids and Potamoidea. Given that no evidence has been found to discount a [Pseudothelphusidae+Potamoidea] clade, such a relationship is considered here to be a good working hypothesis (see also Sternberg et al., 1999).

The objective of this study was to clarify the position of the freshwater crab families within the Eubrachyura, as opposed to resolving relationships within any one freshwater crab group. The general absence of hierarchical freshwater crab generic relationships observed for the batch-1 and batch-2 consensus trees, are undoubtedly due to the high degree of incongruence seen for almost all character states that have been examined (excepting for the four just mentioned above). Numerous other mosaic character state combinations are also found distributed among the

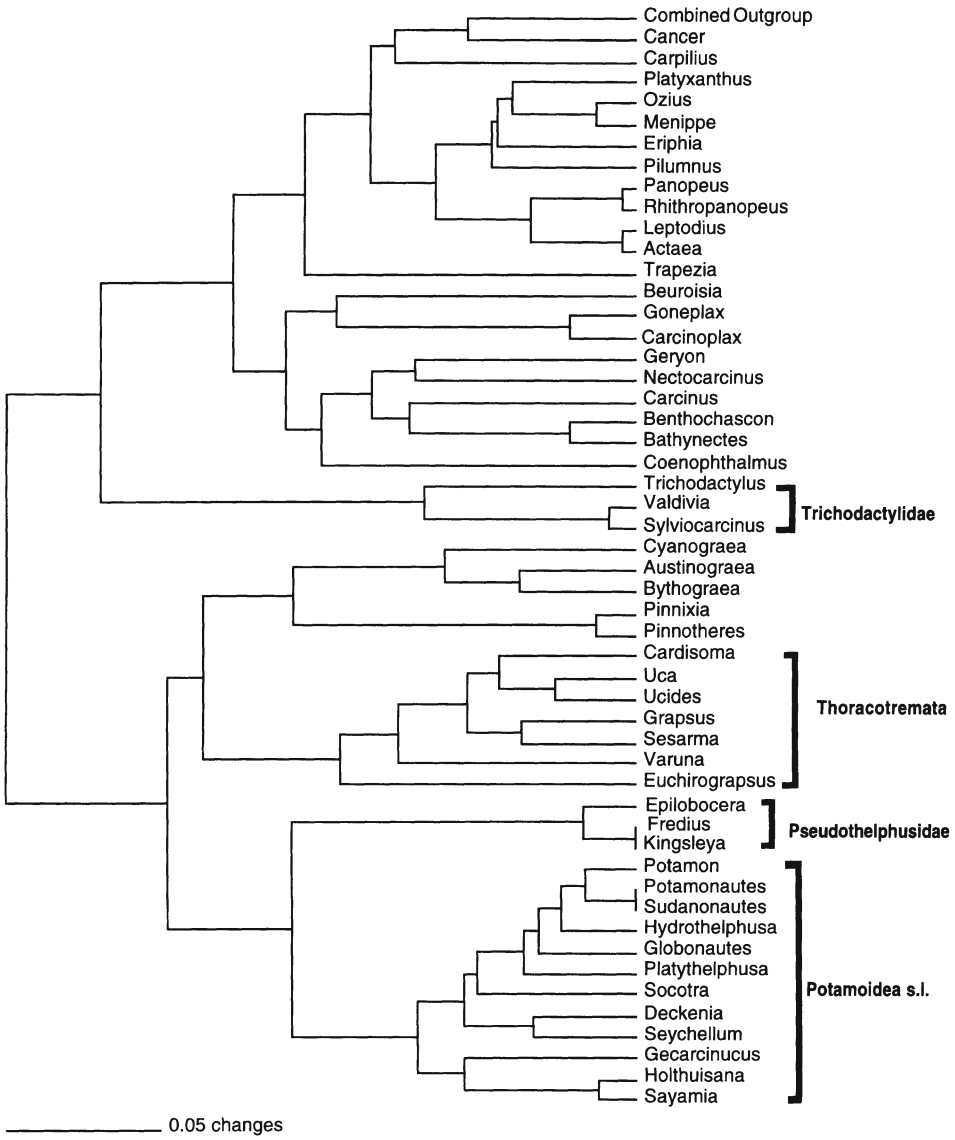

Figure 3. Phylogram derived by UPGMA cluster analysis of Eubrachyuran relationships for 51 ingroup taxa and a combined outgroup (Table 2). Phylogram based on 121 characters generated by PAUP 4.0 (Swofford, 2000). Branch lengths are drawn to scale.

pseudothelphusids and various subclades of potamoids in combinations that support conflicting hypotheses of relationships. For example, pseudothelphusids and the African potamoids have the following characters in common: (a) a horizontally-oriented median projection on the epistome (**44-1**); (b) a male telson which tends to be triangular in outline (**78-1**); (c) a specific conformation of the anterior region of the plastron (sternites 1–5) (see Rodriguez, 1992; Cumberlidge, 1999); and (d) similarities in the subbranchial, suborbital, and pterygostomial regions of the carapace, and in the outline of the buccal frame. Many of these characters need to be investigated further and so have not been included in the present work.

On the other hand, pseudolungs are found in the Pseudothelphusidae (Rodriguez, 1986), the African globonautines (Cumberlidge, 1991), the African deckeniids (unpublished data), *Madagopotamon* (unpublished data), *Seychellum* (unpublished data), and some Australian parathelphusids (Taylor & Greenaway, 1979). Pseudolungs are notably absent from most African and Australasian potamoids, and so this character state distribution would conflict with

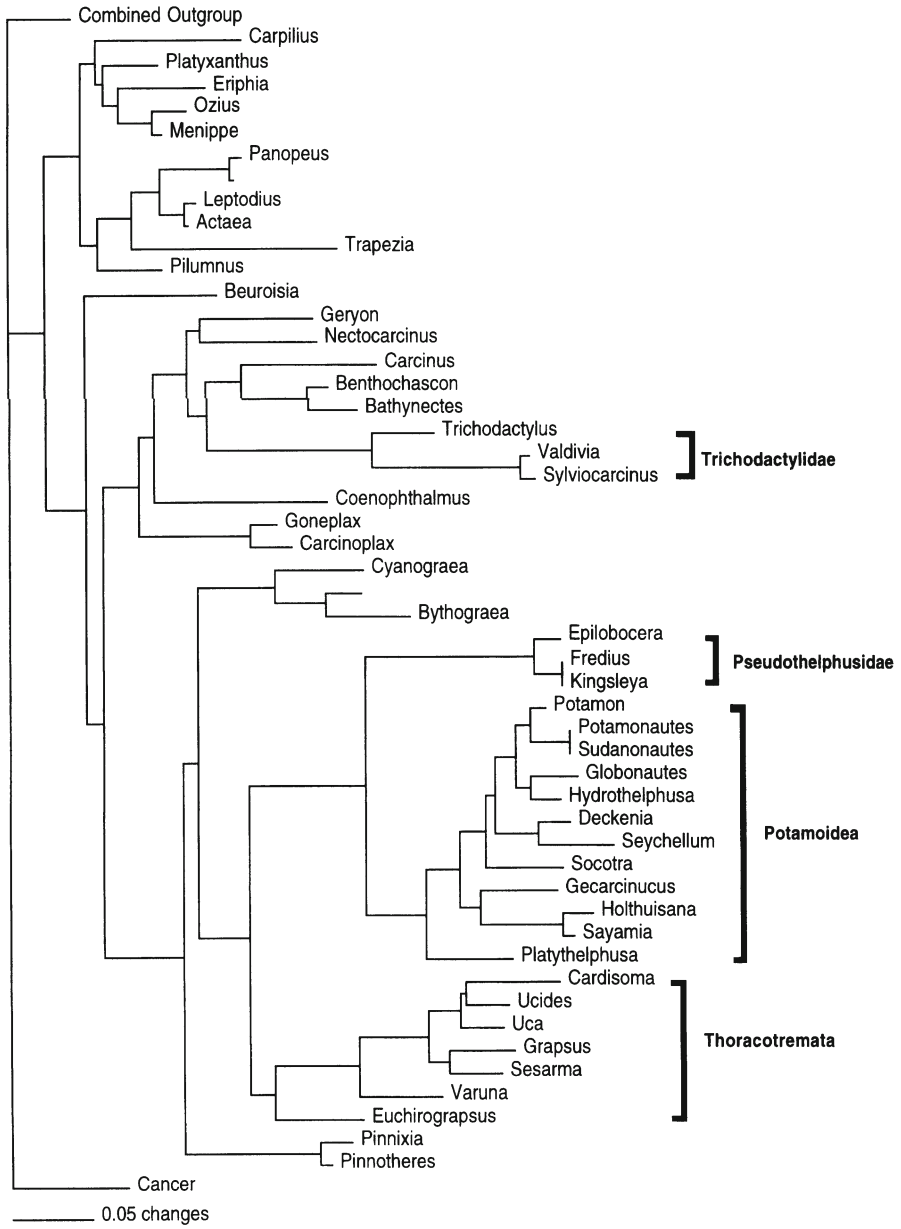

Figure 4. Neighbor-joining phylogeny of Eubrachyuran relationships for 51 ingroup taxa and a combined outgroup (Table 2). Phylogram based on 121 characters generated by PAUP 3.1.1 (Swofford, 1993). Branch lengths are drawn to scale.

a [pseudothelphusid+African potamoid] sister group relationship.

A mandibular palp with a bilobed terminal segment is shared by the Asian and Australian gecarcinucids and parathelphusids, African globonautines, and pseudothelphusids (see Bott, 1970; Rodríguez, 1986; Ng, 1988; Cumberlidge, 1999), and has been considered to be a synapomorphy for a [Pseudothelphusidae [Gecarcinucidae+Parathelphusidae]] lineage (Rodríguez, 1986; Ng et al., 1995). However, the presence of various intermediate conditions of the terminal segment of the mandibular palp such as those found among the African and Madagascan potamonautids undermines confidence in such intercontinental relationships. Moreover, a close relationship between the Pseudothelphusidae, Gecarcinucidae, and Parathelphusidae is contradicted by a number of characters found in the latter two Old World families that are

not found in the pseudothelphusids. These characters include: epigastric crests (**13-1**), postorbital crests (**16-1**), posterolateral carapace carinae (**24-1**), a distinct lateral carina on the extreme posterior margin of the carapace (**25-1**), carapace sidewall carinae (**32-1**), and a first gonopod with a significantly developed terminal article (**99-1**, **100-1**). These features are also shared by a number of other Old World potamoids (e.g. deckeniids, potamids and most potamonautids) with a mandibular palp with a 'simple' (i.e. single) terminal segment. It is clear that relationships among the Old World potamoids must first be resolved before additional character states can be added to the node linking the pseudothelphusids and the Old World potamoids. Whatever the final hypothesis of relationships within and among the various nontrichodactylid freshwater crab groups, homoplasy on a massive scale will have to be accommodated.

A comment must also be made about the taxonomic distribution of the two-segmented mandibular palp in brachyuran crabs. A two-segmented mandibular palp was previously hypothesized to be a synapomorphy linking the pseudothelphusids and potamoids (Sternberg & Cumberlidge, 1999; Sternberg et al., 1999). Insofar as nearly all basal members of the various eubrachyuran families and superfamilies have a three-segmented mandibular palp (a plesiomorphy), it was correct to code the groundpattern of groups such as the eriphiids or xanthids as having the plesiomorphic state. However, it is now apparent that marine crab genera from a diverse range of families have either a two-segmented mandibular palp or a 3-segmented palp that shows incomplete fusion of the proximal and penultimate segments. A two-segmented mandibular palp is by no means an exclusively freshwater crab characteristic, and it is found in many marine crab groups including most portunoids, some corystoids, some trichodactylids, various xanthoids and possibly all majoids (unpublished results). Because parsimony analysis does not support a singular derivation of a two-segmented mandibular palp, it is a strong possibility that this apomorphy is a rampant homoplasy among eubrachyurans. For example, the Portunoidea includes families with a 3-segmented mandibular palp (e.g. the Geryonidae), families with a 3-segmented palp with intermediately fused proximal-penultimate segments (e.g. *Scylla*), and families with a 2-segmented palp. This means that either the apomorphic 2-segmented mandibular palp has repeatedly arisen in the portunoids, or that recurrent reversals to the 3-segmented condition are common.

The strict consensus of the shortest trees in our parsimony searches (Figs 1 and 2) both position the [pseudothelphusid+potamoid] clade in a polytomy with the Thoracotremata, a result that robustly supports the conclusions of Sternberg & Cumberlidge (1999) and Sternberg et al. (1999). However, many of the apomorphies previously thought to be unique to the [Thoracotremata [Pseudothelphusidae+Potamoidea]] lineage have since been identified in members of the Pinnotheridae. For example, pinnotherids lack an endostomial gutter (**48-2**) and some taxa also have pereiopod 2–5 meri which are subtriangular in cross-section (**110-1**), with rugosities on the surface (**111-1**). The presence of states **48-2**, **110-1**, and **111-1** in pinnotherids suggests that this family might either occupy a position basal to the Thoracotremata, with the pseudothelphusids and potamoids as the sister taxon of a pinnotherid+thoracotreme clade, or as the group basal to the [Thoracotremata [Pseudothelphusidae+Potamoidea]] lineage. Guinot (1977, 1979) previously placed the Pinnotheridae in the Thoracotremata although Guinot & Richer De Forges (1997) moved the family to the Heterotremata on the basis that this taxon lacks truly sternal male gonopore openings. It is clear that additional pinnotherid genera must be examined before any firm conclusions regarding sister group relationships of the pinnotherids *vis-à-vis* the thoracotremes and nontrichodactylid freshwater crabs can be reached. This is especially important insofar as some pinnotherid genera (e.g. *Pinnotherelia* and *Tritodynamia*) have a distinctly thoracotreme-like habitus and thus may hold a basal station within the group.

The same applies to the bythograeids which were found to form a polytomy with the pinnotherids, thoracotremes and nontrichodactylid freshwater crabs. The bythograeid groundpattern is remarkably like that of pseudothelphusids, and it seems unlikely that a family associated with deep-sea hydrothermal vents (bythograeids) on the one hand, and a family associated with semiterrestrial habitats and cloud-forest environments (Pseudothelphusidae) on the other, could have attained strikingly similar habitus by 'convergence.' However, more detailed morphological comparisons (and molecular analyses) must be conducted before a definite conclusion can be reached about the placement of the Bythograeidae in the Eubrachyura.

Jamieson et al. (1995) tested the position of the African potamonautid *Potamonautes* within the context of the Brachura through a cladistic study of mainly

spermatozoal characters. *Potamonautes* was placed basal to the xanthid *Pilodius* and the trapeziid *Calocarcinus* in a 50% majority rule consensus tree of 959 shortest cladograms obtained from a parsimony analysis using only spermatozoal characters (Fig. 1a of Jamieson et al., 1995). However, when the 27 spermatozoal characters were combined with 7 non-spermatozoal morphological characters in a heuristic search, the resulting strict consensus tree positioned *Potamonautes* as part of a polytomy with the majids, thoracotremes, *Portunus*, and the xanthoids (Fig. 1a of Jamieson et al., 1995). Since the spermatozoal characters used by Jamieson et al. (1995) cannot resolve relationships within the Heterotremata s.l., that study should not be viewed as incongruent with the results obtained here or elsewhere (Sternberg et al., 1999).

It must be reiterated that a disconcertingly high incidence of character state incongruence was found with the adult eubrachyuran morphological characters used in the present study. Aside from universal support for a Pseudothelphusidae+Potamoidea sister group hypothesis, hierarchical relationships are obscured within the Eubrachyura in general and freshwater crabs in particular. Our preliminary studies (unpublished) indicate that the degree of confidence in any hypothesis of taxic relationships of freshwater crabs decreases dramatically as the number of characters and taxa is increased. This appears to be the result of a 'theme/variation' model of diversification within the Eubrachyura, as opposed to an inappropriate choice of characters or inappropriate character coding. The theme/variation model (Thomson, 1988) posits that whereas a hierarchy of morphotypes or 'morphological themes' can be discerned, the considerable taxic variation observed within a morphotype hinders precise determination of sister group relationships. In other words, it is easier to determine relationships among morphotypes (groundpatterns) than among taxa that share the same morphotype. Presumably this is because the morphotype reflects a preferred domain in morphospace, wherein considerable character state recombination can occur (Thomson, 1988). The fact that very few apomorphies appear to be unique to any one eubrachyuran group suggests that the various crab lineages have differentially parcelled morphological conditions derived from a basic set of shared, potential morphological conditions. Cladistic analyses of eubrachyuran familial relationships using a set of genera from each family (as performed here) would thus be expected to generate much weaker hypotheses compared to those using groundpatterns, because of the 'conflicting' character recombinants found among genera and species within a family or superfamily. A test of the theme/variation model of eubrachyuran morphological relationships hinges upon comparing the parsimony results obtained from using a larger set of characters from a larger and more representative set of marine and freshwater crab genera, selected on the basis of rigorously inferred familial groundpatterns.

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Appendix 1

Listing and current systematic placement of Eubrachyuran taxa used in the cladistic and phenetic studies. Institution acronyms are: FM = Field Museum, Chicago; MNHN = Paris Museum; MT = R.G. Mus. Afr. Centr.; NMU = Northern Michigan Univ. Biol. Dept. Collection; SM = Senckenberg Museum, Frankfurt; UMML = University of Miami Marine Laboratory Invertebrate Museum; and USNM = Smithsonian Institution.

Heterotremata Guinot, 1977
- **Bythograeoidea** Williams, 1980
- **Bythograeidae** Williams, 1980
  - *Austinograea alayseae* Guinot, 1989; exMNHN 24055 (NMU uncatalogued)
  - *Bythograea thermydron* Williams, 1980; FM 5591
  - *Cyanograea praedator* de Saint Laurent, 1984; USNM 239196

Corystoidea Samouelle, 1819
- **Cancridae** Latreille, 1803
  - *Cancer* (*Metacarcinus*) *borealis* Stimpson, 1859; UMML 32.2431

Xanthoidea Macleay, 1838
- **Carpiliidae** Ortmann, 1893
  - *Carpilius corallinus* (Herbst, 1783); UMML 32.839

Eriphiidae MacLeay, 1838
Eriphia gonagra (Fabricius, 1781); UMML 32.1132
Menippe mercenaria (Say, 1818); UMML 32.8217
Ozius reticulatus (Desbonne & Schramm, 1867); NMU uncatalogued
Panopeidae Ortmann, 1893
Panopeus purpureus (Lockington, 1877); NMU uncatalogued
Rhithropanopeus harrisii (Gould, 1841); UMML 32.3160
Pilumnidae Samouelle, 1819
Pilumnus dasypodus Kingsley, 1879; uncatalogued
Pilumnus sayi Rathbun, 1897; uncatalogued
Platyxanthidae Guinot, 1977
Platyxanthus crenulatus A. Milne Edwards, 1879; UMML 32. 7651
Trapeziidae Miers, 1886
Trapezia cymodoce (Herbst, 1801); USNM 286050
Xanthidae Macleay, 1838
Actaea acantha (H. Milne Edwards, 1834); UMML 32.529
Leptodius agassizii A. Milne Edwards, 1880; UMML 32.7286
Portunoidea Rafinesque, 1815
Geryonidae Colosi, 1923
Chaceon quinquedens (Smith, 1879); UMML 32.3950
Portunidae Rafinesque, 1815
Bathynectes superba (Costa, 1853); UMML 32.2450
Benthochascon schmitti Rathbun, 1931; UMML 32.2434
Carcinus maenas (Linnaeus, 1758); NMU uncatalogued
Coenophthalmus tridentatus A. Milne Edwards, 1879; USNM 65037
Nectocarcinus tuberculosus A. Milne Edwards, 1860; USNM 64716
Superfamily Uncertain
Goneplacidae MacLeay, 1838
Beuroisia Guinot & Richer de Forges, 1981 sp.; USNM 371429
Carcinoplax longimanus (de Haan, 1833); USNM 265063
Goneplax sigsbei A. Milne Edwards, 1880; UMML 32.7236, 32.7269
Pinnotheridae de Haan, 1833
Pinnixia cristata Rathbun, 1900; UMML 32.1967
Pinnotheres maculatus Say, 1818; UMML 32.7661
Potamoidea Ortmann, 1896
Deckeniidae Ortmann, 1897
Deckenia mitis Hilgendorf, 1869; NMU III.1990
Gecarcinucidae Rathbun, 1904
Gecarcinucus jacquemonti (H. Milne Edwards, 1844); SM 1763
Globonautes macropus (Rathbun, 1898); NMU 18.VIII.1988
Seychellum alluaudi (A. Milne Edwards & Bouvier, 1893); MT 56.895
Parathelphusidae Colosi, 1920
Holthuisana (*Austrothelphusa*) *transversa* (Martens, 1868); SM 5156
Holthuisana festiva (Roux, 1911); SM 7369
Sayamia sexpunctata (Lanchester, 1906); NMU uncatalogued
Potamidae Ortmann, 1896
Potamon edule (Latreille, 1818); NMU 17.1996
Potamonautidae Bott, 1970
Erimetopus brazzae (A. Milne Edwards, 1886); MNHN BP 71
Hydrothelphusa bombetokensis (Rathbun, 1904); MNHN BP 63
Potamonautes aloysiisabaudiae (Nobili, 1906); NMU VII.1993
Platythelphusa armata A. Milne Edwards, 1887; uncatalogued
Sudanonautes africanus (A. Milne Edwards, 1869); NMU 9.IV.1983B(#37)
Superfamily Unknown
Pseudothelphusidae Rathbun, 1893
Epilobocera sinuatifrons (A. Milne Edwards, 1866); NMU uncatalogued
Fredius reflexifrons (Ortmann, 1897); NMU uncatalogued
Kingsleya latifrons (Randall, 1840); NMU 13.IX.1994
Superfamily Unknown
Trichodactylidae H. Milne Edwards, 1853
Sylviocarcinus pictus Pretzmann, 1968; NMU uncatalogued
Trichodactylus fluviatilis Latreille, 1828; NMU 29.V.1999
Valdivia serrata White, 1847; NMU 30.VI.1983
Thoracotremata Guinot, 1977
Gecarcinoidea Dana, 1851
Gecarcinidae Dana, 1851
Cardisoma guanhumi Latreille, 1825; UMML 32.7414

Grapsoidea Dana, 1851
Grapsidae Dana, 1851
Grapsinae Dana, 1851
Goniopsis pulchra Lockington, 1877; NMU 6.XI.1996
Sesarminae Dana, 1852
Sesarma curacaoense de Man, 1892; UMML 32.1333
Sesarma reticulatum (Say, 1817); UMML 32.1337
Varuninae Alcock, 1900
Euchirograpsus americanus A. Milne Edwards, 1880; uncatalogued
Varuna litterata (Fabricius, 1798); MNHN B 25736
Ocypodoidea Fabricius, 1798
Ocypodidae Fabricius, 1798
Heloeciinae H. Milne Edwards, 1852
Ucides occidentalis (Ortmann, 1898); UMML 32.929, 32.7400
Ocypodinae Fabricius, 1798
Uca pugilator (Bosc, 1802); UMML 32.859
Uca vocator vocator (Herbst, 1804); UMML 32.8680

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Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene

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Key words: phylogeny, systematics, 16S rRNA, Crustacea, Decapoda, *Brachynotus*

Abstract

The crab genus *Brachynotus* de Haan, 1833 is restricted to the intertidal and shallow subtidal of the Mediterranean and northeastern Atlantic. It is presently recognized to consist of four species, of which three (*B. foresti*, *B. gemmellari* and *B. sexdentatus*) are endemic to the Mediterranean. The fourth species, *B. atlanticus*, is found along the Atlantic coasts of northern Africa and southern Europe, but also extends into the western Mediterranean. This high level of endemism suggests that speciation within *Brachynotus* is strongly correlated with the geography and geology of the Mediterranean Sea. A molecular phylogeny based on the mitochondrial large subunit (16S) rRNA gene indicates that the four species of *Brachynotus* form a monophyletic group within Atlantic Varunidae. The DNA sequence data also show that the genus *Brachynotus* can be subdivided into two species groups, one comprising *B. atlanticus* and *B. foresti*, and the other one *B. gemmellari* and *B. sexdentatus*. While *B. atlanticus* and *B. foresti* are clearly genetically distinct, *B. gemmellari* and *B. sexdentatus* are identical in the studied region of the 16S rRNA gene, suggesting a recent separation or continuing gene flow.

Introduction

Crabs of the genus *Brachynotus* de Haan, 1833 are restricted to the western Atlantic and Mediterranean, despite the fact that many species from different parts of the world, which are now classified in *Cyrtograpsus*, *Hemigrapsus*, *Leptograpsodes*, *Tetragrapsus* or *Thalassograpsus*, had been previously placed in this genus (see for example Rathbun, 1893; Tesch, 1918; Tweedie, 1942; Phillips et al., 1984). Today, *Brachynotus* consists of four species. *B. atlanticus* Forest, 1957 is found along the Atlantic coasts of northern Africa and southern Europe extending into the western Mediterranean (García-Raso, 1984; d'Udekem d'Acoz, 1999). The other three species, *B. foresti* Zariquiey Alvarez, 1968, *B. gemmellari* (Rizza, 1839) and *B. sexdentatus* (Risso, 1827), are mostly endemic to the Mediterranean, with occasional findings from the Black Sea, the Suez Canal, and the Gulf of Cádiz (Zariquiey Alvarez, 1968; d'Udekem d'Acoz, 1999). The geographical confinement of its species, makes *Brachynotus* a very interesting genus for the study of speciation and biogeography within the Mediterranean Sea.

The ecological distribution of the crabs belonging to *Brachynotus* is typically the intertidal and shallow subtidal zone of rocky or soft bottom shores (d'Udekem d'Acoz, 1999). The resurrection of the species *B. gemmellari* by Froglia & Manning (1978) was mainly based on bathymetric and morphometric differences of this species and *B. sexdentatus*. Otherwise, these two species have overlapping distributions and a practically identical morphology. The taxonomic status of *B. gemmellari* is widely accepted, and several studies on its distribution, ecology and larval stages, have been published since its recognition (Almaça, 1985; Števčić, 1990; Guerao et al., 1995; Paula, 1996; Atkinson et al., 1997; d'Udekem d'Acoz, 1999).

The remaining species of *Brachynotus* are easily separable on the basis of morphological characters. On

the other hand, the important question about underlying phylogenetic relationships among these mostly sympatric species was never resolved. In this study, we compared a 580 basepair region of the mitochondrial DNA (large subunit rRNA) in order to test whether *Brachynotus* forms a monophyletic group, to help resolve phylogenetic relationships within the genus, and to establish the degree of genetic differentiation between the closely related *B. gemmellari* and *B. sexdentatus*.

Material and methods

For the molecular phylogenetic analysis of *Brachynotus* and allied crabs, we included representatives of all the genera of Varunidae occurring in the Atlantic, i.e. four species (six specimens) of *Brachynotus*, as well as *Cyrtograpsus affinis*, *C. angulatus*, *Chasmagnathus granulatus*, *Cyclograpsus integer*, *Eriocheir sinensis* and *Hemigrapsus penicillatus* (see Table 1). The latter two are East Asian species that have been introduced into European waters during this century (Schnakenbeck, 1924; Noël et al., 1997). 16S mtDNA sequences of *Chasmagnathus granulatus* (EMBL accession number AJ250640), *Cyclograpsus integer* (AJ250639), *Cyrtograpsus affinis* (AJ130801), *Eriocheir sinensis* (AJ250642), *Hemigrapsus oregonensis* (AJ250644), *Sesarma reticulatum* (Sesarmidae) (AJ130799) and *Tetragrapsus jouyi* (AJ250647) had been used in a previous study to show that the genera *Chasmagnathus* and *Cyclograpsus* need to be classified within the Varunidae (see Schubart et al., 2000a). Other sequences obtained from genetic databases and included in the present study were *Eriocheir japonica* (AF105242) and *Grapsus grapsus* (Grapsidae) (AJ250650). The latter species served as an outgroup for the phylogenetic analyses. New sequences were submitted to the EMBL genetic database and can be retrieved under the accession numbers AJ278831 – AJ278836. The crabs specimens used for DNA extraction and sequencing were deposited as museum vouchers (Table 1).

Genomic DNA was extracted from the muscle tissue of walking legs or claws using a phenol-chloroform or Puregene extraction. An approximately 580 basepair region of the mitochondrial large ribosomal subunit rRNA (16S rRNA) gene was amplified by polymerase-chain-reaction (PCR) (38–40 cycles; 1 min 94°/1 min 48–55°/2min 72° denaturing/annealing/extension temperatures) with the primers listed in Table 2. PCR products were purified and sequenced by dideoxy chain termination with S35 radioactive labeling (at the Pennsylvania State University) or with the ABI Prism 310 Genetic Analyzer using the ABI BigDye terminator mix (at the University of Louisiana at Lafayette). All sequences were aligned manually using the multisequence editing program ESEE (Cabot & Beckenbach, 1989). Distance matrices of sequence divergence were analyzed using Kimura 2-parameter distances and neighbor joining (NJ) (Saitou & Nei, 1987) with the program MEGA (Kumar et al., 1993). Maximum parsimony (MP) analyses were carried out with PAUP (Swofford, 1993), using the heuristic search method with tree bisection and reconnection branch swapping. Gaps were treated as missing and the tree was rooted by a user-defined outgroup. Statistical significance of groups within inferred trees was evaluated by bootstrapping with 2000 replications.

Results

The complete alignment of the sequenced 16S rRNA gene region consisted of 580 positions. Of these, 186 were variable and 114 parsimony-informative. Pairwise transition to transversion ratios ranged between 0.74 (outgroup vs. ingroup) and 6.5 (closely related species). The MP heuristic search, with transversions versus transitions weighted 3/1, resulted in two most parsimonious trees. The MP bootstrap analysis (2000 replicates) yielded a consensus tree of the length 722 with the following tree-fit values: CI: 0.665, RI: 0.569, RC: 0.378 (Fig. 1). Results obtained by NJ agreed in the tree topology with MP (only considering bootstrap values $> 50\%$) and are, therefore, combined in Fig. 1.

All phylogenetic analyses suggest that *Brachynotus* forms a monophyletic group within the other Varunidae tested in this analysis (bootstrap values of 100 / 100). The sister group of this eastern Atlantic and Mediterranean genus cannot be determined with certainty, due to low nodal support (lower than 50%). According to our results, the genus *Brachynotus* can be further subdivided into two species groups: *B. atlanticus* and *B. foresti* (bootstrap values of 88 / 91), as well as *B. gemmellari* and *B. sexdentatus* (100 / 100) (Fig. 1). While *B. atlanticus* and *B. foresti* are clearly genetically distinct, *B. gemmellari* and *B. sexdentatus* turn out to be identical in the 16S mtDNA region that was analyzed. This was confirmed after comparing sequences of additional specimens of both species

Table 1. Localities, dates of collection and genetic database accession numbers of the specimen of *Brachynotus* (4 species), *Hemigrapsus penicillatus*, *Cyrtograpsus angulatus* and *Eriocheir sinensis* used for genetic comparisons. Abbreviations of museums where animals were deposited as voucher specimens: BMNH: British Museum of Natural History, London; SMF: Senckenberg Museum und Forschungsinstitut, Frankfurt a. M.; ULLZ: University of Louisiana at Lafayette Zoological Collection, Lafayette; USNM: United States National Museum, Smithsonian Institution, Washington

	Brachynotus sexdentatus (Risso, 1827) (EMBL AJ278832)
*	Spain: Cádiz: Puerto de Santa María: El Toruño, in aquaculture ponds up to 1.5 m depth;14. June 1999; coll. A. Rodríguez; SMF 25794
*	Greece: Gulf of Amvrakikos: Menidi; '0.5 m – 1.5 m deep muddy bottom with sea grasses", 17. July 1993; coll. C. d'Udekem d'Acoz; SMF 25795
	Brachynotus gemmellari (Rizza, 1839) (EMBL AJ278833)
*	Italy: Ancona, 3 miles off, 15 m depth; 7. July 1963; coll. Froglia; USNM 172093
*	England: Swansea: Queens Dock, March–June 1957; coll. E. Naylor; BMNH 1957.11.11.1-6
	Brachynotus atlanticus Forest, 1957 (EMBL AJ278831)
*	Spain: Cádiz: Cabo de Trafalgar; June 1996; coll. J.A. Cuesta; SMF 25706
	Brachynotus foresti Zariquiey Alvarez, 1968 (EMBL AJ278834)
*	Greece: Gulf of Amvrakikos: Agia Triada, 4 July 1993; coll. C. d'Udekem d'Acoz; SMF 25796
	Hemigrapsus penicillatus (de Haan, 1835) (EMBL AJ278835)
*	France: La Gironde Estuary, Talmont (~ 45° 32′ N - 0° 54′ W); 9 May 1996; coll. P. Noël; SMF 25798
	Cyrtograpsus angulatus Dana, 1851 (EMBL AJ278836)
*	Argentina: Mar Chiquita; January 1996; coll. K. Anger; SMF 24546
	Eriocheir sinensis H. Milne Edwards, 1853 (EMBL AJ250642)
*	U.S.A. California: San Francisco Bay: Byron; State Fish facility; 11.11.1996; coll. K. Hieb; ULLZ 4175

from different localities (see Table 1), which rendered identical results.

The present results also show significant support for other phylogenetic groupings. The Varunidae (sensu Schubart et al., 2000a) is confirmed as a monophyletic family (Fig.1: node 'VAR'; 99 / 99) (see also Cuesta & Schubart, 1997; Schubart & Cuesta, 1998). Congeneric species belonging to two other varunid genera were grouped together with relatively high bootstrap values: *Cyrtograpsus* (98 / 98) and *Eriocheir* (100 / 100), thereby confirming current taxonomy. The monotypic genus *Tetragrapsus* from the Gulf of California is nested within two species of the genus *Hemigrapsus*. If future results confirm that the eastern Pacific *Hemigrapsus* (e.g. *H. oregonensis*) are closer related to *Tetragrapsus* than to the western Pacific *Hemigrapsus* (e.g. *H. penicillatus*), the genus *Hemigrapsus* has to be considered paraphyletic.

Table 2. Primers used for PCR amplification and sequencing of parts of the 16S rRNA gene

16Sar: 5′- CGCCTGTTTATCAAAAACAT -3′
16L2 5′- YGCCTGTTTATCAAAAACAT -3′
16L15: 5′- GACGATAAGACCCTATAAAGCTT -3′
1472 5′- AGATAGAAACCAACCTGG -3′
16Sbr: 5′- CCGGTCTGAACTCAGATCACGT -3′
16H16: 5′-TTATCRCCCCAATAAAATA-3′

Discussion

In this study, the monophyly of the crab genus *Brachynotus* was supported by a phylogeny based on a 580 basepair region of the 16S rRNA gene. This finding is not only important in terms of corroborating present taxonomic classification. It is also useful for understanding evolutionary relationships among

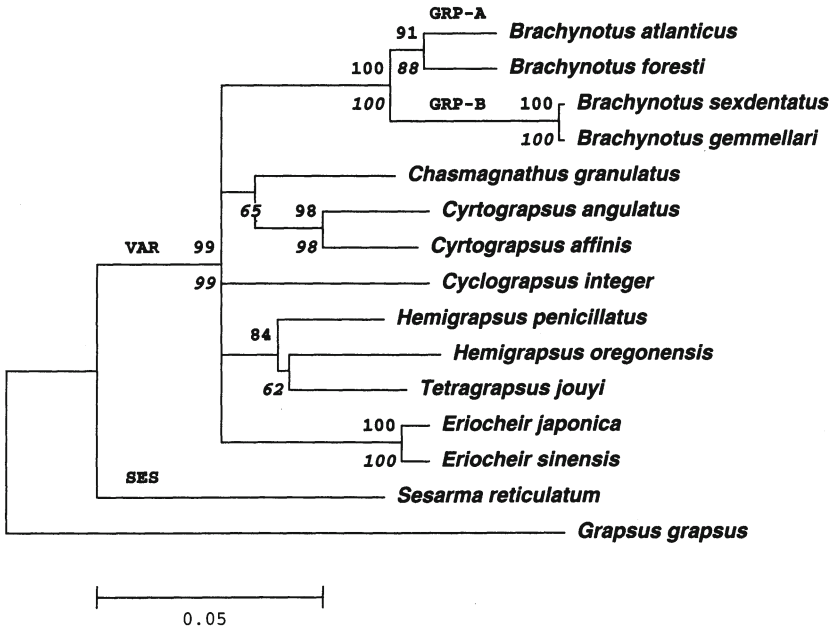

Figure 1. Molecular phylogeny of the genus *Brachynotus* and other representatives of the Varunidae based on 580 basepairs of the 16S rRNA gene. Upper values: Kimura 2-parameter distances, neighbor joining, 2000 bootstrap replications. Lower values in italics: maximum parsimony, 2000 bootstrap replications (transversions/transitions weighted 3/1). Only bootstrap values above 50% are shown. Abbreviations: VAR: Varunidae, SES: Sesarmidae, GRP: Group.

the comprised species and for developing a model of speciation.

Three species of *Brachynotus* are almost exclusively found in the Mediterranean Sea (d'Udekem d'Acoz, 1999). It is, therefore, most likely that they also evolved there. The fourth species, *B. atlanticus*, has an eastern Atlantic distribution from southern Spain to Mauritania (d'Udekem d'Acoz, 1999). In the Mediterranean, it is only found in the westernmost part (Sea of Alborán) (García-Raso, 1984). The geology of the Mediterranean Basin gives evidence for at least one major isolation event from Atlantic waters (Por, 1989). Assuming that crabs survived in the Mediterranean, despite possible water level decrease and hypersaline conditions (e.g. Messinian crisis in the Pliocene), allopatric speciation from the Atlantic form would have been the logical consequence.

According to our data, the oldest split within *Brachynotus* is the separation of *B. atlanticus* and *B. foresti* (Group-A) from *B. sexdentatus* and *B. gemmellari* (Group-B) (Fig. 1). Assuming that the documented speciation events occurred by Atlantic-Mediterranean allopatric differentiation, we suggest that the ancestor of Group-A was originally isolated as an Atlantic population from its Mediterranean counterpart (Group-B). After reconnection of Atlantic and Mediterranean waters took place, introgression of Group-A into parts of the Mediterranean, and a subsequent second isolation event could explain speciation of the Atlantic *B. atlanticus* and the Mediterranean *B. foresti*. The chronologically last split took place when the Mediterranean Group-B separated into *B. sexdentatus* and *B. gemmellari*. This separation most likely occurred within the Mediterranean Sea, but the completion and mechanisms of this possible speciation event are unconfirmed.

Based on our speciation model, most species did only slightly redisperse after reconnection of Atlantic and Mediterranean waters. Today's distribution of species shows only marginal redispersal beyond the Gibraltar Straits (*B. sexdentatus* in Bay of Cádiz, *B. atlanticus* in Sea of Alborán). The sister species *B. atlanticus* and *B. foresti* even seem to exclude each other, since the latter species is absent from the Sea of Alborán, which represents the westernmost distribution of *B. atlanticus* (see García-Raso, 1984; García-Raso et al., 1987). The lack of redispersal is probably due to long-term adaptation to different local conditions encountered in the Atlantic Ocean and the Mediterranean Sea during isolation (e.g. temperature, salinity, substratum). Occasionally, some of the Mediterranean species of *Brachynotus* have been recorded from local-

ities clearly outside their normal distributionary range. *B. sexdentatus* has been reported from Swansea, U.K. (Naylor, 1957) and the French Atlantic coast (Noël et al., 1997; d'Udekem d'Acoz, 1999), but never established breeding populations (except at the warmed docks of Swansea). We found that specimens from Swansea deposited in the Bristish Museum of Natural History were labeled as *B. sexdentatus gemmellari*. This identifcation was confirmed by C. Froglia (pers. comm., 1999) and they are thus here considered to belong to *B. gemmellari* (see Table 1).

Four specimens of *Brachynotus sexdentatus* and *B. gemmellari* were included in this study (Table 1), and all of them shared the same 16S mtDNA haplotype. This gene is normally variable enough to be used for population studies in marine crabs (e.g. Cuesta & Schubart, 1998; Schubart et al., 2000b). The lack of variation between the two species of *Brachynotus* is thus an indication for a very recent separation or for continuing gene flow. A close relationship between *B. gemmellari* and *B. sexdentatus* can already be inferred from the fact that adults of both species can only be separated on the basis of morphometry and bathymetry (Froglia & Manning, 1978). New results on comparative larval morphology of these two species reveal only minor differences in setation of appendages that are known to vary intraspecifically (Cuesta et al., 2000).

Morphological and molecular comparisons of many populations of *Brachynotus sexdentatus* and *B. gemmellari* throughout the Mediterranean need to be undertaken to determine how consistently these two 'forms' can be separated and how likely it is that they represent good species. These studies can be supported by crossbreeding experiments in the laboratory in order to determine whether *B. sexdentatus* and *B. gemmellari* can produce fertile offspring. As far as future molecular work is concerned, we will use a second, more variable gene (cytochrome oxidase subunit I, COI) to compare a large number of different populations. Preliminary results revealed differences in 4 out of 640 positions between two specimens of *B. sexdentatus* (Greece and Spain). Unfortunately, specimens of *B. gemmellari* had probably been preserved in formalin (judging from tissue and PCR sucess), and amplification of the long COI fragment was so far unsuccessful. Based on the present results, it seems at least possible that *Brachynotus gemmellari* (subtidal) and *B. sexdentatus* (intertidal to shallow subtidal) represent different ecophenotypes of a single species.

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Austinogebia, a new genus in the Upogebiidae and rediagnosis of its close relative, *Gebiacantha* Ngoc-Ho, 1989 (Crustacea: Decapoda: Thalassinidea)

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Key words: Crustacea, Thalassinidea, *Austinogebia*, new genus, taxonomy, Indo-Pacific

Abstract

New material described recently permits the separation of six upogebiid species into the new genus *Austinogebia*, for which the diagnostic characters and a key are presented. The new taxon is compared to its close relative, *Gebiacantha* Ngoc-Ho, 1989, and the opportunity is taken to rediagnose the latter.

Abbreviations: cl – carapace length measured from the tip of the rostrum to the posterior border of the carapace; tl – total length measured from the tip of the rostrum to the posterior border of the telson; AMS – Australian Museum, Sydney; BLT – Biological Laboratory, Shikoku Women's University, Japan; MNB – Museum für Naturkunde, Berlin; MNHN – Muséum national d'Histoire naturelle, Paris; NTOU – Graduate School of Fisheries, National Taiwan Ocean University; SMF – Senckenberg Museum, Frankfurt.
'Teeth' – refers to structures mainly rounded at base, with a blunt tip which is sometimes corneous. These are found on the upper parts of the rostrum, the carapace and the lateral ridges of the gastric region; 'Spines' – refer to structures often not rounded at base with a pointed, non-corneous tip; 'Upper' and 'lower' – considered as equivalent to 'dorsal' and 'ventral', respectively, are used where they seem more appropriate, especially in the description of appendages

Introduction

In 1995, Sakai and Türkay studied two upogebiid species collected in the Persian-Arabian Gulf, both with infrarostral spines: *Upogebia nobilii* Sakai & Türkay, 1995 and *Upogebia plantae* Sakai, 1982 (the latter was placed in the genus *Gebiacantha* by Ngoc-Ho, 1989). *Upogebia spinifrons* (Haswell, 1881) was also briefly discussed. To these authors '*it got clear that all three species treated herein are quite similar and cannot be separated generically*.' They concluded: '*therefore we prefer to leave the species treated herein in Upogebia s.l. and suppose that Gebiacantha should be treated as a synonym of Upogebia*'.

I have now re-examined all upogebiid species with infrarostral spines and compared *Upogebia nobilii* and *Upogebia plantae.* The excellent figures provided by Sakai & Türkay (1995) show several important differences between them (Figs 1 and 2). I believe these species represent two similar upogebiid groups that are distinct from each other and from the remaining species of the Upogebiidae. In this contribution, both groups are given generic rank. *Austinogebia* gen. nov. and *Gebiacantha* are diagnosed and their type species figured.

Seven American upogebiid species with infrarostral spines have now been described: *U. affinis* (Say, 1818), *U. spinistipula* Williams & Heard, 1991, *U. felderi* Williams, 1993, *U. paraffinis* Williams, 1993, *U. pillsbury* Williams, 1993, *U. schmitti* Williams, 1993 and *Upogebia bermudensis* Williams, 1993. All of these are excluded from *Austinogebia* and also from *Gebiacantha,* except for *U. bermudensis.*

Likewise, two other species with infrarostral spines are excluded from both *Austinogebia* and *Gebiacantha;* they are: *Upogebia poensis* De Saint Laurent

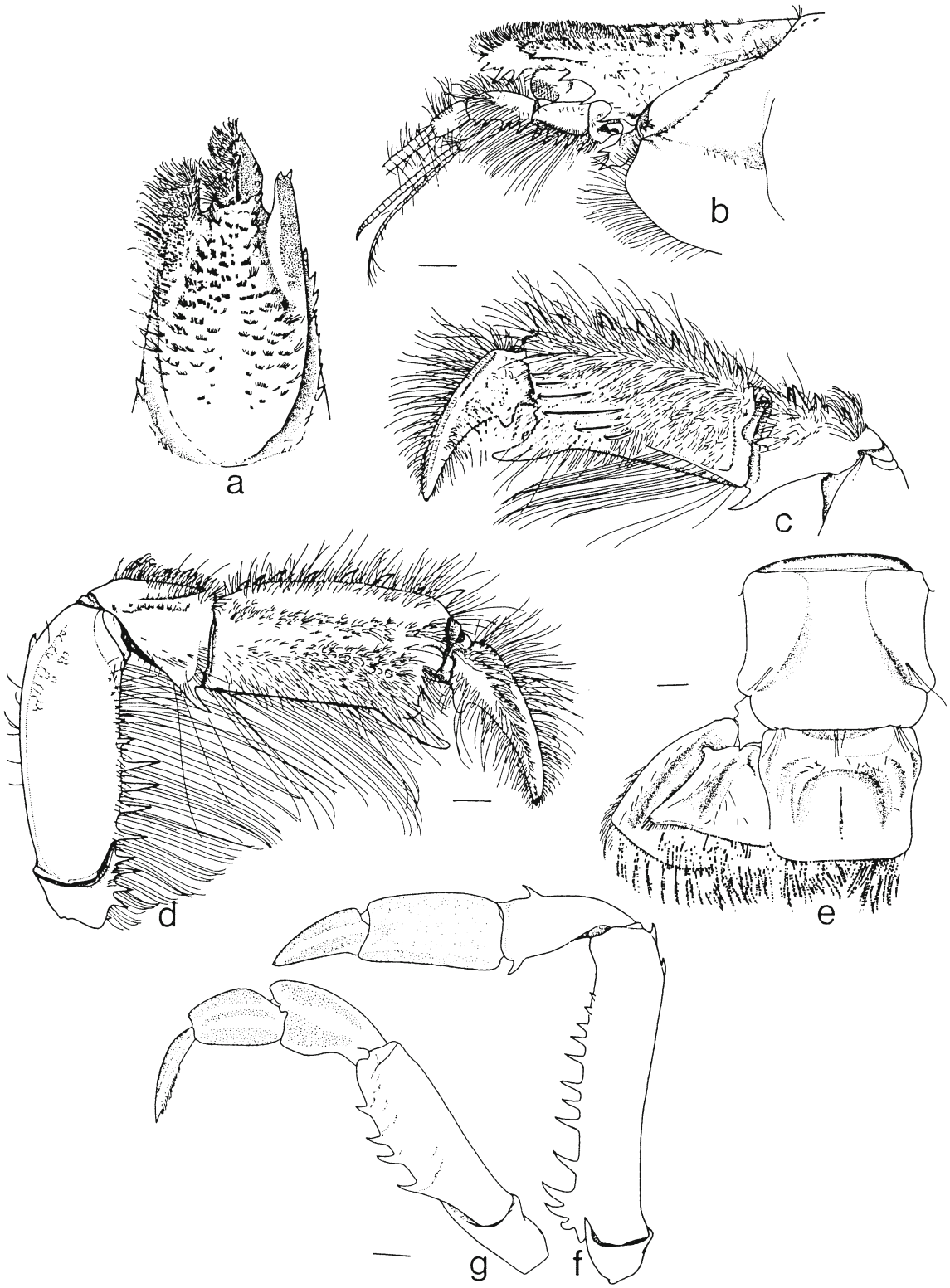

Figure 1. Upogebia nobilii (Sakai & Türkay, 1995): (a) and (b) anterior part of body, dorsal and lateral view, respectively; (c) distal part of male peropod 1, mesial view; (d) male pereiopod 1, lateral view; (e) telson and uropods; (f) and (g), pereiopod 2 and 3, respectively. Scale line: 1 mm.(from Sakai & Türkay, 1995).

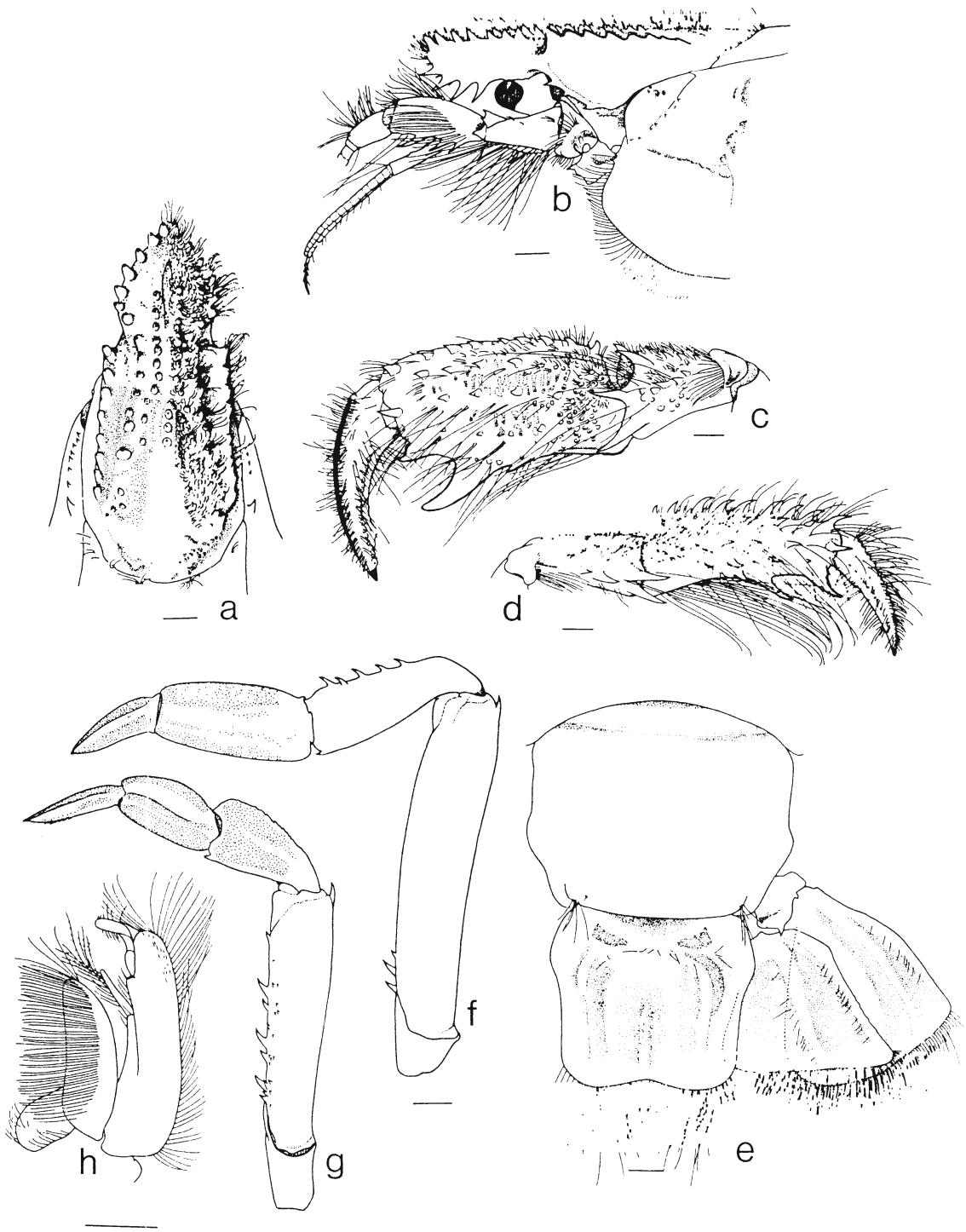

Figure 2. Upogebia plantae (Sakai, 1982): (a) and (b) anterior part of body, dorsal and lateral view, respectively; (c) and (d) distal part of male and female peropod 1, respectively, mesial view; (e) telson and uropods; (f) and (g) pereiopod 2 and 3, respectively; (h) first maxilliped. Scale line: 1 mm. (from Sakai & Türkay, 1995).

& Ngoc-Ho, 1979, from Fernando Poo Island (Gulf of Guinea) and *Upogebia snelliusi* Ngoc-Ho, 1990 from Indonesia. These are discussed.

Astinogebia gen. nov.

Type-species: *Upogebia narutensis* Sakai, 1986, by present designation. *Species included*: *Austinogebia spinifrons* (Haswell, 1881), *Austinogebia wuhsienweni* (Yu, 1931), *Austinogebia narutensis* (Sakai, 1986), *Austinogebia edulis* (Ngoc-Ho & Chan, 1992), *Austinogebia nobilii* (Sakai & Türkay, 1995) and *Austinogebia takaoensis* (Sakai & Türkay, 1995).

Diagnosis: Two to four infrarostral spines, several spines on anterolateral border of carapace (Fig. 3b). Lateral ridge of gastric region projecting forwards, upper distal half thickened and densely setose, with 1–3 lower distal spines (Figs 3a, b). Mandible without mesioanterior tooth; maxillipeds 1 and 3 both with epipod; arthrobranchs consisting of a series of large lamellae on either side of the rachis. Pereiopod 1 propodus with spines and spinules on upper border, lower border unarmed (Fig. 3d). Pereiopod 2 merus with 2–3 large proximal lower spines (Fig. 3e). Pereiopod 3 merus about three times as long as broad, with 2–4 large lower spines and one or more lateral curved rows of setae (Fig. 3f). Latero-external border of uropodal endopod with prominent knob on proximal shoulder (Fig. 3c).

Etymology: The genus is named in honour of the late Dr Austin B. Williams in recognition of his numerous contributions to the study of the Upogebiidae.

Remarks: Species of this genus are recognisable by the infrarostral spines, the projecting lateral ridges of the gastric region with lower distal spines, the morphology of pereiopod 3 and the knob on the proximal shoulder of the uropod endopod.

Characters they share with species of *Gebiacantha* are: 1. infrarostral spines (Figs 1b and 2b). 2. numerous spines on anterolateral border of carapace (Figs 1b and 2b) and pereiopod 1 (Figs 1c, d and 2c, d) and 3. (for a few species of *Gebiacantha*) the uropod exopod approximately as long as telson (Figs 1e and 2e). They differ especially by the projecting lateral ridges of the gastric region carrying lower distal spines. Other differences are summarised in Table 1.

Some species of this new taxon are figured in dorsal view with a spine at the tip of the rostrum (Figs 1a and 3a). This is actually not an upper rostral spine but an infrarostral spine that is visible dorsally. Similarly, the spine(s) shown at the tip of the lateral ridge of the gastric region in dorsal view belong(s) to the lower distal border of the ridge (Figures 1a and 3a).

All known American upogebiid species with infrarostral spines, as well as *Upogebia poensis* and *Upogebia sneliusi,* are excluded from this genus for having no lower distal spines on the lateral ridges of the gastric region and no knob on the proximal shoulder of the uropod endopod.

Key to the species of *Austinogebia*:

1. Anterior half of rostrum and anterior half of gastric ridge unarmed dorsally.....2
- Anterior half of rostrum with teeth, anterior half of gastric ridge with or without teeth.....5
2. Rostrum narrow, about 2.5 times as long as broad at base. Lower border of antennular and antennal peduncle unarmed ..... *Austinogebia takaoensis* (Sakai & Türkay, 1995)
- Rostrum about 1.2–1.5 times as long as broad at base 3
3. Lower border of antennal peduncle with 1 spine; small spines on pereiopods 1 and 2; pereiopod 2 merus with 1 upper distal spine ..... *Austinogebia narutensis* (Sakai, 1986)
- Lower border of antennal peduncle with numerous spines; large spines on pereiopods 1 and 2; pereiopod 2 merus with 2 upper distal spines 4
4. Rostrum 1.3 times as long as broad; telson approximately quadrate ..... *Austinogebia nobilii* (Sakai & Türkay, 1995)
- Rostrum 1.7 times as long as broad; telson broader than long ..... *Austinogebia spinifrons* (Haswell, 1881)
5. Anterior half of gastric ridge with 6–9 spiniform teeth, basis of pereiopod 1 with sharp spine *Austinogebia wuhsienweni* (Yu, 1931)
- Anterior half of gastric ridge unarmed or with 1–2 tubercles, basis of pereiopod 1 with blunt tooth ..... *Austinogebia edulis* (Ngoc-Ho & Chan, 1992)

Austinogebia narutensis (Sakai, 1986) n. comb. (Fig. 3)

Upogebia spinifrons - Sakai, 1984: 209, Figs. 1–3.
Upogebia narutensis Sakai, 1986: 25, pl. 1; Ngoc-Ho, 1994b: 198, Figs 4, 5 (in part).

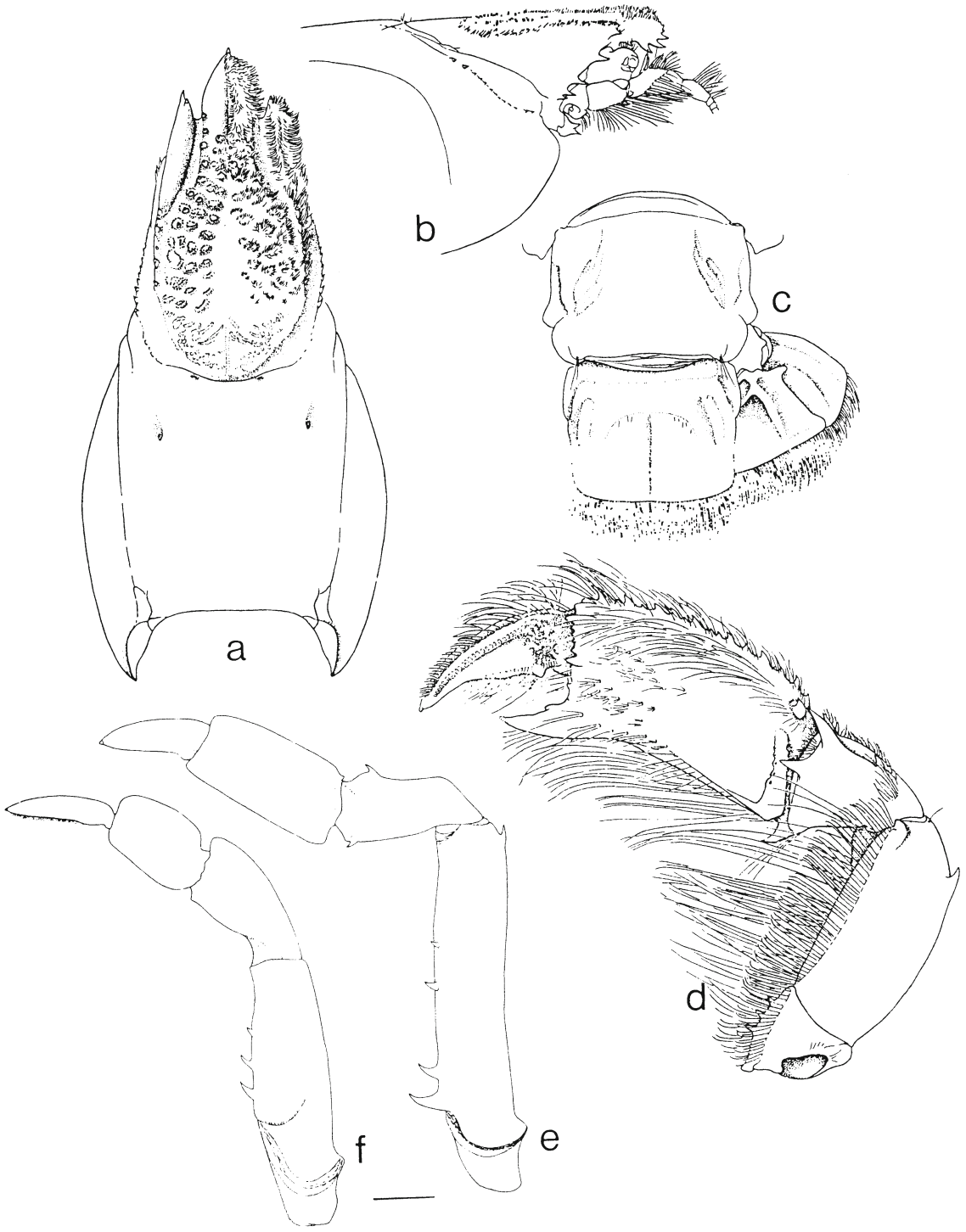

Figure 3. Austinogebia narutensis (Sakai, 1986): (a) carapace, dorsal view; (b) and (b'), anterior part of body, lateral view; (c) telson and uropods; (d) male pereiopod 1, mesial view; (e) and (f) pereiopod 2 and 3, respectively; Scale line: 1 mm (a–d, from Sakai, 1984, scale not indicated; e–f, from Ngoc-Ho, 1994b; b', male paratype, BLT 1718).

Table 1. Differences between *Austinogebia* gen. nov. and *Gebiacantha* (Ngoc-Ho, 1989)

Characters	*Austinogebia*	*Gebiacantha*
Lateral ridges of gastric region	projecting forward, thickened and densely setose on anterior half, lower distal spines present (Fig. 1a)	not projecting forward, same thickness and setation through out, lower distal spines absent (Fig. 2a)
First maxilliped	with large epipod	without epipod (Fig. 2h)
Lower border of pereiopod 1 propod posterior to fixed finger	unarmed (Fig. 1c, d)	with large spine often accompanied by small one (Fig. 2c, d)
Pereiopod 2 merus	2–3 large proximal lower spines (Fig. 1f)	1 or 2 small proximal lower spines (Fig. 2f)
Pereiopod 3 merus	about 3 times as long as broad, 2–4 large lower spines, lateral curved row(s) of setae (Fig. 1g)	about 4 times as long as broad, small lower spines, no lateral curved rows of setae. (Fig. 2g)
Posterior border of telson	straight (Fig. 1c)	medially concave (Fig. 2e)
Lateral external border of uropod endopod	prominent knob on proximal shoulder (Fig. 1e)	no knob on proximal shoulder (Fig. 2e)

Holotype: Male, from Ryuguno-Iso, Ohge-Jima, Naruto, Japan (BLT 1066).

Distribution: Japan, Taiwan.

Remarks: This is the only species of the genus in which the male pereiopod 1 propodus has no (presumably sound producing) crest on the mesial surface. Instead, the mesial proximal surface of the dactylus bears several round tubercles that may have the same function. Males have been reported as having genital openings on coxae of both pereiopods 3 and 5 (Sakai, 1984: 213; Ngoc-Ho, 1994 b: 201).

Austinogebia spinifrons (Haswell, 1881) n. comb.

Gebia spinifrons Haswell, 1881: 762; 1882: 165, pl.3, Figure 5.

Upogebia spinifrons - De Man, 1927: 53–56, pl. 6, Figure 20; 1928: 23, 46; Poore & Griffin, 1979: 305, Figures 53–54; Sakai, 1982: 58 (in part, not Figs 11c, 12 d–e, 13 e–f, pls F1, F3 = *Austinogebia takaoensis*); Sakai & Türkay, 1995: 202, Figure 4.

Syntype: One female (AMS P. 1544). Port Stephens, New South Wales.

Distribution: Queensland, New South Wales (Australia).

Austinogebia nobilii (Sakai & Türkay, 1995) n. comb. (Fig. 1)

Upogebia nobilii Sakai & Türkay, 1995: 198, Figures 1–3.

Holotype: Male, RV 'Akademik', St. PG-14, 54 m, 11.12.1991 (SMF 22169).

Distribution: Persian-Arabian Gulf.

Remarks: This species is very similar to *A. spinifrons*, but the two are probably distinct, given the distance between their distribution ranges. According to Sakai & Türkay (1995: Table 1), *A. nobilii* can be differentiated from *A. spinifrons* by a more slender pereiopod 1 and by having 6 oblique carinae on the mesial surface of the pereiopod 1 propodus in males (7 in *A. spinifrons*). These characters are however subject to variation. By contrast, the differences given for

the rostrum and telson of the two species seem more reliable: *A. nobilii* has a shorter rostrum compared to *A. spinifrons* (1.3 times as long as broad vs 1.7 times as long as broad in *A. spinifrons*) and an approximately quadrate telson (1.2 times as broad as long vs 1.3–1.4 times as broad as long in *A. spinifrons*).

Males and small females (Sakai & Türkay, 1995: 201) are provided with genital ducts on coxae of both pereiopods 3 and 5; larger females bear genital ducts on coxae of pereiopods 3.

Austinogebia wuhsienweni (Yu, 1931) n. comb. (Fig. 4)

Upogebia Wuhsienweni Yu, 1931: 89, Figure 2.

Upogebia wuhsienweni - Liu, 1955: 68, Figures 7–12.- Ngoc-Ho & Chan, 1992: 38, Figure 4. - Not Sakai, 1993: 92, Figures 1 and 2 [= *A. edulis* (Ngoc-Ho & Chan)].

Upogebia (*Upogebia*) *wuhsienweni* - Sakai, 1982: 59 (in part, not Figs. 11d, 12f–g, 13 g–h, pls. G1-2, and samples USNM 59070, 59071, 59072, 59073 [= *A. edulis* (Ngoc-Ho & Chan)].

Synypes: One male tl. 31 mm, 1 female, tl. 46 mm from Kiaochow bay, Northern China; no depository stated.

Distribution: China, Hong-Kong, Taiwan.

Remarks: *Austinogebia wuhsienweni* is similar to *A. edulis* (Ngoc-Ho & Chan, 1992), and some materials of the latter species were assigned to the former (Sakai, 1982; 1993).

Differentiating characters between the two are given by Ngoc-Ho & Chan (1992: 38), and the following can readily be used: (a) Distal half of lateral ridges of gastric region with 6–9 spiniform teeth dorsally in *A. wuhsienweni* (Fig. 4a) (unarmed or with 1–2 tubercles in *A. edulis*). (b) Basis of pereiopod 1 with a large and sharp spine in *A. wuhsienweni* (Fig. 4b, c) (with a blunt tooth in *A. edulis*).

The distal half of the lateral ridges of the gastric region bearing teeth and the presence of a spine on the pereiopod 1 basis are clearly figured in the original description (Yu, 1931: Fig. 2), as well as in Liu (1955: Fig. 9).

Austinogebia edulis (Ngoc-Ho & Chan, 1992) n. comb.

Upogebia edulis Ngoc-Ho & Chan, 1992: 33, Figures 1–4; Lin, 1995: 1, Figure 2, pls. 1–2.

Upogebia (*Upogebia*) *wuhsienweni* - Sakai, 1982: 59 (in part), Figures 11d, 12f–g, 13g–h, pls. G1–2, and lots USNM 59070, 59071, 59072, 59073).

Upogebia wuhsienweni - Sakai, 1993: 92, Figures 1 and 2.

Types: Holotype, male, from Luk-Kong, Chang-Hua County, Taiwan (MNHN-Th 1234). Paratypes: from Luk-Kong, Chang-hua County, Taiwan (MNHN-Th 1235–1239) and (NTOU 1900-1-13, NTOU 1900-10-9); from Mali County (NTOU 1901-5-11).

Distribution: Taiwan, Vietnam.

Remarks: This species is a traditional food in Taiwan and represents one of the very few upogebiid species of some economic importance. It is also unusual in having a polymorphism of the male pereiopod 1 in the late mature phase. This appendage, which is of similar morphology in small males, differs strongly in some larger specimens and can be of two types: a 'stout type' with the propodus about twice as long as broad, the fixed finger strong, erect and subdistal; and a 'slender type' resembling that of the female, with the propodus about three times as long as broad, the fixed finger short and distal. The meaning of this polymorphism is not known.

Males, in the stout as well as in the slender type, have been found bearing gonopores on coxae of both pereiopods 3 and 5 (Ngoc-Ho & Chan, 1992).

Austinogebia takaoensis (Sakai & Türkay, 1995) n. comb. (Fig. 5)

Upogebia(Upogebia) spinifrons - Sakai, 1982: 58 (in part), Figures 11c, 12 d–e, 13 e–f, pls F1, F3.
Upogebia takaoenis Sakai & Türkay, 1995: 203.

Holotype: Male, from Takao, Formosa (Taiwan) (MNB 12664).

Distribution: Taiwan.

Remarks: Examination of the holotype (male, cl. 15 mm, tl. 41 mm) confirms the view of Sakai & Türkay (1995) about distinguishing the species from *A. spinifrons* and gives support for it being included in the new genus.

A. takaoensis differs from the other *Austinogebia* species by its narrow, unarmed rostrum (Fig. 5a), the unarmed antennular and antennal peduncles (Fig. 5b) and the pereiopod 1 dactylus bearing a longitudinal upper row of quadrate plates (Fig. 5d). The holotype (the only known specimen) is at present soft, with a thin cuticule and the telson unusually flattened. Con-

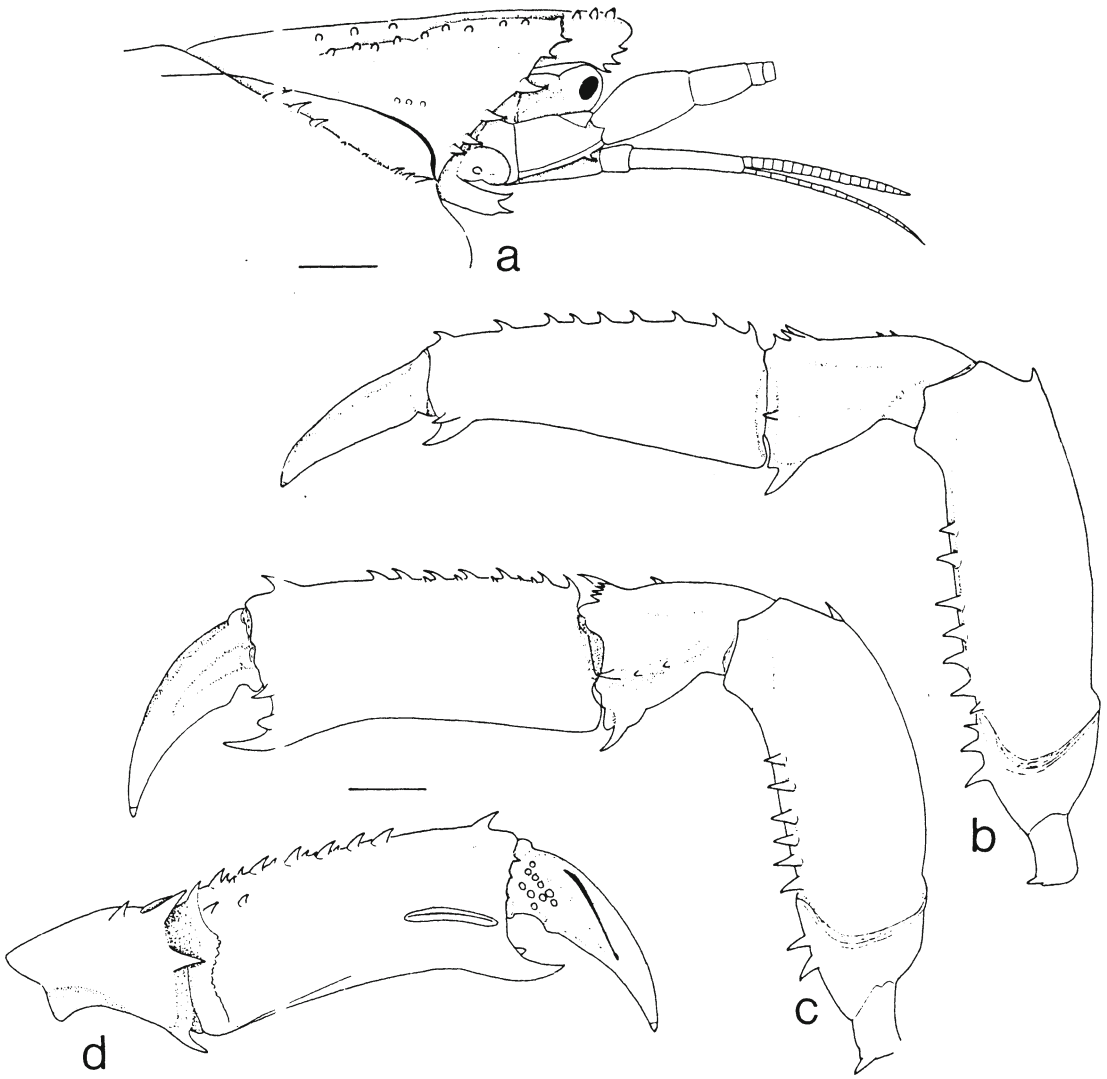

Figure 4. Austinogebia wuhsienweni (Yu, 1931): (a) anterior part of body, lateral view; (b) and (c) female and male pereiopod 1, respectively, lateral view; (d) distal part of male pereiopod 1, mesial view. Scale line: 1 mm (from Ngoc-Ho, 1994).

sequently, the shape of the telson shown in Figure 5e is probably not acurate.

Gebiacantha Ngoc-Ho, 1989

Gebiacantha Ngoc-Ho, 1989: 118.- Poore, 1994: 105 (key).

Upogebia - Sakai & Türkay, 1995: 198 (part).

Type-species: *Upogebia talismani* Bouvier, 1915 by original designation (Fig. 6).

Species included: *Gebiacantha talismani* (Bouvier, 1915), *G. ceratophora* (De Man, 1905), *G. monoceros* (de Man, 1905), *G. acanthochela* (Sakai, 1967), *G. acutispina* (de Saint Laurent & Ngoc-Ho, 1979), *G. plantae* (Sakai, 1982), *G. arabica* Ngoc-Ho, 1989, *G. laurentae* Ngoc-Ho, 1989, *G. lagonensis* Ngoc-Ho, 1989, *G. reunionensis* Ngoc-Ho, 1989, *G. richeri* Ngoc-Ho, 1989, *G. priochela* Sakai, 1993, *G. lifuensis* Ngoc-Ho, 1994, *G. multispinosa* Ngoc-Ho, 1994, *G. poorei* Ngoc-Ho, 1994, *G. bermudensis* (Williams, 1993).

Diagnosis (adapted from Ngoc-Ho, 1989, 1994 a): Rostrum approximately ovoid, bordered with teeth or

spines, one or many infrarostral spines; antero-lateral border of carapace with 2 or more spinules (Fig. 6a, b). Posterior border of telson medially concave (Fig. 6f).

Mandible without acute anterior tooth. Maxilliped 1 without epipod (Fig. 6e); maxilliped 3 with small epipod or (rarely) without. Gill filaments relatively narrow and undivided, making single row each side of rachis. Pereiopod 1 (Fig. 6c, d) subcheliform, carpus and propodus with numerous spines, lower border of propodus with 1–2 large spines posterior to fixed finger; fixed finger short, not exceeding half length of dactylus. Coxae of pereiopods 1–3 or 1–4 with spines or spinules. Uropod exopod equal or longer than telson (Fig. 6f).

Remarks: Examination of previously described *Gebiacantha* specimens and material more recently collected reveals two important characters that were missing in the diagnoses of the genus given in 1989 and 1994. These concern the shape and the spination of the rostrum as well as the median spine on the lower border of the pereiopod 1 propodus. The uropod exopod is not always longer than the telson as previously stated. These features are added or redefined in the new diagnosis.

Among those mentioned in the diagnosis, the most important characteristics of *Gebiacantha* are: 1. presence of infrarostral spines. 2. rostrum approximately ovoid, bordered with teeth or spines. 3. maxilliped 1 without epipod. 4. lower border of pereiopod 1 propodus with 1–2 large spines (often 1 large and 1 small) posterior to fixed finger. 5. posterior border of telson medially concave.

Characters 4. and 5. readily distinguish species of this genus from those of *Austinogebia.* Regarding the spinous structures on the lower border of the pereiopod 1 propodus in *Gebiacantha* species, it is questionable which one should be considered as the fixed finger. These structures are actually very similar or nearly identical in most species but in a few, such as *G. reunionensis* (Ngoc-Ho, 1989: Fig. 2f, g, l, m) and *G. lagonensis* (Ngoc-Ho, 1989: Fig. 3h, i), the fixed finger is well indicated: it is distal with the cutting edge bearing small teeth. Similarly, the fixed finger is considered as distal in the pereiopod 1 of other *Gebiacantha* species. It is longer in young male or female specimens, but very small in large adult males in numerous species (Figs 2c and 6c). *Gebiacantha* includes 16 species at present. Ngoc-Ho (1994a: 60) provided a key but an American species, *G. bermudensis* (Williams, 1993) was unfortunately omitted. The holotype, and only known specimen of this species, is a small male, of 5 mm in carapace length and the figure given by Williams (1993: Fig. 9) shows most characteristics of a *Gebiacantha.* Examination of the specimen (slightly stained with chlorazol black) also reveals that the first maxilliped is devoid of an epipod. Except for this species, all other American upogebiids with infrarostral spines bear a large epipod on the first maxilliped. This feature, considered as plesiomorphic, excludes them from *Gebiacantha.*

U. poensis and *U. snelliusi* are set apart from species of *Gebiacantha* by several important characters. Firstly, both species have a straight posterior border of the telson, and *U. poensis* can be distinguished also by having no spine on the lower border of the pereiopod 1 propodus, behind the fixed finger. *U. snelliusi* has a large epipod on the first maxilliped, and differs from *Gebiacantha* species, as well as all other upogebiids with infrarostral spines, by its rostrum bearing a large infrarostral and 4 large upper rostral spines.

Discussion

Males of several *Austinogebia* species have been reported to bear gonopores on the coxae of both the pereiopods 3 and 5, but whether they are functional has never been determined. Therefore, they must be referred to as 'intersex' rather than 'hermaphrodite', as hermaphroditism should be defined on a functional basis. In *Austinogebia edulis* from Taiwan, Lin (1995) found 4 males (out of 979 males collected between 1992 and 1994) with the pereiopod 1 enlarged but also with first pleopods. One of these (captured on 23 February 1994) had a very small ovary while there was no trace of it in the other three (collected on 26 January 1992, 25 June 1994 and 20 November 1994), which means at least at that time, they were not functional females.

Like *Gebiacantha, Austinogebia* appears to comprise a group of species belonging to the Indo-Pacific. Except for *G. talismani* from the European coasts, and *G. bermudensis* from Bermuda, both genera are also restricted to this area. Nevertheless, while *Gebiacantha* species are distributed all over the Indo-Pacific, those of *Austinogebia* seem to be confined to the eastern part, from the east coast of Australia in the south to Vietnam, Taiwan, Japan and China in the north. The exception is *A. nobilii* collected in the Persian-Arabian Gulf. Species of *Austinogebia* must be considered localised in their distribution as they are all limited to rather small areas.

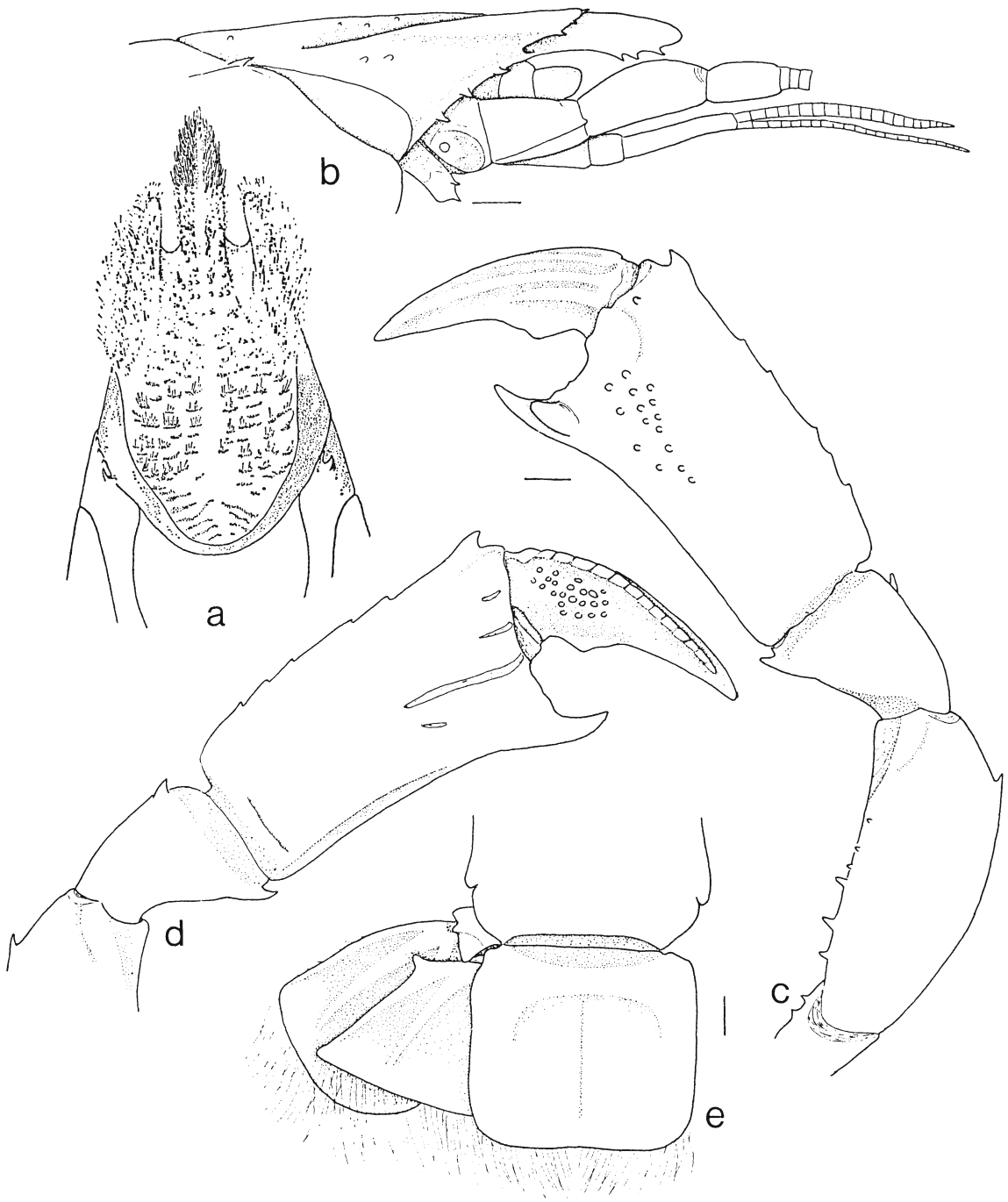

Figure 5. Austinogebia takaoensis (Sakai & Türkay, 1995), holotype, male: (a) carapace, dorsal view; (b) anterior part of body, lateral view; (c) pereiopod 1, lateral view; (d) distal part of pereiopod 1, mesial view; (e) telson and uropods. Scale line: 1 mm (a, from Sakai, 1982).

It is interesting to note that, with a few exceptions, species of *Austinogebia* occur in areas where those of *Gebiacantha* have not been found. As their first maxilliped bears a large epipod, which is regarded as a plesiomorphic feature and is absent in *Gebiacantha*, *Austinogebia* species possibly represent a more prim-

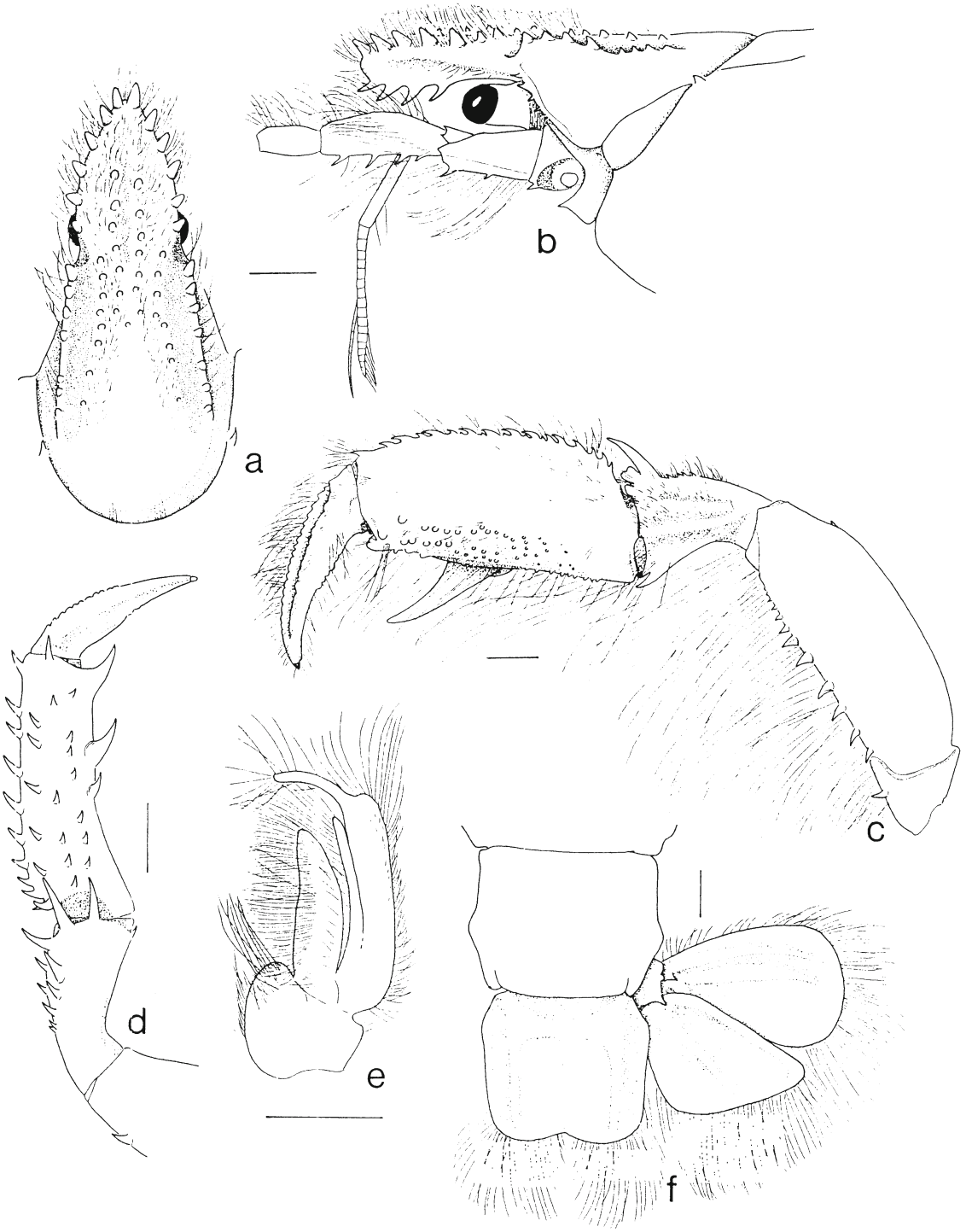

Figure 6. Gebiacantha talismani (Bouvier, 1915) (MNHN-Th 1352): (a) and (b) anterior part of body, dorsal and lateral view, respectively; (c) male pereiopod 1, lateral view; (d) distal part of female pereiopod 1, mesial view; (e) first maxilliped; (f) telson and uropods. Scale line: 1 mm.

itive group. They are of larger size and inhabit colder waters than do most species of *Gebiacantha*. Intersex males have been reported in many of them, while there has been no account of these for *Gebiacantha*.

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Addendum: *Upogebia imperfecta* Sakai, 1982 (Sakai, 1982: 63) though provided with infrarostral spines, is excluded from both *Austinogebia* and *Gebiacantha*.

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J.P.M. Paula, A.A.V. Flores & C.H.J.M. Fransen (eds), Advances in Decapod Crustacean Research.

Recent samples of mainly rare decapod Crustacea taken from the deep-sea floor of the southern West Europe Basin

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Key words: NE-Atlantic, deep-sea floor, Crustacea, Decapoda, Reptantia, Natantia

Abstract

During cruise 198 of F.R.V. 'Walther Herwig III' in August/September 1998, eight successful hauls were made in the southern part of the West Europe Basin from the bottom in a depth of about 4700 m. Seven samples were taken around 46° N, 17° W and one at 46° N, 13° W. In the same region, two decapod crustaceans were dredged during cruise 175 in 1996 and one in 1993. The following species of typical deep-sea decapods, most of them rarely recorded, were collected: *Willemoesia leptodactyla* (Willemoes-Suhm, 1873); *Parapagurus abyssorum* (Filhol, 1885); *Munidopsis crassa* Smith,1885; *Munidopsis parfaiti* (A. Milne-Edwards & Bouvier, 1894); *Heterogenys microphthalma* (Smith, 1885); *Glyphocrangon atlantica* Chace, 1939; *Benthesicymus brasiliensis* Bate, 1881; *Benthesicymus iridescens* Bate, 1881; *Plesiopenaeus armatus* (Bate, 1881). For most of these species of Reptantia and Natantia, our knowledge on vertical and/or geographic range is extended considerably by the new records and some new records enable us to enlarge our knowledge on morphological variations within these species.

Abbreviations: Cpb – breadth of carapace; Cpl – length of carapace; Sl – shield length; Tl – total length

Introduction

The present investigation deals with deep-sea decapods of the southern West Europe Basin collected during a cruise in 1993, cruise 175 in 1996, and cruise 198 in 1998 by F.R.V. 'Walther Herwig III' (Table 1). In August/September 1998, eight successful hauls with an opening/closing Agassiz-trawl near the position 46° N/17° W and 46° N/13° W were taken from a depth around 4700 m (Fig. 1). Deep-sea decapods have not been recorded for this region before. As few species and individuals seem to occur in the West Europe Basin as in other deep-sea regions. Türkay (1975) mentioned this phenomenon for the northern Iberian Basin too.

All specimens are deposited in the collection of the Zoologische Staatssammlung, Munich, Germany.

The following species were collected:

Reptantia:	Polychelidae:	*Willemoesia leptodactyla* (Willemoes-Suhm, 1873)
	Parapaguridae:	*Parapagurus abyssorum* (Filhol, 1885)
	Galatheidae:	*Munidopsis crassa* Smith, 1885
		Munidopsis parfaiti (A. Milne-Edwards & Bouvier, 1894)
Natantia:	Oplophoridae:	*Heterogenys microphthalma* (Smith, 1885)
	Glyphocrangonidae:	*Glyphocrangon atlantica* Chace, 1939
	Aristeidae:	*Benthesicymus brasiliensis* Bate, 1881
		Benthesicymus iridescens Bate, 1881
		Plesiopenaeus armatus (Bate, 1881)

Table 1. Station-list of successful samples in the southern part of the West Europe Basin. UTC=time when samples were taken; coordinates when gear was on the bottom

Station	Haul	Data	UTC	Coordinates	Depth (m)
198/07	1	30/08/1998	00:45:27	46° 03.34′ N/16° 43.15′ W	4719.50
			04:42:56	45° 57.43′ N/16° 43.45′ W	4684.50
198/13	2	31/08/1998	00:01:27	46° 03.23′ N/16° 42.65′ W	4723.50
			02:48:09	45° 58.04′ N/16° 41.24′ W	4701.25
198/18	3	01/09/1998	00:02:16	46° 02.60′ N/17° 06.63′ W	4714.25
			03:50:37	46° 05.71′ N/17° 14.67′ W	4702.75
198/24	4	02/09/1998	00:18:47	46° 03.93′ N/17° 10.12′ W	4725.75
			04:00:28	45° 57.26′ N/17° 09.95′ W	4699.00
198/29	5	03/09/1998	00:06:11	46° 03.18′ N/16° 43.40′ W	4718.50
			03:45:02	45° 57.02′ N/16° 43.81′ W	4684.75
198/40	7	04/09/1998	23:50:38	46° 00.63′ N/17° 05.53′ W	4707.25
		05/09/1998	03:45:08	45° 58.50′ N/17° 14.85′ W	4723.00
198/50	8	08/09/1998	00:10:34	46° 02.69′ N/16° 44.95′ W	4635.75
			04:00:02	45° 55.75′ N/16° 41.40′ W	4704.25
198/59	9	09/09/1998	23:56:31	46° 01.24′ N/13° 06.93′W	4785.50
		10/09/1998	03:45:06	46° 02.61′ N/13° 15.75′ W	4785.75
175/77		04/09/1996	–	46° 02.81′ N/16° 42.98′ W	4650.00
				45° 55.05′ N/16° 41.66′ W	
–		06/04/1993	–	46° 12.0′ N/17° 08.0′ W	4900.00

RESULTS

Reptantia

Polychelidae

Willemoesia leptodactyla (Willemoes-Suhm, 1873)

(Figs 1 and 2)

Material examined: station 198/13, haul 2: 1 male (Cpl 33.7 mm, Cpb 23.1 mm, Tl 70.0 mm); 1 female (Cpl 40.8 mm, Cpb 30.0 mm, Tl not measurable); station 198/18, haul 3: 1 female (Cpl 43.1 mm, Cpb 31.0 mm, Tl 94.8 mm); station 198/24, haul 4: 2 females (Cpl. 47.2 mm, Cpb 24.9 mm, Tl 104.8 mm; Cpl 33.8 mm, Cpb 23.7 mm, Tl 73.0 mm); station 198/50, haul 8: 1 carapace (sex cannot be identified) (Cpl 41.0 mm); station 198/59, haul 9: 1 female (seriously damaged) (Cpl 46.0 mm, Cpb 31.0 mm); station 175/77: 1 female (telson broken off) (Cpl 50.4 mm, Cpb 35.2 mm).

Remarks: *Willemoesia leptodactyla* was described by Willemoes-Suhm (1873) as *Deidamia leptodactyla*. Grote, G.-R., 1873 set up the genus *Willemoesia* (according to Bouvier, 1917) and attributed to it the type-specimen (according to Sund (1920a), the female of the 'Challenger'-station 13). Eight species of this genus are known "occurring in widely separated geographical areas" (Gore, 1984). Gore (1984) gives in his Table 2a "Comparison of morphological characters in the deep-sea polychelid lobsters, genus *Willemoesia*" for *Willemoesia leptodactyla* the following characters based on former authors: "Carapace Ornamentation: minute spinules, stiff hair; lateral spines

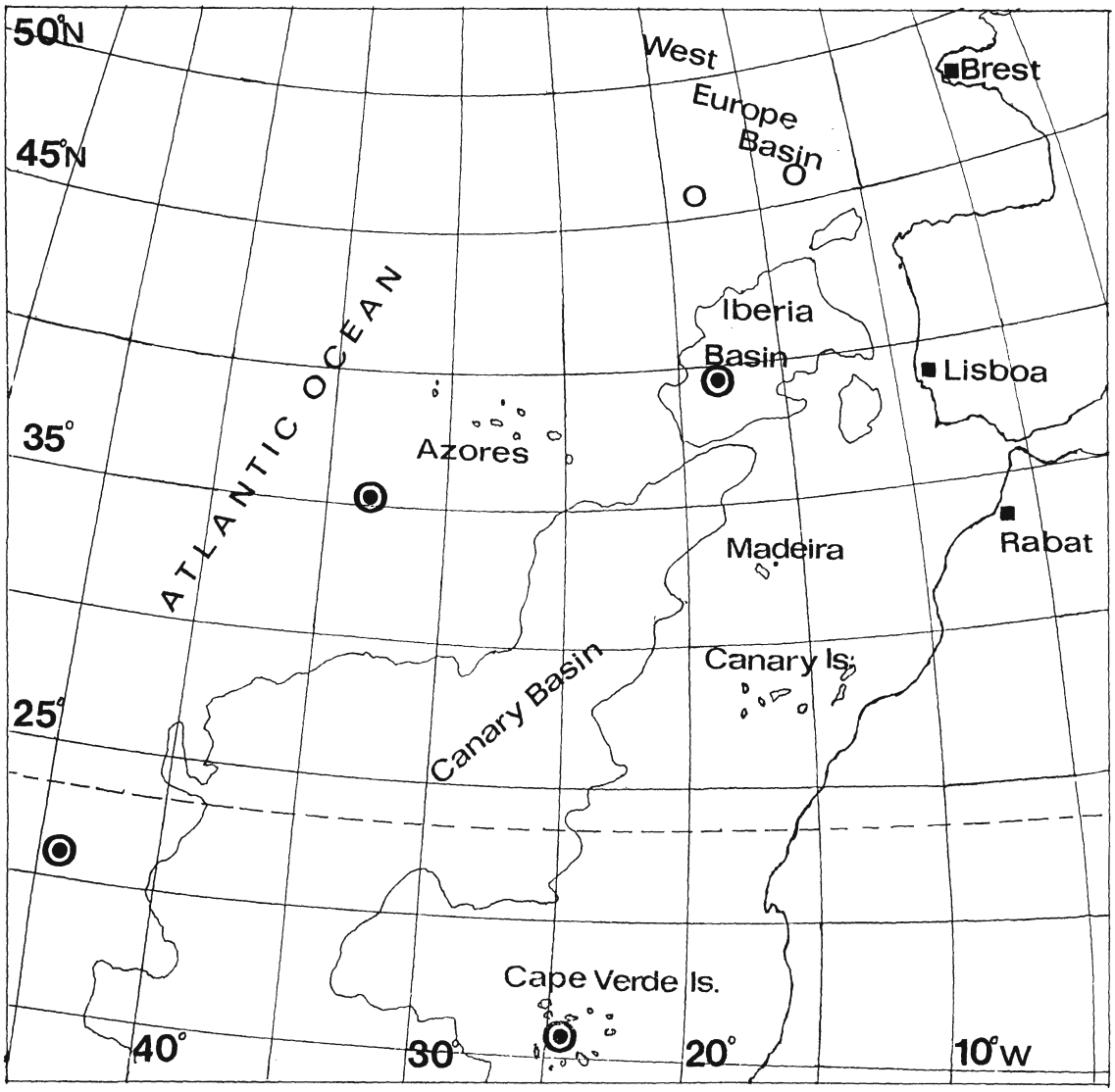

Figure 1. Map of the NE-Atlantic showing the known distribution of *Willemoesia leptodactyla* (Willemoes-Suhm, 1873) (circles with middle point) and the areas of the present records (open circles).

6–9/3–5/15–20; frontal margin nearly transverse; orbital sinus present: postero-medial carinae spined; abdomen somite 6 smooth, or slight keel". Firth & Pequegnat (1971) described the lateral margins of the carapace as subparallel, but this does not correspond with the present specimens in which the margins of the carapace are anteriorly moderately rounded.

The spine-formula of the lateral margin was adopted by Firth & Pequegnat (1971) from the former authors as 6–9/ 4–6/ 15–22, probably not taking into account the spine-formula found by Sivertsen & Holthuis (1956): 6–9/4–7/15–22. The present material shows a spine-formula of 6–10/3–8/14–23. The spine-formula of the median dorsal carina of the carapace varies in the present material 1.(5–8).2.1./2.2.(3–5). Bate's (1888) specimen from 'Challenger', station 13, shows the median carina spine-formula 1.(4).2.1.1./2.1.(3), Bouvier (1917) specified 1.(5).2.1./2.(3). and Sivertsen & Holthuis (1956) report 1.(6).2.1./2.2.(4). The specimens recorded here closely agree with the spine-formula of the specimens recorded by Sivertsen & Holthuis (1956). I believe that the differences with the specimens referred to by the other authors are caused by the two often indistinct spines situated on the ridge just behind the cervical groove. The spines of the gastro-orbital carina

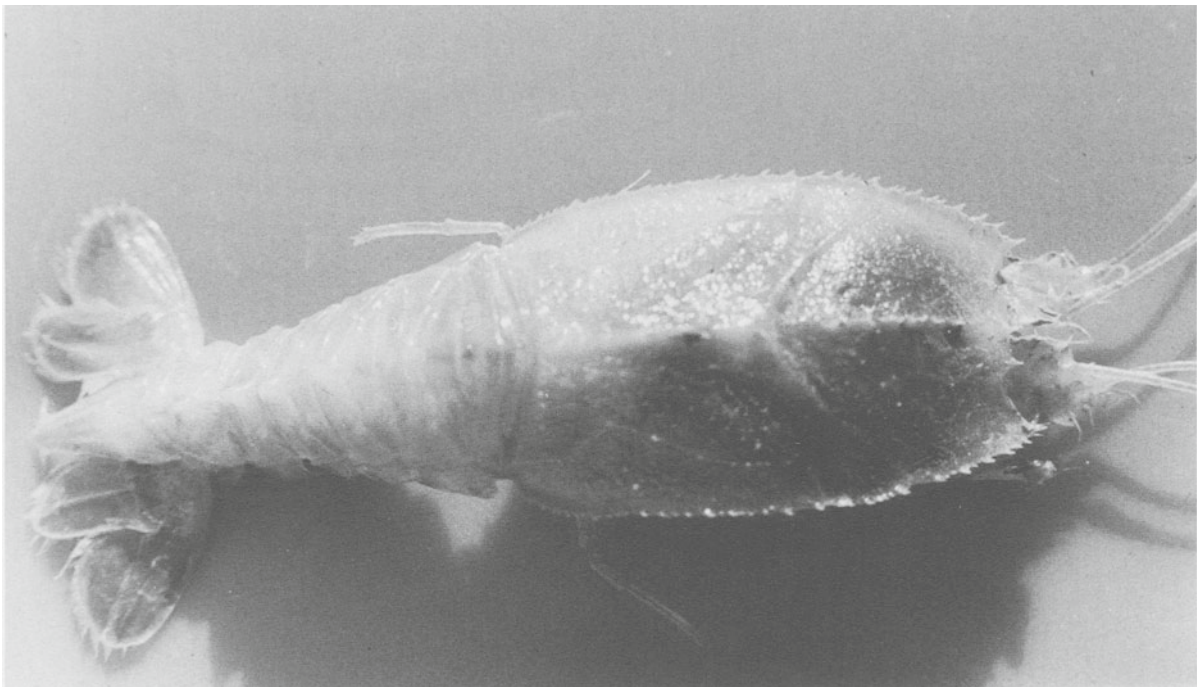

Figure 2. Willemoesia leptodactyla (Willemoes-Suhm, 1873). Male of station 198/13, haul 2, photographed on board of F.R.V. 'Walther Herwig' immediately after the haul.

and superior branchial carina in the present specimens are often inconspicuous. I am not sure if they are important diagnostic characteristics of the species.

Only the female of haul 2 possesses a cheliped at the left side. The dactylus does not bear any denticles on its outside, the palm shows a double row of spinules on the upper and lower surface. The double row on the upper side is not as distinct as the lower one. Beyond the base of the fixed finger this row becomes single and ends at about 2/3 of the length of the finger. The fixed finger shows the strong subdistal spine which is characteristic for the genus *Willemoesia*. The two spines at the merus near the articulation to the carpus as figured by Bate (1888) are distinctly present here. Bouvier (1917) does not draw these spines. Measurements of the cheliped: dactylus 20.4 mm; propodus 35.0 mm; carpus 34.9 mm; merus 41.3 mm; ischium 17.6 mm.

Figure 2 shows the male of station 198/12, haul 2, immediately after the haul. The gastrical region of the carapace is red coloured below the surface. In the adjacent regions, the darker viscera can be seen through the integument. The antennae and antennulae, including their peduncles, the border of the carapace, the pereiopods and the complete dorsal surface of the abdominal summits including the uropodes are tinged pale rosy. The eyes are reduced. Only six specimens of this species are known till now. The type-specimen (according to Sund's (1920a) separation of Bate's (1888) '*Willemoesia leptodactyla*' (see above)) was collected in 1873 by H.M.S. Challenger in the Central-Atlantic from a depth of 3475 m (the depth was measured as 1900 fathoms. 1 fathom=1.829 m. Some authors multiplicated with only 1.8 and therefore calculated 3420 m). Bouvier (1917) described two males, one of which was collected by the yachts 'Hirondelle' and 'Princesse Alice' in 1896 (station 753; depth 4360 m) in the central part of the Iberian Deep-Sea between Portugal and the Azores, the other specimen was collected in 1901 (station 1150, depth 3890 m) near the isle Sta. Luzia of the Cape Verde Islands. Unfortunately Bouvier notes down in the text for both localities 'Açores', which is cited by later authors without considering the list of stations with their coordinates (see Bouvier, 1917: 124–127). In 1910, during the "Michael Sars North Atlantic Deep-Sea Expedition" three further females of *Willemoesia leptodactyla* were captured from a depth of 2615 m, SW of the Azores (Sivertsen & Holthuis, 1956). The new records from the West Europe Basin are therefore the most northern and eastern localities for this species

(Fig. 1), and the maximal depth is extended to 4785 m, illustrating that the adult specimens of *Willemoesia leptodactyla* are typical abyssobenthic animals.

Parapaguridae

Parapagurus abyssorum (Filhol, 1885)

Material examined: station 198/24, haul 4: 2 males (Sl 7.3 mm, 12.9 mm), 1 female (Sl 10.2 mm); station 198/40, haul 7: 1 male (Sl 11.9 mm), 2 ovigerous females (Sl 9.8 mm, 11.0 mm); station 198/50, haul 8: 1 male (Sl 11.9 mm).

Remarks: The identification of these specimens, belonging to the most successful genus of deep-sea hermit crabs, is very easy, using the key "for the western Atlantic species of *Parapagurus* Smith" by Lemaitre (1989). The meri, carpi and propodi of the pereiopods are distinctly armed with small spines, which is characteristic for this species. Their dactyli are nearly twice as long as the propodi. The left legs are slimmer than the right ones. In all other respects, the specimens are in agreement with the detailed description of Lemaitre.

The small male from station 198/24, haul 4, inhabited a snail-shell of *Gymnobela frielei* (Verrill, 1885), a typical lower bathyal and abyssal gastropod of the Northern Atlantic. [The new record for this snail is one of the deepest known till now (comp. Bouchet, Ph. & A. Waren, 1980)]. The female caught in the same haul was associated with a colony of six individuals of *Epizoanthus* spec. (order Zoantharia). The other specimens mentioned above were living in a shelter formed by actinians producing a soft chitinous pseudo-shell covering the inner wall of the home of the hermit crab. In the larger males and females the carpus and chela of the chelipeds are covered with a fur of simple and plumose setae. In younger specimens, the fur is more dense than mentioned by Lemaitre (1989).

With these new records, the known distribution of the species is extended towards the European continent. Lemaitre (1989) provides a distributional map and reports the locality nearest to the present record to be about 200 sm to the SW, about halfway between the Azores ("Talisman; 24.8.1883; 42° 19′ N/23° 36′ W; depth 4100 m") and the locality of the present record. The vertical range as given by Lemaitre (1989) from 2500 to 4360 m (see also A. Milne-Edwards & E.-L. Bouvier, 1899, as *P. pilosimanus* Smith; var. *abyssorum* A. Milne-Edwards) is extended by more than 350 to 4725.25 m. *Parapagurus abyssorum* appears to be a very rare deep-sea species. However, this can be due to the fact that we possess comparatively few deep-sea hauls.

Galatheidae

Munidopsis crassa Smith,1885

Material examined: station 198/13, haul 2: 1 ovigerous female (Cpl 65.9 mm, Cpb 41.8 mm, Tl 125.8 mm, egg 2.8×3.0 mm (more than 100)); station 198/18, haul 3: 1 male (Cpl 30.2 mm, Cpb 19.1 mm, Tl 54.1 mm); 1 female (Cpl 37.0 mm, Cpb 23.0 mm, Tl 67.0 mm); station 198/29, haul 5: 1 male (with parasite) (Cpl 47.0 mm, Cpb 29.8 mm, Tl 84.8 mm); 4 females (Cpl 51.0 mm, Cpb 30.0 mm, Tl 92.1 mm; Cpl 34.0 mm, Cpb 20.2 mm, Tl 61.9 mm; Cpl 22.0 mm, Cpb 13.1 mm, Tl 41.0 mm; small specimen, carapace missing); station 198/40, haul 7: 2 males (Cpl 50.9 mm, Cpb 30.5 mm, Tl 93.0 mm; Cpl 29.4 mm, Cpb 19.1 mm,Tl 53.0 mm); station 198/50, haul 8: 1 male (Cpl 23.9 mm, Cpb 14.8 mm, Tl 44.2 mm); 2 females (Cpl 40.0 mm, Cpb 26.0 mm, Tl 74.0 mm; Cpl 16.0 mm, Cpb 9.8 mm, Tl 28.5 mm).

Remarks: This species of deep-sea squat lobsters was identified with the use of the key provided by Zariquiey Alvarez (1968). The present specimens of *Munidopsis crassa* are 'chalk-coloured' as described by Murray & Hjort (1912, cited in Sivertsen & Holthuis, 1956). The fingers of the chelae are characteristically spoonlike shaped. The tips of the dactyli of the pereiopods are really rusty to black coloured as mentioned by Bouvier (1922) which, however, is not visible in his coloured figure. The short blond hairs on the distal third of the propodus of the pereiopods are distinct in the present specimens. The lateral outer spines at the eyes vary from conspicuous to extremely reduced. The rostrum is slightly curved up. All other characteristics agree very well with the drawing in Bouvier (1922).

The ovigerous female of station 198/13, haul 2, measures 125.8 mm from the tip of the rostrum to the tip of the telson. It seems to be the largest known specimen of *Munidopsis crassa*. There are more than 100 eggs measuring 2.8×3.0 mm. Their colour of the living animal was deep red.

The male (Tl 84.8 mm) of station 198/29, haul 5, is parasitized by a bopyrid (Isopoda, Bopyridae). The left branchial region of the carapace is voluminously swollen. Gore (1983) mentioned 7 specimens of *Munidopsis crassa* which had either rhizocephalan or bopyrid parasites.

Figure 3. Munidopsis parfaiti (A. Milne Edwards & Bouvier, 1894). Ovigerous female of station 198/50, haul 8.

Munidopsis crassa Smith, 1885 ranges from the western North Atlantic from SE Georges Bank (SE off Boston) down to the Venezuela Basin in depths of 3000–5012 m (Williams & Turner, 1986). Gore (1983) mentioned a depth-record of 2514 m in the western Atlantic by Mayo (Mayo, B.S., 1974 unpublished, fide Gore, 1983). From the eastern North Atlantic, *Munidopsis crassa* is known from the Bay of Biscaya, the Iberian Basin between Portugal and the Azores, and from the Canary Islands (A. Milne-Edwards & E.-L. Bouvier, 1899; Sivertsen & Holthuis, 1956; Türkay, 1975; Gore, 1983; Williams & Turner, 1986). The record by Bouvier (1922) of the Bay of Biscaya ("station 2964: 46° 17′ 30″ N/5° 42′ W, depth 4380 m") is situated in the eastern part of the West Europe Basin and is together with Türkay's record from the Iberian Basin the closest to the present one. The male mentioned by Türkay (preserved at the Zoologische Staatssammlung, Munich) was captured in a depth of 5315 m (position 42° 44.5′ N/13° 34.3′ W) and is the deepest record for the species. The depth in which the present specimens were found ranges from 4635.75 to 4723.50 m.

***Munidopsis parfaiti* (A. Milne-Edwards & Bouvier, 1894)**

(Figs 3 and 4)

Material examined: station 198/7, haul 1: 1 male (Cpl 41.0 mm, Cpb 30.1 mm, Tl 77.0 mm); station 198/13, haul 2: 1 male (Cpl 35.0 mm, Cpb 24.9 mm, Tl 64.0 mm), 1 female (Cpl 36.1 mm, Cpb 25.9 mm, Tl 68.0 mm), one carapace (not measurable); station 198/18, haul 3: 1 male (Cpl 40.4 mm, Cpb 28.1 mm, Tl 66.5 mm); station 198/24, haul 4: 8 males (Cpl 43.0 mm, Cpb 30.0 mm, Tl 78.1 mm; Cpl 41.0 mm, Cpb 28.0 mm, Tl 76.0 mm; Cpl 41.9 mm, Cpb 27.8 mm, Tl 75.9 mm; Cpl 41.0 mm, Cpb 28.0 mm, Tl 74.0 mm; Cpl 40.0 mm, Cpb 27.5 mm, Tl 73.8 mm; Cpl 39.8 mm, Cpb 27.1 mm, Tl 72.2 mm; Cpl 38.1 mm, Cpb 26.5 mm, Tl 71.0 mm; Cpl 37.5 mm, Cpb 25.1 mm, Tl 69.1 mm); station 198/29, haul 5; 1 male (Cpl 36.9 mm, Cpb 26.2 mm, Tl 69.0 mm); station 198/40, haul 7: 6 males (Cpl 43.5 mm, Cpb 30.0 mm, Tl 82.5 mm (with parasite); Cpl 42.3 mm, Cpb 27.0 mm, Tl 77.1 mm (with parasite); Cpl 42.1 mm, Cpb 28.4 mm, Tl 76.9 mm; Cpl

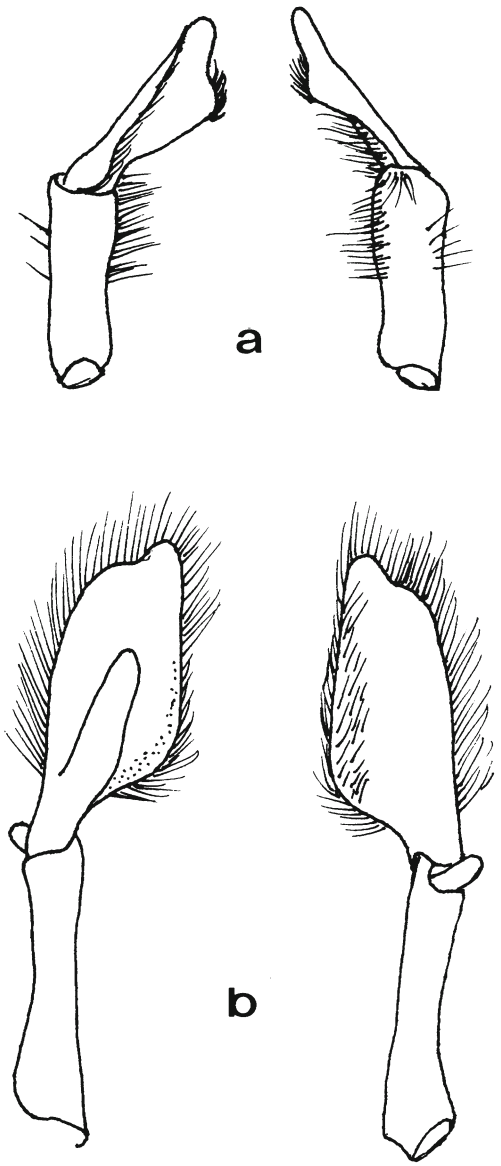

Figure 4. Munidopsis parfaiti (A. Milne Edwards & Bouvier, 1894) male of station 198/59, haul 9. Terminal joints of (a) gonopod 1; (b) gonopod 2.

40.8 mm, Cpb 28.0 mm, Tl 76.0 mm; Cpl 41.5 mm, Cpb 26.1 mm, Tl 75.5 mm; Cpl 38.0 mm, Cpb 26.7 mm, Tl 70.0 mm), 1 female (Cpl 40.0 mm, Cpb 28.2 mm, Tl 75.8 mm); station 198/50, haul 8: 1 ovigerous female (Cpl 40.0 mm, Cpb 28.8 mm, Tl 74.9 mm, egg-size 3.2×3.8 mm (about 50)); station 198/59, haul 9: 1 male (Cpl 50.0 mm, Cpb 35.0 mm, Tl 92.9 mm), 1 female (Cpl 40.5 mm, Cpb 27.0 mm, Tl 73.0 mm).

Remarks: A. Milne-Edwards & E.L. Bouvier (1894) described this species as *Orophorhynchus Parfaiti* on the basis of a single specimen captured by the 'Talisman' from a depth of 4255 m at 44° 20′ N/19° 31′ W (according to Nobre, 1936), which is close to the position on which the present specimen was collected. In 1899, the same authors described one male and one female from a depth of 4360 m at 39° 50′ N/20° 18′ W. Faxon (1895) noted: "*Orophorhynchus* has already been united with *Elasmonotus* by Henderson" and he "united *Elasmonotus* and *Munidopsis* as one genus". Both Doflein & Balss (1913) as well as Isabella Gordon (1955) cited the above-mentioned three specimens. Gordon (1955) stated for the genus *Munidopsis* "only four of this species occur below 4000 m, namely *Munidopsis abyssorum* A. Milne-Edwards & Bouvier, *M. antonii* A. Milne-Edwards, *M. crassa* Smith and *M. parfaiti* (A. Milne-Edwards)". Zariquiey Alvarez (1968) mentioned the same authorities. Here can be added Türkay's *Munidopsis thieli* Türkay, 1975 from the Iberian Deep-Sea Basin. In 1975, Türkay described two male specimens of *Munidopsis parfaiti* collected by an Agassiz-trawl during cruise 3 of the German R.V. 'Meteor' in 1966 from the Iberian Deep-Sea Basin (positions and depths: 42° 4.1′ N/14° 55.6′ W, depth 5275 m; 42° 55.4′ N/14° 7.9′ W, depth 5260 m). These specimens apparently represent the most recent and deepest records.

The specimens could be easily identified using the key of Zariquiey Alvarez (1968) for the identification of eastern Atlantic *Munidopsis* species. The median dorsal anterior directed spines on the second, third and fourth abdominal tergites are characteristic for this eastern Atlantic species (Fig. 3).

The new records (compare list of stations and Fig. 1) of 19 males (Tl 64.0–92.9 mm) and 4 females (Tl 68.0–75.8 mm) give us the largest number of specimens of this species till now. The depth on which they were found ranges between 4635.75 and 4785.75 m. The male (Tl 92.9 mm) of station 198/59, haul 9, and the female (Tl 75.8 mm) of station 198/40, haul 7, are the largest specimens of the species known till now. The female (Tl 74.9 mm) of station 198/50, haul 8, is the first known to be ovigerous. Its about 50 dark orange coloured eggs show a diameter of 3.2–3.8 mm. The largest male (Tl 82.5 mm) of station 198/40, haul 7, is parasitized by *Peltogaster* sp. (Rhizocephala, Peltogasteridae).

In consideration of this new material, a more extended description of *Munidopsis parfaiti* is given. The specimens appear rather robust. The carapace, in-

cluding the rostrum, measures 35.5–50.0 mm×24.4–35.0 mm corresponding to a ratio of about 3:2. The dorsal surface of the carapace is densely granulated. The regions can be well distinguished from each other. The rostrum is triangular, one-third of the width of the carapace at its base, with angles at two thirds, and its tip curved up. Dorsally it shows a distinct carina which is beset with one row of granules. This carina continues in the frontal region and in two thirds of the mesogastric region with a double row of granules. In the branchial, cardiac and intestinal regions as well as on the pleura of the abdominal segments 2–4, the granules are replaced by short, more closely set ridges. Spines are not present on the surface of the carapace. A supra-antennal spine, as a single strong spine, is not present. It is replaced by a small patch of granules. Gastric spines and hairs are not present on the carapace nor on the margins, but granules are. The linea anomurica is distinct.

The eye-stalks are strongly broadened, placed against the lower surface of the rostrum, without a spine at the basis, connected by a bridge as described for *Munidopsis thieli* by Türkay (1975), and apparently not movable. The corneae are small, lacking pigment and apical spines. The antennular peduncles are armed with two distinct spines. The basal segments of the antennae are provided with one spine on the outer side.

Basis and ischium of the third maxillipeds are provided with a row of denticles. Only the chelipeds possess epipods. Epipods are missing from the second to fifth pereiopods. The chelipeds possess the characteristic spoonlike fingers. In pereiopods 2–5, we find a remarkable, narrow, brushlike row of plumose hairs on the proximal side of the propodus. The dactyli bear a row of small spines on the ventral margin. The fifth pereiopods are reduced in the characteristic manner for Galatheidae and show 2/3–3/4 of the distal part of the merus granulated. The terminal joints of the first gonopods of the males are folded. On the distal double-lobed margin, we find only a short row of short hairs (Fig. 4a). This is quite different from *Munidopsis crassa* and *M. bermudezi* as drawn by Türkay (1975). The terminal joints of the second gonopods are wide with a double-lobed distal border (Fig. 4b).

The whole animal is chalk coloured except for the blond to dark brown coloured tips of the dactyli of pereiopods 2–5.

Natantia

Oplophoridae

Heterogenys microphthalma (Smith, 1885)

(=*Acanthephyra microphthalma* Smith, 1885)

Material examined: station 198/13, haul 2: 1 female (Cpl 25.4 mm); station 198/24, haul 4: 1 female (Cpl. 22.8 mm).

Remarks: Chace, Jr. (1986) considered the differences of this species in comparison with the other members of the genus *Acanthephyra* as so important that he set up the new genus *Heterogenys* with the only species *Heterogenys microphthalma*. The identification of the species is easy because of several characteristic features. The rostrum bears fewer teeth dorsally than ventrally; the eyes have the corneae smaller and narrower than the eyestalks; the abdominal segments 3–6 are dorsally carinate, the third segment bears a slender, very long median dorsal spine which reaches beyond the fourth abdominal segment. Unfortunately, this spine is broken off, just as the rostrum is, but the other features still allow a positive identification.

Chace (1986) noted: " ... the number of published records of *H. microphthalma* is relatively low". Without regard to the few specimens reported "from Bay of Bengal, the Celebes Sea and from the southern Pacific Ocean" (Sivertsen & Holthuis, 1956), added by the records from the western North Pacific (Aizawa, 1974; fide Chace, 1986), I found in literature only 38 specimens reported for the Atlantic (from off the east coast of the U.S.A. (Smith, 1886), W of the Cape Verde Islands (Crosnier & Forest, 1973), SW of the Azores (Sivertsen & Holthuis, 1956), off Madeira (Fransen, 1991), from off Portugal (Coutière (1911a) fide Crosnier & Forest, 1973) and from two localities mentioned by Domanski (1986), which are situated around 31° 17′ N/25° 24′ W (Madeira Abyssal Plain) and 41° 30′ N/20° 30′ W (Kings Trough) (I believe that the position indicated by Domanski "30° W" is a mistake. The map "Fig. 1" in his publication justifies this correction). For a long time, the deepest record of the species was reported by Smith (1886) with '2.620 fathoms' (=4792 m). Domanski (1986) reports a new depth-record from the Madeira Abyssal Plain of 5440 m.

Both present records of this species extend the known distribution in the Atlantic considerably to the north and confirm with 4699.00 and 4725.75 the

known depth range. *Heterogenys microphthalma* apparently seems to be a bathypelagic to abyssopelagic species which often lives within the abyssobenthic zone. The female of station 198/13 (haul 2) apparently is the largest specimen of the species known till now. Unfortunately small pieces of the rostrum and the telson of both specimens are broken off, therefore it is not possible to report the exact total length.

Glyphocrangonidae

Glyphocrangon atlantica Chace, 1939

Material examined: station 198/29, haul 5: 1 male (Cpl 21.8 mm); station 198/40, haul 7: 1 male (Cpl 11.1 mm); 1 female (Cpl 11.6 mm); station 175/77: 1 male (Cpl 24.0 mm); 1993: 1 female (Cpl 21.8 mm).

Remarks: Of this typical heavily armoured deep-sea genus, only *Glyphocrangon atlantica* Chace, 1939, *G. longirostris* (Smith, 1882) and *G. sculpta* (S.I. Smith, 1882) are known from the eastern Atlantic. Holthuis (1971) gives very detailed descriptions and clear figures of these species. Using his key to the Atlantic species, the present specimens could easily be identified. However, the "two distinct teeth behind the branchiostegal spine" (Holthuis, 1971: key p. 277) of the anterior lateral carina of the carapace are not as "distinct" in the present species, but merely represented as two protuberances as illustrated by Holthuis (1971: Fig. 5). Holthuis (1971) describes this feature as follows: "The anterior lateral carina has two large, but not very high, teeth with blunt tops" and further on "In the French specimen, the sculpture of the carapace is far less distinct than in the PILLSBURY specimen, on which the above description is based ... ". The two distinct teeth at the end of the pleura of the fifth abdominal segment separate *G. atlantica* without any doubt from *G. sculpta*, showing three teeth there. Holthuis' description of *G. atlantica* is based on Chace's type-specimen (a female) "from south of Santa Clara Province, Cuba" (20° 47′ 30″ N/80° 24′ 30″ W; depth 3885 m) from the ATLANTIS-Expedition in 1938, sta. 2966, on an ovigerous female from the PILLSBURY-Expedition in 1967, sta. 575, NW of Swan Island off Honduras (17°43' N/84° 20′ W–17° 48′ N/84° 25′ W, depth 6373–6364 m) and a female from the Bay of Biscay, France, collected by the Centre Océanologique de Bretagne CHO4-sta. BO17 Put 115 in 1969 (45° 13′ N/05° 30′ W; depth 4665 m) (Holthuis, 1971). These three specimens were the only known of this species for a long time, till Gore (1985a) described 48 specimens (males and females) collected from USNS 'Bartlett' Cruise 1301-82, in October–December 1981, at 11 stations in the Venezuela Basin. This material allowed him to make many additions to the known variability, reproductive biology, alimentation, parasitism and ecology of this species. Gore confirmed that the above-mentioned two teeth of the anterior lateral carina "were usually sufficiently developed to distinguish the species. In 2 instances even these teeth were reduced to sinuounities", which agrees with the present specimens.

Apart from the record from the Bay of Biscay (Holthuis, 1971), *Glyphocrangon atlantica* was only known from the Caribbean Sea in the western Atlantic. Here it was found in a depth-range of 3885–6364 m. "The deepest 'Bartlett' specimens came from Stn. 97, 5055–5060 m" (Gore, 1985a). The one female from the Bay of Biscay from a depth of 4665 m was the only specimen known from the eastern Atlantic till now (Holthuis, 1971). The present specimens from 46° N, 17° W in the Western Europe Basin have a depth range of 4650–4900 m. *Glyphocrangon atlantica* therefore seems to be a typical abyssobenthic shrimp.

Aristeidae

Benthesicymus brasiliensis Bate, 1881

Material examined: station 198/7, haul 1: 1 male, 1 specimen (sex cannot be identified; both not measurable); station 198/18, haul 3: 1 male, 1 female (both not measurable).

Remarks: Although the specimens are partly damaged they could be positively identified as belonging to this rare species using the work of Crosnier & Forest (1973). The presence of the dorsomedian spines at the abdominal segments 3–6 is usually distinct.The new records are apparently the most northern ones. The closest records of the species found in literature are those of the Kings Trough and Madeira Abyssal Plain (Domanski, 1986), and off Morocco (Bouvier, 1908). Bouvier recorded the species as *Benthesicymus moratus* S.-I. Smith, 1886 from the cruise with the yacht 'Princesse Alice' in 1894, station 443, position 34° 04′ N/8° 58′ 45″ W, depth 3745 m. Sund (1920b) recorded the species from the Canary Islands at a depth of 2603 m caught with the 'Michael Sars' in 1910, stating that the species "has formerly been taken in the Southern Pacific and the South Atlantic, in depth between 600 and 4300 m". The present records

in a depth between 4684.50 and 4719.50 m confirm, that this dark red *Benthesicymus brasiliensis*, is an abyssopelagic animal living near the deep-sea bottom.

Benthesicymus iridescens Bate, 1881

Material examined: station 198/7, haul 1: 4 specimens (sex cannot be identified; size not measurable); station 198/13, haul 2: 4 females (size not measurable), 1 female (Cpl 45.2 mm); station 198/18, haul 3: 1 specimen (sex cannot be identified; size not measurable); station 198/24, haul 4: 1 female (size not measurable), 1 female (Cpl 41.2 mm); station 198/29, haul 5: 1 specimen (sex cannot be identified; size not measurable); station 198/40, haul 7: 1 specimen (sex cannot be identified; size not measurable); station 198/50, haul 8: 1 male, 2 specimens (all not measurable).

Remarks: All specimens listed are damaged and their identification was sometimes difficult. The absence of medio-dorsal spines in the abdominal segments 3–6 was not always clear at first sight. As in some of the specimens of *B. brasiliensis*, the characteristic spines are broken off, they could easily have been mistaken for the present species. However, with the key of Crosnier & Forest (1973) the separation of these two species was clear. *Benthesicymus iridescens* seems to be a rare, but typical species for the zone near the deep-sea floor. Under the name *Benthesicymus longipes*, Bouvier (1908) described one male and one female of this species from station 1150 of the cruises by the yachts 'Hirondelle' and 'Princesse Alice' in 1901, southwest of the Cape Verde Islands (16° 12′ N/24° 43′ 45″ W) from a depth of 3890 m. A large female (Bouvier, 1922) was recorded under the same name from station 2994 of the cruise in 1910 off Cape Finisterre (Spain) (44° 08′ N/10° 44′ W) from a depth of 5000 m. This last locality is very close to the present ones. Sund (1920b) reports two males from the 'Michael Sars'-Expedition in 1910 taken from a haul SW of the Azores and one damaged specimen from near the Canary Islands. Hanström (1933) noted the deepest record of the species till now with 6500 m from the Dana-Expedition in 1931 at station 4180 in the Canarian Basin west of Madeira (32° 56′ N/23° 47′ W). Domanski (1986) records 5 specimens of this species from Kings Trough and 233 specimens from the Madeira Abyssal Plain in a depth of 5440 m. This is the largest number of specimens known from these three localities.

Plesiopenaeus armatus (Bate, 1881)

Material examined: station 198/7, haul 1: 1 female (Cpl 58.3 mm); station 198/29, haul 5: 2 females (Cpl 62.1 mm; 81.3 mm); station 198/40, haul 7: 1 female (Cpl 68.8 mm); station 198/50, haul 8: 1 female (Cpl 65.0 mm); station 198/59, haul 9: 2 females (Cpl 41.2 mm; 54.8 mm).

Remarks: This large, completely deep scarlet coloured penaeid shrimp, could be easily identified with the figures by Crosnier & Forest (1973). Gore (1985b) reported about 50 specimens of this species from samples by USNS 'Bartlett' in the Venezuela Basin. His deepest record in this region was from 5060 m. Bouvier (1908) mentioned a young male from the cruise of the yacht 'Princesse Alice' in 1904 (station 1787: 31° 07′ N/24° 03′ W), which was captured NW of the Canaries at a depth of 5413 m. This record between the Azores and the Canaries is one of the deepest and one of the most northern in the eastern Atlantic till now. The present records from a depth between 4635.75 and 4785.75 m confirm that *Plesiopenaeus armatus* is a truly abyssopelagic shrimp living near the bottom of the deep-sea. Crosnier & Forest (1973) designated this species as "exclusivement benthic".

Discussion

The nine decapod species treated in this report were obtained by an opening/closing Agassiz-trawl together with holothurians, sea stars and mainly alepocephalid and macrourid fishes in the southern part of the West Europe Basin. All these species live truly abyssobenthic or abyssopelagic near the deep-sea floor. They seem to form an abyssobenthic association. Except for *Munidopsis crassa* and *Plesiopenaeus armatus*, all species mentioned here are considered quite rare till now. Both *Munidopsis crassa* and *Plesiopenaeus armatus* appear to be widely distributed in the Atlantic, seem to be comparatively common, and sometimes occur in larger populations (see Gore, 1983, 1985b).

The species of Reptantia,*Willemoesia leptodactyla*, *Parapagurus abyssorum* and *Munidopsis parfaiti* on the contrary, are considered extremely rare. However, if we look at the 8 specimens of *Willemoesia leptodactyla*, and especially at the 24 specimens of *Munidopsis parfaiti* which we found in the eight hauls in 1998 in this rather small area in the NE-Atlantic,

I believe that we are not allowed to speak of extremely rare species. The same holds for the Natantia recorded here. 'Rare' in respect to species from the abyssobenthal, respectively, abyssopelagial should be replaced by "seldom found till now". Before Gore (1985a), for instance, only three specimens of *Glyphocrangon atlantica* were known. 11 deep-sea trawls of USNS 'Bartlett' from the Caribbean Sea in October–December 1981 obtained 48 specimens of this species and gave Gore the possibility for "observations on variation in morphological features". The 5 specimens reported here extend our knowledge further. The same is expected for *Heterogenys microphthalma*, *Benthesicymus brasiliensis* and *Benthesicymus iridescens*, if new records become available. In conclusion, the species are not primarily rare, but the number of hauls are.

Nevertheless, this does not mean that the populations of these species are dense. Thiel (1972) points out that the "animal density does not decrease steadily from nearshore to offshore biocoenosis, i.e. generally with increasing depth" but ... "the decrease is more pronounced for the macrofauna ... ". And further on: "The structure of the biocoenosis of the deep-sea floor is characterized by the meiofauna living on and in the sediment and by the dominance of sediment feeders in the macrofauna".

Polychelidae are sediment feeders and occasionally necrophagous as Tiefenbacher (1995) described for *Polycheles typhlops typhlops* (Heller, 1862) and *Stereomastis sculpta* (Smith, 1880) and which suggests that this holds for *Willemoesia leptodactyla*, too. For both species of *Munidopsis*, with their spoonlike shaped chelae, we may suppose the same. Gore (1983) stated for *Munidopsis crassa*: "This type of chelipeds seems well adapted for both scraping away the relatively soft sponge tissue, or spooning up the detrital material on the sea floor". Thompson (unpublished; fide Gore, 1985a) stated for the Glyphocrangonids that they "are omnivores, subsisting on gastropod molluscs, fish scales, annelid setae, and other detrital-like particles" and Gore (1985a) added: ... "they may be rather agile epibenthic predators above the abyssobenthic substrata". According to Gore (1985b), we may state that the Aristeids *Benthesicymus brasiliensis*, *B. iridescens* and *Plesiopenaeus armatus* subsist in a similar way.

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Data on the family Pandalidae around the Canary Islands, with first record of *Plesionika antigai* (Caridea)

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Key words: Pandalidae, *Plesionika antigai*, biology, zoogeography, Canary Islands

Abstract

Of the 20 pandalid shrimps species and subspecies reported for the Eastern Central Atlantic (26–36° N), 16 were found in one or more Macaronesian archipelagos (Azores, Madeira, Canary Islands and Cape Verde Islands) (14–40° N), and 11 of them were recorded to date in the Canary Island waters (27° 30′–29° 30′ N): *Bitias stocki* Fransen, 1990; *Heterocarpus ensifer ensifer* A. Milne-Edwards, 1881; *Heterocarpus grimaldii* A. Milne-Edwards & Bouvier, 1900; *Heterocarpus laevigatus* Bate, 1888; *Plesionika edwardsii* (Brandt, 1851); *Plesionika ensis* (A. Milne-Edwards, 1881); *Plesionika holthuisi* Crosnier & Forest, 1968; *Plesionika martia martia* (A. Milne-Edwards, 1883); *Plesionika narval* (J.C. Fabricius, 1787); *Plesionika williamsi* Forest, 1964; and *Stylopandalus richardi* Coutière, 1905. In the present work, *Plesionika antigai* Zariquiey Álvarez, 1955 is recorded for the first time from the Canary Islands. As a result of many fishing surveys around the Canary Islands at 27–1550 m depth between 1985 and 1998, information on bathymetric distribution, habitat, size and biology of the 12 Canarian pandalid species is given. The geomorphologic, geographic and oceanographic characteristics of the Canary Islands marine ecosystems could explain the great diversity in the biogeographic patterns of the pandalid species inhabiting this area. The distribution patterns found were: Macaronesian (1 spec.), Atlanto-Mediterranean (1 spec.), Eastern Atlantic warm-temperate (1 spec.), amphi-Atlantic warm (2 spec.), amphi-Atlantic warm-temperate (1 spec.), pantropical (5 spec.), and cosmopolitan (1 spec.).

Introduction

Of the 20 pandalid shrimps species reported for the Eastern Central Atlantic Ocean (26–36° N), 16 were found in one or more Macaronesian archipelagos (Azores, Madeira, Canary and Cape Verde Islands) and 11 of them (*Plesionika antigai* Zariquiey Álvarez, 1955 not included) have been recorded to date in the Canary Island waters (Crosnier & Forest, 1973; Fransen, 1990, 1991; Martins & Hargreaves, 1991; Biscoito, 1993; González, 1995; González & Santana, 1996; d'Udekem d'Acoz, 1999). The genus *Physetocaris* Chace, 1940, often assigned to the family Physetocarididae Chace, 1940, has been recently transferred to the family Pandalidae Haworth, 1825 by d'Udekem d'Acoz (1999).

Information on bathymetric distribution, habitat, size and/or biology of these 20 Eastern Atlantic species were given by González (1995), González & Santana (1996) and d'Udekem d'Acoz (1999). For the Canarian species, data reported by González (1995) included several colour photographs, while information given by González & Santana (1996) compiled results from surveys until 1994. In the present work, all this ecological and biological information is updated to 1998, *Plesionika antigai* is recorded for the first time from the Canary Island waters, and a zoogeographical analysis for the Canarian pandalid species is provided.

Materials and methods

The surveys conducted around the Canary Islands in the Eastern Central Atlantic encompassed the area between 27° 30′–29° 30′ N and 13° 00′–18° 15′ W. Operations included crustacean bottom traps, multiple shrimp traps, bottom trawling and dredges on the insu-

Table 1. Origin of the pandalid samples studied

Cruise	Date month/year	Island	Collecting method	Depth interval (m)	Main reference
AGAMENÓN 7406	06/1974	P	CBT	10–1000	Santaella et al. (1975)
CANCAP	1977–80	L, F, C, T, G, H, P	CBT, D	160–1800	Fransen (1991)
CANARIAS 85	1985	L, F, C, T, G, H, P	CBT	27–1025	González et al. (1988)
MOGÁN 8701	01/1987	C	CBT, MST	1370–548	Santana et al. (1987)
MOGÁN 8710	10/1987	C	CBT	135–410	Lozano et al. (1990)
MOGÁN 8802	02/1988	C	CBT	118–313	Lozano et al. (1990)
MOGÁN 8804	04/1988	C	CBT	110–384	Lozano et al. (1990)
MOGÁN 8806	06/1988	C	CBT, MST	127–270	Lozano et al. (1990)
TFMC ZM-90	1990	T	CBT	84–262	Hernández et al. (1991)
GOMERA 9009	09/1990	G	CBT	50–1100	González & Santana (1996)
NASAS 9112	12/1991	C	CBT	846–1406	González & Santana (1996)
CANARIAS 9206	06/1992	T	CBT, BT	25–1500	López Abellán et al. (1994)
TFMC BM-92	1992	G	CBT	1100–1550	Hernández & Jiménez (1993)
TALIARTE 9301	01/1993	C, T	CBT	140–935	González & Santana (1996)
GRAN CANARIA 9307 (I)	07/1993	C	CBT	650–865	González & Santana (1996)
CANARIAS 9310	10/1993	T	CBT	420–1450	González & Santana (1996)
TALIARTE 9401	01/1994	C, F	CBT	266–842	González & Santana (1996)
TALIARTE 9402	02/1994	C	CBT	231–465	González & Santana (1996)
TALIARTE 9403	03/1994	C	CBT	250–627	González & Santana (1996)
TALIARTE 9406	06/1994	C	CBT	74–285	González & Santana (1996)
DBAULL9612	12/1996	T	CBT	500–600	This work
CAMARÓN 9701	01–02/1997	C	MST	111–417	This work
CAMARÓN 9704	04–05/1997	C	MST	190–386	This work
GIPECAN 9705	05/1997	T	CBT	713–1070	This work
TALIARTE 9709	09/1997	C	CBT	498–598	This work
CAMARÓN 9801	01–03/1998	T	MST	196–479	This work
CAMARÓN 9804	04/1998	C	MST	138–433	This work
TALIARTE 9812	12/1998	T	CBT	388–460	This work

L=Lanzarote, F=Fuerteventura, C=Gran Canaria, T=Tenerife, G=La Gomera, H=El Hierro, P=La Palma; CBT=crustacean bottom traps, MST=multiple shrimp traps, BT=bottom trawl, D=dredge

lar shelves and slope regions from 10 to 1800 m depth. Twenty eight cruises carried out on board of several research vessels and artisanal fishing boats from 1974 to 1998 form the basis of this work (see Table 1).

In most samples, carapace length (CL) was measured with calipers to the nearest 0.1 mm. Sex and ovigerous condition of the females were also noted.

Some of the specimens reported have been deposited in the collection of the Instituto Canario de Ciencias Marinas (ICCM).

Results and discussion

First record of Plesionika antigai *Zariquiey Álvarez, 1955 from the Canary Islands*

Material examined: 1 non-ovigerous specimen, 9.0 mm CL (ICCM colec.: no. Pant1ICCM): Sta. 11 "Canarias 9512"; Morro Jable, south coast of Fuerteventura, 28° 02.348′ N, 14° 27.625′ W; depth 330–425 m; in stomach content of the zeid fish *Cyttopsis roseus*; 2.xii.1995.

Geographic distribution: Atlanto-Mediterranean species known from the West African coasts (from Morocco to Mauritania) and the Western Mediterranean coasts (from Spain to Sicily and the west coast of Italy, and from Morocco to Tunisia) (Holthuis, 1987; García-Raso, 1996). The species is now recorded for the first time from the Canary Islands and has not been previously reported from the Macaronesian archipelagos.

Habitat: Benthic, on muddy bottoms, sand of shell

Table 2. Pandalid species found around the Canary Islands

Species	Habitat	Depth interval (m)	Carapace length (mm)	Ovigerous females	
				Minimum size observed (mm, CL)	Observed in months
Plesionika narval (J.C. Fabricius, 1787)	B, N, s, r	20–476	2.0–30.0	80	I–XII
Plesionika edwardsii (Brandt, 1851)	B, M, s, r	54–649	4.5–40.0	126	I–XII
Heterocarpus ensifer ensifer A. Milne-Edwards, 1881	B, M, s	88–821	7.0–42.0	96	I–XII
Stylopandalus richardi Coutière, 1905	P, diel vertical migrations	100–650	55	–	–
Plesionika ensis (A. Milne-Edwards, 1881)	B, M, s, r	155–700	7.0–25.0	132	I–IV, XII
Plesionika williamsi Forest, 1964	B, M, r	238–900	11.0–37.0	180	I–III, VI–VII, XI
Plesionika holthuisi Crosnier & Forest, 1968	B, M	261–420	8.0–15.0	–	VI
Plesionika martia martia (A. Milne-Edwards, 1883)	B rarely P, M	245–1004	8.0–26.0	179	IX, XII
Plesionika antigai Zariquiey Álvarez, 1955	B, M	330–425	90	–	–
Heterocarpus grimaldii A. Milne-Edwards & Bouvier, 1900	B, M, s	620–1450	14.0–44.0	350	V–XII
Heterocarpus laevigatus Bate, 1888	B, M, s	714–1450	14.0–63.0	160	V–VI, X–XI
Bitias stocki Fransen, 1990	B, r	1004	70	–	–

Information on habitat, depth distribution, size range and reproduction taken from González & Santana (1996) and this work. B=benthic, P=pelagic, M=mud, s=sand, r=rocks

and shell remains, at depths between 120 and 800 m, mainly at 330–370 m (Holthuis, 1987; García-Raso, 1996).

Remarks: The predator *Cyttopsis roseus* (Lowe, 1843) (Osteichthyes, Zeidae), which was caught during an experimental survey with trammelnets in deep-sea waters, is now also recorded for the first time for the Canaries. This fact can partially explain the finding of *P. antigai* in the Canary Island waters, and the collection of more material will be necessary in order to confirm the presence of a stable population of the pandalid species in this archipelago.

Check-list of pandalid species found around the Canary Islands

Updated information on habitat, depth distribution, size range and reproduction aspects (notably, smallest ovigerous female observed and reproduction period) for the 12 pandalid species found around the Canary Islands is given in Table 2. Species listed have been sorted by depth interval, *P. narval* (20–476 m) and *B. stocki* (1004 m) were found to be the shallowest and deepest forms respectively. *P. narval* (2.0–30.0 mm CL) and *H. laevigatus* (14.0–63.0 mm CL) were observed to be the smallest and largest species respectively. Ovigerous females minimum size observed varies from 8.0 mm CL (*P. narval*) to 35.0 mm CL (*H. grimaldii*). Females bearing eggs of *P. narval*, *P. edwardsii* and *H. ensifer ensifer* occur year-round off the Canary Islands (Table 2).

Canarian pandalid species: distribution and biogeography

Table 3 shows the world distribution (detailed by zoogeographical regions) of the 12 pandalid species found around the Canary Islands.

Table 3. World distribution of the pandalid species found around the Canary Islands

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Plesionika narval (J.C. Fabricius, 1787)	X	X	X	X		X	X	X	X	X					X	
Plesionika edwardsii (Brandt, 1851)	X	X	X				X	X	X	X	X	X			X	X
Heterocarpus ensifer ensifer A. Milne-Edwards, 1881		X	X	X					X	X	X	X			X	
Stylopandalus richardi Coutière, 1905	X	X	X	X					X			X	X		X	X
Plesionika ensis (A. Milne-Edwards, 1881)		X	X				X		X	X	X	X			X	X
Plesionika williamsi Forest, 1964		X	X						X	X	X	X			X	X
Plesionika holthuisi Crosnier & Forest, 1968			X								X	X			X	
Plesionika martia martia (A. Milne-Edwards, 1883)	X	X	X				X	X	X	X	X	X	X	X	X	
Plesionika antigai Zariquiey Álvarez, 1955			X				X	X	X	X	X			X		
Heterocarpus grimaldii A. Milne-Edwards & Bouvier, 1900	X	X	X	X					X	X	X	X				
Heterocarpus laevigatus Bate, 1888		X	X	X						X			X		X	X
Bitias stocki Fransen, 1990	X		X	X												

1. Azores, 2. Madeira, 3. Canary Islands, 4. Cape Verde Islands, 5. Ascension Island, 6. St. Helena Island, 7. Western Mediterranean, 8. Eastern Mediterranean, 9. Iberian-Moroccan region, 10. Western Sahara, 11. Mauritania-Senegal, 12. Senegal-Angola, 13. Angola-South Africa, 14. English Chanel-Bay of Biscay, 15. Western Atlantic, 16. Indo-Pacific

Physetocaris microphthalma Chace, 1940, usually placed into the Physetocarididae Chace, 1940, has been assigned to the Pandalidae by d'Udekem d'Acoz (1999). Foxton & Herring (1970) reported this species from the Eastern Atlantic, based on the record of four females from the area between 10° 46′–10° 31′ N and 19° 54′–19° 58′ W and "a single male caught some 600 miles further north off the island of Fuerteventura in the Canary Islands" at 22° 30′ N–21° 31′ W. However, the Foxton & Herring's (1970) finding in fact corresponds to a situation between the Cape Barbas (22° 10′ N) and Río de Oro Peninsula (23° 40′–24° 00′ N) in the Western Sahara, therefore very far (further south) off the Canary Islands area (27° 30′–29° 30′ N).

Crosnier & Forest (1973) reported *Plesionika heterocarpus* (A. Costa, 1871) "au sud des Canaries (26° 03′ N–15° 00′ W)". In fact, this situation corresponds to a locality to the south of the Cape Bojador in the Western Sahara, therefore further south off the Canary Islands area.

At present, we will not include both these species in the Canary Islands pandalids inventory. D'Udekem d'Acoz (1999: 129) questioned the presence of *Plesionika holthuisi* Crosnier & Forest, 1968 in the Canary Islands on the basis of a dubious colour photograph given by González (1995: 95, photo 48). This species was certainly first caugth by us in the Canaries (off W Gran Canaria, Veneguera, 405 m, 2 non-ovigerous individuals, 31.i.87) (González et al., 1990; González, 1995; González & Santana, 1996) and the material was identified by Mr. C.H.J.M. Fransen, curator of crustaceans in the National Museum of Natural History at Leiden.

The 12 pandalid shrimps known from the Canaries living essentially benthic (adults) include 1 Macaronesian species (*Bitias stocki*), 1 Atlanto-Mediterranean species (*Plesionika antigai*), 1 Eastern Atlantic warm-temperate species (*Heterocarpus grimaldii*), 2 amphi-

Atlantic warm species and subspecies (*Heterocarpus ensifer ensifer* and *Plesionika holthuisi*), 1 amphi-Atlantic warm-temperate subspecies (*Plesionika martia martia*), and 5 pantropical species (*Heterocarpus laevigatus*, *Plesionika edwardsii*, *P. ensis*, *P. narval* and *P. williamsi*). The Canary Islands represent the northernmost limit of the distribution area in the Eastern Atlantic for *Plesionika holthuisi*.

The only exclusively pelagic pandalid shrimp known from the Canaries, *Stylopandalus richardi*, is a cosmopolitan species occurring in all major tropical and temperate seas.

The volcanic characteristics of the Canary Islands are manifest by the absence of wide insular shelves, with a bottom depth of 180–200 m near the coast. Therefore, many deep-sea species are dependent on the dynamics of these insular ecosystems. On the contrary, deep-sea species inhabiting the continental shelf tend to reside farther from the coast. Moreover, the Canarian archipelago is situated relatively close to the continents of Africa and Europe, but separated from them by great depths. The Canary Islands are also under the influence of the subtropical gyre of the Eastern Central Atlantic, which would facilitate the transport of shrimp larvae to the archipelago from the American, European and Northwest African coasts (Aguilera et al., 1994; González & Santana, 1996). These geomorphologic, geographic and oceanographic particularities of the Canary Islands could explain the great diversity in the biogeographic patterns of the pandalid species inhabiting this area.

The absence of Guinean (tropical and subtropical) pandalid species off the Canaries may be the result of the absence of a consistent current flow from the south, and to the thermal barrier created by the Cape Blanc upwelling cell. Transport of such species to the Canary Islands is possible by means of the occasional displacement of African source water, generated by winds from the south when the ascending strong coastal current (reverse current) extends past Cape Blanc (20°46′ N, 17° 03′ W) towards Cape Bojador (26°08′ N, 14° 30′ W). These conditions appear during winter (Mittelstaedt, 1983).

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Crustacea Decapoda of the Paripe River Estuary, Pernambuco, Brazil

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Key words: Crustacea, Decapoda, estuary, Brazil

Abstract

The Paripe river estuary is located in Itamaracá Island, north coast of Pernambuco, northeastern Brazil. Its Crustacea Decapoda fauna was studied during several years. In the estuary there are mangroves, mud- and sandbanks, and tidal creeks. The fauna comprises a total of 64 species in 20 families. The coexistence of these species confirms the ecological importance of this small estuary, only 1.6 km long and 0.5 km wide. The ecology of each species in the estuary is presented.

Introduction

The estuary of the Paripe River is located in the south of Itamaracá Island, state of Pernambuco, 50 km north of the city of Recife (7° 47′– 7° 51′ S and 34° 50′– 34° 52′ W). This river flows to the Santa Cruz Channel, which separates Itamaracá Island from the continent (see Fig. 1). The Paripe River has 4 km of extension and is 0.55 km in the widest part. The estuary has a length of 1.6 km length (Coelho & Santos, 1990). As a non-inhabited area, the anthropic action is minimum. The local population lives basically of fishing and of subsistence agriculture.

The total area is 37.3 ha of which 29.4 ha are swamp area and 7.9 ha exposed soil according to J. D.V. Silva (pers. comm.). The mangrove is formed mainly by *Rhizophora mangle*. In smaller proportion, *Avicennia schaueriana*, *Laguncularia racemosa* and *Conocarpus erectus* are present. The littoral vegetation is composed by *Annona glabra* besides species of the Juncaceae, Pomaceae and Blechmaceae families (Coelho & Santos, 1990). The climate is according to the Köppen classification: hot and humid with fall-winter rains. The wet season occurs from September to February, the dry season from March to August. The average air temperature is around 27 °C (minimum 26 °C and maximum 31 °C). The salinity varies from 37.1% to 3.5%, with average values around 30%, during the dry season. The water temperature varies between 24.3 °C and 30.3 °C.

Methodology

Samples were obtained along the estuary by hand collecting. After sampling, the animals were preserved in alcohol 75% and send to the Benthic Laboratory of the Departamento de Oceanografia da Universidade Federal de Pernambuco for identification.

Results

The fauna comprises 64 species of Decapoda, distributed in 2 suborders, 20 families and 42 genera.

The suborder Dendrobranchiata comprises the infraorder Penaeidea including five species of which three belonging to the family Penaeidae: *Farfantepenaeus notialis* (Pérez Farfante, 1967), *F. subtilis* (Pérez Farfante, 1967), and *Litopenaeus schmitti* (Burkenroad, 1936) and two to the family Sicyoniidae: *Sicyonia typica* (Boeck, 1864) and *S. laevigata* Stimpson (1871). These Penaeidea belongs to the sublittoral vagile fauna, in bottoms of sand or mud.

The suborder Pleocyemata corresponds to 92% of the fauna, including the Caridea (17%), Thalassinidea (8%), Anomura (11%) and Brachyura (56%).

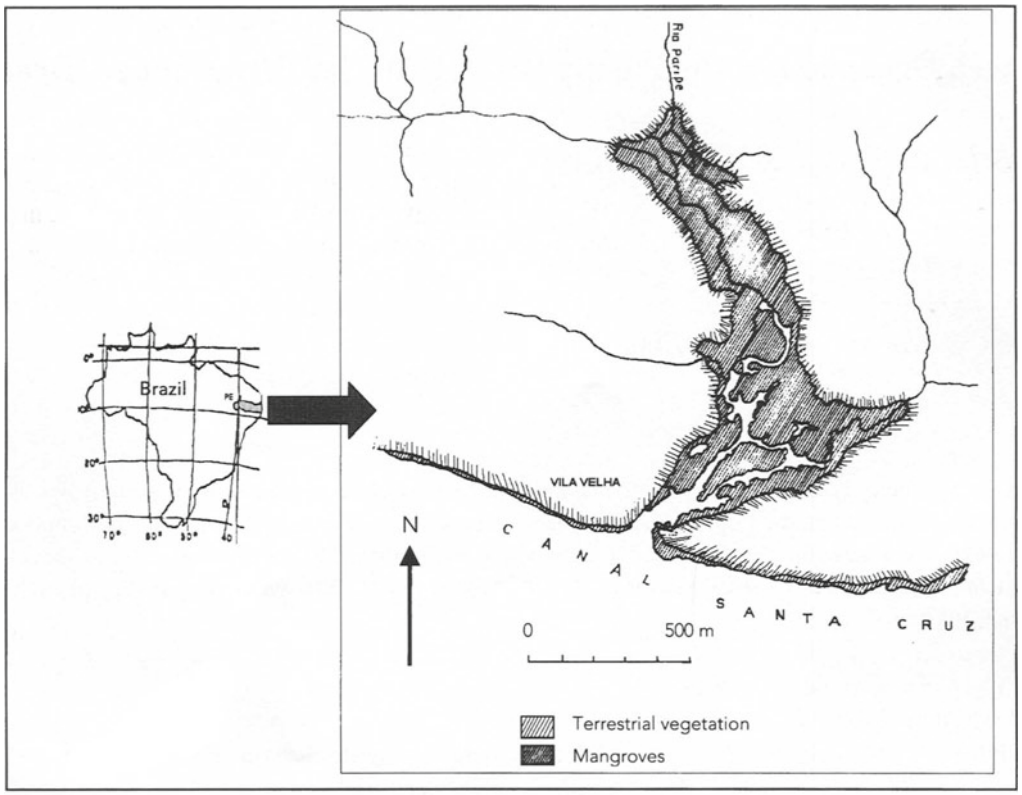

Figure 1. Map of the Paripe River Estuary.

The infraorder Caridea is represented by four families and 11 species, including both freshwater and marine species. In the Atyidae, a freshwater species, *Potimirim potimirim* (Müller, 1881), was found. The Palaemonidae presents five marine species: *Leander tenuicornis* (Say, 1818), *Palaemon northropi* (Rankin, 1898), *P. pandaliformis* (Stimpson, 1871), *Periclimenes americanus* (Kingsley, 1878) and *P. longicaudatus* (Stimpson, 1860) and one freshwater species: *Macrobrachium acanthurus* (Wiegmann, 1836); these palaemonids are found on plants or on sand or mud bottoms. The Alpheidae are represented by *Alpheus estuariensis* Christoffersen (1984), *Salmoneus ortmanni* (Rankin, 1898) and *Leptalpheus petronii* Ramos-Porto & Souza (1994); they are sedentary, found in sand or mud bottoms. The hippolytid *Merguia rhizophorae* (Rathbun, 1900) is found on mud bottoms.

The infraorder Thalassinidea is probably represented by a larger number of families and species than presently known. At present, five species in three families have been recorded: three species of Callianassidae: *Neocallichirus rathbunae* (Schmitt, 1935), *Sergio guassutinga* (Rodrigues, 1971) and *Lepidophthalmus siriboia* (Felder & Rodrigues, 1993), one species of Laomediidae: *Axianassa australis* Rodrigues & Shimizu (1992), and one species of Upogebiidae: *Upogebia omissa* Gomes Corrêa, (1968); these Thalassinidea live in galleries found in mud and sand bottoms.

The infraorder Anomura is represented by seven species in three families. The family Paguridae is represented by *Pagurus criniticornis* (Dana, 1852), the Diogenidae by *Clibanarius antillensis* Stimpson, 1862, *C. sclopetarius* (Herbst, 1796) and *C. vittatus* (Bosc, 1802) and the Porcellanidae by *Minyocerus angustus* (Dana, 1852), *Petrolisthes armatus* (Gibbes, 1850) and *P. galathinus* (Bosc, 1801). The Paguridae and Diogenidae are found in mud and sand bottoms, the Porcellanidae live under rocks or are commensals of the sea-star *Luidia senegalensis* (Lamarck).

The infraorder Brachyura, with 36 species in eight families, accounts for more than half of the known decapod species. Some families are represented by one or two species, like the marine Calappidae with *Calappa ocellata* Holthuis, 1958, the

terrestrial Gecarcinidae with *Cardisoma guanhumi* Latreille, 1825, and the estuarine Portunidae with *Callinectes danae* Smith, 1869 and *C. exasperatus* (Gerstaecker, 1856). Majidae and Pinnotheridae are represented by three species each; the Majidae with the marine spider crabs *Epialtus bituberculatus* H. Milne Edwards (1834), *Inachoides forceps* A. Milne Edwards (1879) and *Microphrys bicornutus* (Latreille, 1825) and the Pinnotheridae with the commensal crabs *Pinnixa chaetopterana* Stimpson (1860), *Zaops ostreum* (Say, 1817) and *Tumidotheres maculatus* (Say, 1818). The Grapsidae, with seven, and the Ocypodidae with nine species, dominate the environment. The terrestrial or intertidal Grapsidae comprise: *Aratus pisonii* (H. Milne Edwards, 1837), *Armases angustipes* (Dana, 1852), *Cyclograpsus integer* H. Milne Edwards (1837), *Goniopsis cruentata* (Latreille, 1803), *Pachygrapsus gracilis* (Saussure, 1858), *P. transversus* (Gibbes, 1850) and *Sesarma rectum* Randall (1840). The Ocypodidae are burrowing species found in sand or mud bottoms: *Uca burgersi* Holthuis (1967), *U. cumulanta* Crane (1943), *U. leptodactyla* Rathbun (1898), *U. maracoani* (Latreille, 1802–1803), *U. mordax* (Smith, 1870). *U. rapax* (Smith. 1870), *U. thayeri* Rathbun (1900), *U. vocator* (Herbst, 1804) and *Ucides cordatus* (Linnaeus, 1763). The Xanthidae form the largest group of crabs with 10 species: *Cyrtoplax spinidentata* (Benedict, 1892), *Eurytium limosum* (Say, 1818), *Hexapanopeus angustifrons* (Benedict & Rathbun, 1891), *Hexapanopeus caribbaeus* (Stimpson, 1871), *Hexapanopeus schmitti* Rathbun (1930), *Menippe nodifrons* Stimpson (1859), *Panopeus americanus* Saussure (1857), *P. bermudensis* Benedict & Rathbun (1891), *P. lacustris* Desbonne (1867) and *P. occidentalis* Saussure (1857). The Xanthidae live in sand or mud bottoms. In holes dug by *C. spinidentata*, a diverse fauna is found, with species of Decapoda (Alpheidae, Callianassidae, Laomediidae) and a Stomatopoda, *Chloridopsis dubia* (H. Milne Edwards, 1837).

Comments

Although high in diversity, the Paripe River Estuary decapod fauna corresponds to 76% of the estuarine decapod fauna of Pernambuco listed by Coelho & Ramos-Porto (1994, 1995). The decapods are found in several bottoms types, as sand (26% of the species), mud (12%), sand and mud (36%), plants (7%), plants, mud and sand (9%), rocks (3%) or are commensal (7%). About 75% of the species is sublittoral, 12% is supratidal or terrestrial.

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Intertidal habitats and decapod faunal assemblages (Crustacea: Decapoda) of Socotra Island, Republic of Yemen

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Key words: Socotra Island, intertidal habitats, decapod fauna, zoogeography, Indian Ocean

Abstract

The Socotra Archipelago, situated in the north-western part of the Indian Ocean at the entrance of the Gulf of Aden, has a unique zoogeographical position, as the transition between the Arabian and Red Seas and East African shores. The Socotran marine environment, however, is as yet poorly studied, with only sparse and incomplete reference to the field of crustaceans. The current work presents results from a survey of the intertidal decapod assemblages of Socotra Island conducted in spring 1999.

The information from 185 sites sampled around the island is summarized in a map with short descriptions of representative intertidal habitats, their relative area and distribution. Both rocky shores and cobble beaches have the largest diversity of decapods. Sandy beaches are dominated mainly by *Ocypode saratan* and *Coenobita scaevola*, whilst rocky shores are dominated by *Grapsus albolineatus*, *G. tenuicrustatus*, *Plagusia tuberculata*, *Pachygrapsus minutus*, *Metopograpsus messor* and *Eriphia smithii*. In cobble beaches, *Pseudozius caystrus*, *Leptodius exaratus*, *Xanthias sinensis*, *Clibanarius signatus* and *Clibanarius virescens* are the most common species. *Cardisoma carnifex* and *Uca inversa* are common bordering mud flats and coastal lagoons. As fishing pressure is low, mud flats surrounding wadis and coastal lagoons host small undisturbed populations of *Scylla serrata* and *Fenneropenaeus indicus*. There is only a reduced number of mangrove trees and area of mangrove, most of which is already destroyed or under severe human and environmental pressure. The largest and most representative stand has an unusual structure: species diversity is strikingly low, it is disconnected from the sea by a sand bar or dune, and is completely dry.

Interesting zoogeographical findings are detailed, and a list of intertidal decapod fauna, relating each species to its common habitat is presented. This list is compared with previous studies, and other intertidal decapod assemblages from the Arabian Gulf, Red Sea and East Africa.

Introduction

The Socotran Archipelago consists of four main islands: Socotra, Abd al-Kuri and two smaller islands of Samha and Darsah, also known as 'The Brothers', together with rock outcrops Kal Farun and Sabuniyah (Fig. 1). Socotra is situated in the north-western Indian Ocean approximately 350 km south of Ras Fartak on the Yemeni mainland, 700 km to the south-east of Aden and 225 km off the 'Horn of Africa' (Cape Guardafui).

Very little information exists concerning the marine biodiversity of the Socotran Archipelago. Several expeditions took place early in the 20th century, which principally studied the terrestrial botany and zoology of the islands with only sparse data on the marine fauna. Results from these expeditions are summarised in Wranik (1998). More recently, Van Belle & Wranik (1996) provided a comprehensive study on the chitons of Socotra, and still in 1996, Saad (1996a) presented a mollusc species list based on several Russian scientific cruises, with further additions by Wranik (1998). The

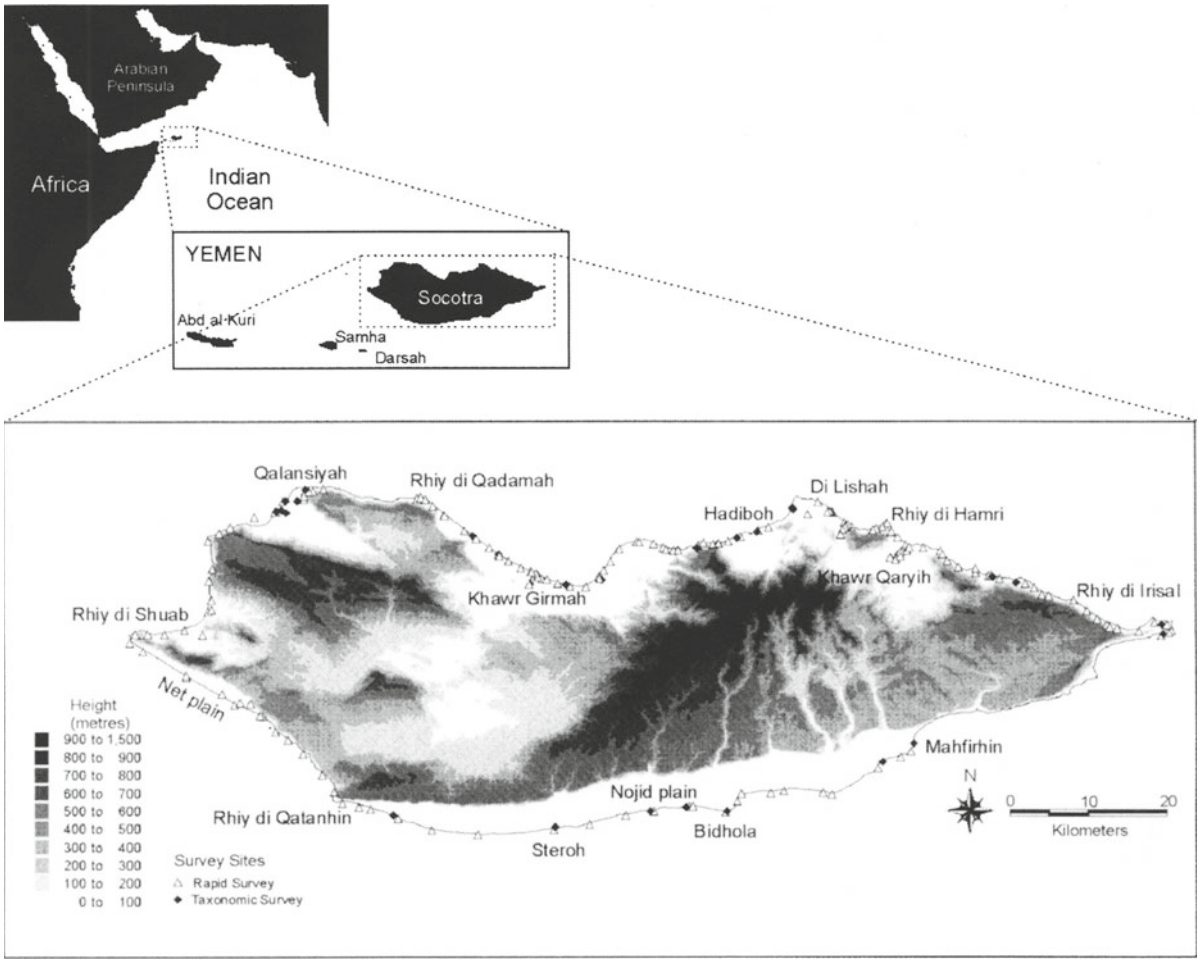

Figure 1. Location of the Socotra Archipelago and detailed map of Socotra with positions of rapid and taxonomic survey sites. Names of localities are taken from the map of Socotra (1:125 000) published by the Royal Geographical Society (1978).

pilot survey for the United Nations Global Environmental Facilities (GEF) programme (MacAlister et al., 1996) produced a report with preliminary data on the fish community associated with corals on Socotra, later validated by Kemp (1998a). Kemp (1998b) also studied the occurrence of a macroalgae species in the Socotran Archipelago and correlated this with local upwelling in the Arabian Sea.

The first to publish a decapod crustacean from Socotra was Hilgendorf (in Taschenberg, 1883), describing a new species of freshwater crab (*Telphusa socotrensis*) along with a record of the land crab *Cardisoma carnifex*. Pocock et al. (1903) published some material collected by Balfour in 1880 and Forbes in 1899 on Socotra and Abd al-Kuri. Since both expeditions, however, focussed on the terrestrial flora and fauna, the collections contain only few common estuarine and littoral species. Thus, the number of decapod species known from the archipelago by then was only 11 and the marine crustacean fauna of the archipelago remained basically unknown until very recently. Apart from information on the occurrence of economically important lobsters by Saad (1996b), the only lists of crustaceans species are by Wranik (1998) and MacAlister et al. (1996), which was the first to describe both the sub-littoral and littoral environments of Socotra including biological and physical characteristics. Present work further extends this list of species, and classifies and describes in detail the intertidal habitats of Socotra and the associated decapod fauna (Table 1).

The marine environment of the entire Socotra Archipelago has been identified as having 'very high priority' for protection on the basis of its location at the boundary between the seasonal Somali upwelling

Table 1. List of 137 intertidal decapod species found at Socotra Island

Family	Species	N sites species	*	**	***	Habitat
Penaeidae	*Fenneropenaeus indicus* (H. Milne Edwards, 1837)	3	+			Khawrs
	gen. sp.	1	+			seagrass bed, Qalansiyah lagoon
Alpheidae	*Alpheidae* spp. indet.	22	+			
	Athanas sp.			+		
	Alpheus djeddensis Coutière, 1897			+		
	Alpheus lottini Guérin, 1829			+		
	Alpheus frontalis H. Milne Edwards, 1837 (?)			+		
	Alpheus deuteropus Hilgendorf, 1879			+		
	Alpheus lobidens de Haan, 1850			+		
	Alpheus sp.			+		
	gen. sp.			+		
	Racilius compressus Paulson, 1875			+		
Thalassinidae	gen. sp.	1	+			rocky shore
Callianassidae	*Neocallichirus* sp.	1	+			under stones on sand
	Podocallichirus sp. nov.	4	+			mud & muddy sand; mangroves
Albuneidae	*Albunea* cf. *steinitzi* Holthuis, 1958	1	+			
Hippidae	*Emerita holthuisi* Sankolli, 1965	29	+			sandy beaches
	Hippa pacifica (Dana, 1852)	1	+			sandy beaches
Coenobitidae	*Coenobita scaevola* (Forskål, 1775)	80	+	+	+	sandy & cobbly beaches, khawrs
Diogenidae	*Aniculus erythraeus* Forest, 1984	2	+			rocky inter- & shallow subtidal
	Calcinus gaimardii (H. Milne Edwards, 1848)	2	+			rocky & cobbly shores
	Calcinus guamensis Wooster, 1984	3	+			rocky coasts
	Calcinus laevimanus (Randall, 1839)	16	+			rocky & sandy shores, tide pools
	Calcinus latens (Randall, 1839)	13	+			rocky hores, tide pools
	Calcinus tropidomanus Lewinsohn, 1981	5	+			rocky shores
	Ciliopagurus strigatus (Herbst, 1804)	1	+			rocky shores
	Clibanarius eurysternus (Hilgendorf, 1879)	2	+			rocky/cobbly shore & sand on beachrock
	Clibanarius signatus Heller, 1861	40	+	+		rocky & cobbly shores
	Clibanarius virescens (Krauss, 1843)	47	+	+		rocky & cobbly shores
	Dardanus lagopodes (Forskål, 1775)	1	+			rocky shores
	Dardanus sp. ?	1	+			Qalansiyah lagoon: beach rock with sand
	Dardanus tinctor (Forskål, 1775)	2	+			sand on beachrock & exposed sandy beach
	Diogenes avarus Heller, 1865	3	+			sandy beaches
	Diogenes sp. 1	2	+			sandy beaches
	Diogenes sp. 2	1	+			sandy beaches
	Diogenes sp. 3 [aff. *gardineri* Alcock, 1905]	1	+			Qalansiyah lagoon: seagrass beds
Porcellanidae	*Pachycheles natalensis* (Krauss, 1843)	1	+	+		rocky shores
	Pachycheles tomentosus Henderson, 1893	2	+			rocky shores
	Petrolisthes sp. 1 [aff. *leptocheles* (Heller, 1861)]	3	+			rocky & cobbly shores
	Petrolisthes carinipes (Heller, 1861) (?)			+		
	Petrolisthes lamarckii (Leach, 1820)	3	+			rocky & cobbly shores
	Petrolisthes leptocheles (Heller, 1861)	14	+	+	+	rocky & cobbly shores

Continued on p. 84

Table 1. Continued

Family	Species	N sites species	*	**	***	Habitat
	Petrolisthes cf. *militaris* (Heller, 1862)			+	+	
	Petrolisthes ornatus Paulson, 1875	11	+	+	+	rocky & cobbly shores
	Petrolisthes pubescens Stimpson, 1858	3	+			
	Petrolisthes rufescens (Heller, 1861)	4	+			rocky & cobbly shores
	Petrolisthes unilobatus Henderson, 1888			+	+	
	Petrolisthes virgatus Paulson, 1875	2	+			rocky & cobbly shores
	Aliaporcellana pygmaea (De Man, 1902)				+	
Dromiidae	*Cryptodromia fallax* (Lamarck, 1818)	2	+			
	Epigodromia granulata (Kossmann, 1878)	1	+			
Calappidae	*Ashtoret lunaris* (Forskål, 1775)	2	+			sandy beaches
	Calappa gallus (Herbst, 1803)	1	+			sand
Leucosiidae	*Leucosia* sp.	1	+			
	Philyra cancella (Herbst, 1783)	3	+			sandy beaches
	Philyra sp. [aff. *platycheir* de Haan, 1841]	1	+			sandy beaches
	Philyra sp.			+		
Majidae	*Achaeus* sp. indet.	1	+			cobble beach & beachrock
	Cyphocarcinus sp.			+		
	Huenia cf. *heraldica* de Haan, 1837	1	+			rocky shore, amongst macroalgae
	Huenia grandidierii A. Milne Edwards, 1865	1	+			rocky shore, amongst macroalgae
	Huenia sp. ?	2	+			rocky shore, amongst macroalgae
	Menaethiops contiguicornis (Klunzinger, 1906)	1	+			sand with rocks
	Menaethiops nodulosa (Nobili, 1905)	1	+			rocky shore, amongst macroalgae
	Menaethiops sp. [aff. *fascicularis* (Krauss, 1843)]	1	+			sand with rocks
	Menaethius monoceros (Latreille, 1825)	3	+	+		rocky shore, amongst macroalgae
	Menaethius orientalis (Sakai, 1969)	1	+			rocky shore, amongst macroalgae
	Menaethius sp. 1			+		
	Menaethius sp. 2			+		
	Micippa platipes Rüppell, 1830	3	+			rocky shore
	Micippa thalia (Herbst, 1803)	1	+			rocky shore
	Pseudomicipe griffini Kazmi & Tirmizi, 1999	1	+			rocky shore
	Simocarcinus pyramidatus (Heller, 1861)	1	+			rocky shore, amongst macroalgae
	Stilbognathus erythraeus von Martens, 1866	1	+			rocky shore
	gen. sp. ?	6	+			
Parthenopidae	*Heterocrypta petrosa* Klunzinger, 1906	1	+			sand with rocks
Portunidae	*Carupa tenuipes* Dana, 1852	1	+			
	Charybdis sp. 1 [aff.*orientalis* Dana, 1852]	5	+			sand with cobbles
	Portunus pelagicus (Linnaeus, 1758)		+	+		sandy shores, Qalansiyah lagoon
	Portunus sp.			+		
	Scylla serrata (Forskål, 1775)	20	+			Khawrs
	Thalamita admete (Herbst, 1803)	8	+	+		sand & rocks/cobbles
	Thalamita crenata Rüppell, 1830	11	+			Qalansiyah lagoon, Khawrs, mangrove
	Thalamita spinifera Borradaile, 1902			+		
	Thalamita stephensoni Crosnier, 1962	1	+			under stones on sand/shingle flat

Continued on p. 85

Table 1. Continued

Family	Species	N sites species	*	**	***	Habitat
Xanthidae	*Actaea* sp.			+		
	Actaeodes tomentosus (H. Milne Edwards, 1834)	2	+			cobble beach
	Etisus anaglyptus H. Milne Edwards, 1834	1	+			sand and rock
	Etisus electra (Herbst, 1801)	2	+			sand and rock
	Forestia depressa (White, 1847)	2	+			rocky shore
	Lachnopodus subacutus (Stimpson, 1858)	1	+			rocky shore, amongst macroalgae
	Leptodius exaratus (H. Milne Edwards, 1834)	29	+	+		rocky & cobbly shores
	Leptodius gracilis (Dana, 1852)			+		
	Leptodius sanguineus (H. Milne Edwards, 1834)	7	+			rocky & cobbly shores
	Liomera rugata (H. Milne Edwards, 1834)	2	+			rocky & cobbly shores
	Paractaea rufopunctata (H. Milne Edwards, 1834)	1	+			dead coral (subtidal)
	Paractaeopsis quadriareolatus (Takeda & Miyake, 1968)	1	+			beachrock and pebble beach
	Pilodius areolatus (H. Milne Edwards, 1834)	5	+			rocks & cobbly shores
	Pilodius paumotensis Rathbun, 1907 (?)			+		
	Pilodius sp.			+		
	Pilodius spinipes Heller, 1861	2	+	+		rocks & cobbly shore
	Platypodia anaglypta (Heller, 1861)	1	+	+		sand & rocks
	Pseudoliomera remota (Rathbun, 1907)	4	+			rocky coasts
	Xanthias sinensis (A. Milne Edwards, 1867)	15	+			rocky & cobbly beaches
	Zozymodes cavipes (Dana, 1852)	7	+			rocky shores
	Zozymodes xanthoides (Krauss, 1843)	3	+			rocky platforms
Menippidae	*Epixanthus corrosus* (A. Milne Edwards, 1873)	2	+			cobbly beach
	Epixanthus frontalis (H. Milne Edwards, 1834)	2	+			pebbles & rocks
	Epixanthus sp.			+		
	Eriphia smithii MacLeay, 1838	42	+		+	rocky & cobbly shores
	Lydia tenax (Rüppell, 1830)	2	+			rocky shore
	Menippe rumphii (Fabricius, 1798)	1	+			Khawr
Pilumnidae	*Actumnus setifer* (De Haan, 1833)	1	+			rocky shore
	gen. sp. 1			+		
	Pilumnopeus sp. 1	6	+			rocky & cobbly shores
	Pilumnus vespertilio (Fabricius, 1793)			+		
	Pilumnus sp. 1	1	+			rocks on sand
Goneplacidae	*Pseudozius caystrus* (Adams & White, 1848)	38	+	+		cobble beaches
Grapsidae	*Cyclograpsus integer* H. Milne Edwards, 1837	2	+			rock pool
	Geograpsus crinipes Dana, 1851	4	+			rocky coasts, boulders & cobbles
	Grapsus albolineatus Lamarck, 1818	83	+	+	+	rocky coasts
	Grapsus granulosus H. Milne Edwards, 1853	18	+			rocky coasts & cobble beaches
	Grapsus longitarsus Dana, 1851 (?)			+		
	Grapsus tenuicrustatus (Herbst, 1783)	58	+			rocky coasts
	Grapsus sp. 1			+		
	Grapsus sp. 2			+		
	Helice leachii Hess, 1865	2	+			sandy & muddy banks of Khawrs
	Metopograpsus messor (Forskål, 1775)	28	+	+		sandy, muddy & rocky shores, mangroves
	Metopograpsus thukuhar (Owen, 1839)	11	+			sandy/muddy shores, mangroves

Continued on p. 86

Table 1. Continued

Family	Species	N sites species	*	**	***	Habitat
	Pachygrapsus minutus A. Milne Edwards, 1873	26	+			rocky coasts, cobble/shingle beaches
	Percnon guinotae Crosnier, 1965	1	+			rocky coasts
	Percnon planissimum (Herbst, 1804)	4	+			rocky coasts
	Plagusia tuberculata Lamarck, 1818	19	+			rocky coasts
	Thalassograpsus harpax (Hilgendorf, 1892)	6	+	+		cobbles on sand/mud, beachrock with sand
Ocypodidae	*Dotilla sulcata* (Forskål, 1775)	4	+			sand flats
	Macrophthalmus boscii Audouin & Savigny, 1825	2	+		+	rock flats/platforms
	Ocypode cordimanus Latreille, 1818	28	+	+		sandy beaches, in dunes
	Ocypode ryderi Kingsley, 1881	1	+			sandy beaches
	Ocypode saratan (Forskål, 1775)	69	+	+	+	sandy beaches
	Ocypode cf. *rotundata* Miers, 1882			+		
	Uca annulipes albimana (Kossmann, 1877)	13	+			sand/mud flats
	Uca inversa (Hoffmann, 1874)	31	+		+	sand/mud flats
Gecarcinidae	*Cardisoma carnifex* (Herbst, 1796)	32	+		+	Khawrs, sandy & muddy banks

Relation to habitat and comparison with previously reported occurrences. *Present survey; **MacAlister et al. (1996); ***Wranik (1998). N sites/species represents the number of sites where each species was found.

to the south and the Gulf of Aden to the North (Chiffings, 1995). Improved knowledge of the intertidal marine habitats present around the archipelago, and the associated crustacean species, fills an important gap in available information on the zoogeography of Western Indian Ocean and Arabian intertidal taxa, and will assist regional marine conservation planning.

Materials and methods

Rapid assessment and taxonomic surveys were conducted at 190 sites along the coast of Socotra island between February and April 1999, either at regular intervals or where topography changed drastically (Figs 1 and 2). Most sites were surveyed from the shore, although many rock cliffs were surveyed by snorkelling from a boat. Rapid assessment surveys targeted the identification, physical and biotic description, distribution and relative extension of representative intertidal habitats. Taxonomic surveys thoroughly examined pre-identified key intertidal habitats. Specimens collected during both surveys were pooled and present work is confined to results obtained on decapod crustacean community identification and distribution.

Rapid assessment survey

Detailed information concerning the presence and absence of key biota for each zone of the intertidal, together with the geological topography/morphology of the area was collected at each site. Observers walked perpendicular to the shore line from the littoral fringe to the shallow sublittoral (or vice-versa) along a 10–15 m width band recording the presence of key species during 30–50 min. Survey time per site depended on uniformity of the coast and access, with sandy beaches generally taking less time than rocky or cobble beaches which contain numerous microhabitats. Despite the fact that certain stretches of coast had more survey points per km, the final number of sites can be used as a rough estimate of site abundance. Exception should be made for the mud flats that, due to their uniqueness, had more time invested and sites surveyed. Their abundance may be, therefore, overestimated. However, in terms of area, they are equally important. Key species were defined as conspicuous macroscopic organisms or clear signs of their presence, such as calcareous structures, cemented shells, burrows, egg masses, tracks, etc. On sandy shores, 6–10 spades of sand (approx. 0.2 m^2, 10–20 cm deep) were sieved through standard 5 and 2 mm sieves and the tailings quickly inspected. Sand was sampled in both the upper and lower eulittoral. Unidentified specimens were collected, immediately

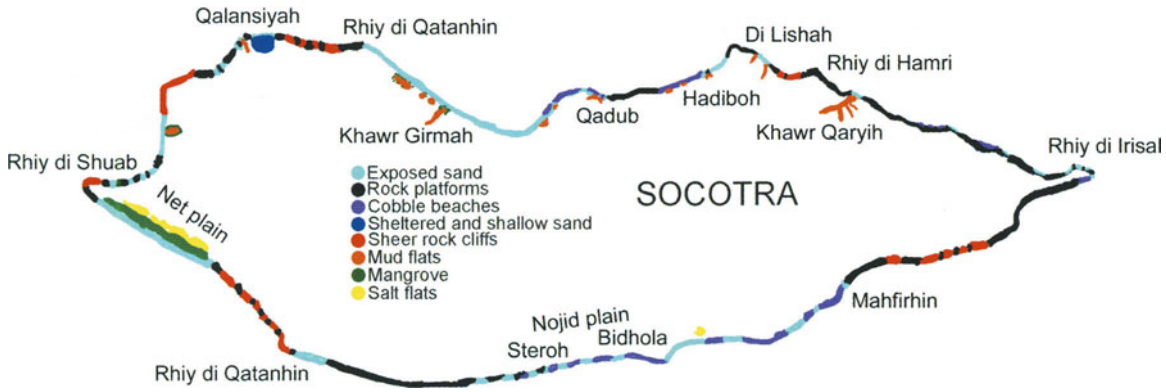

Figure 2. Distribution and extension of intertidal habitats of Socotra Island. Names of localities are taken from the Map of Socotra (1:125 000) published by the Royal Geographical Society (1978).

preserved and added to the taxonomic survey reference collection. Salinity was measured with a hand refractometer and the detailed position was recorded using a GPS (Trimble Navigation 'Scout' or Garmin '48'). A general profile was sketched and, where possible, the widths of the characteristic biotic zonation were measured using a level, a 1.4 m wood pole ruler and a 50 m measuring tape. The substrate classification presented in Table 3 and a general shore habitat morphology using 4 categories (rocky, sandy, muddy and cobble shores) were used to describe each of the four intertidal zones (sub, lower, upper and supra littoral). The information from the 4 zones was pooled for each site due to high correlation between morphology codes at each intertidal zone. General wave exposure was estimated from intertidal band height and classified using a ranking scale with 4 categories. Crab burrow abundance was estimated using metal quadrates of 0.1 and 2.5 m^2. Photographs of key species and important topographic features were taken at most sites. These pictures are organised in a database of images available from the GEF project, Sana'a, Yemen.

Taxonomic surveys

Specimens were collected by hand, snorkelling or using a sieve for fine sediments. Collecting was also done at night, although not at all stations. Following a preliminary identification in the field, a thorough taxonomic examination of collected specimens was done by one of the authors (MA). Preliminary identification was mainly based on pictorial guides as Jones (1986) and Richmond (1997) along with several papers on the regional decapod fauna (e.g. Vannini & Valmori, 1981a, b; Galil & Vannini, 1990; Apel & Spiridonov, 1998). For final identification, however, all relevant taxonomic literature has been considered and specimens have been identified to species level where possible. An exception are the Alpheidae, which are at present under study by A. Anker, and several small groups like the Thalassinidae. Specimens were collected in plastic or glass vials and preserved in 4–8% formaldehyde solution until arrival at the Senckenberg Research Institute, where the material was washed and transferred to 70% ethanol for examination and long-term storage. Notes on the substrate and shore height where specimens were found were recorded together with the accurate site location. While a small reference collection of common species has been deposited on Socotra, the main part of the collections is presently stored at the Senckenberg Research Institute and Natural History Museum Frankfurt (SMF) and will be partly returned to an institution in the Republic of Yemen that will be selected by the relevant authorities.

Statistical analysis

Species were grouped using presence/absence at each site and variable clustering (instead of observation clustering). Cluster analysis used Ward's linkage and squared euclidean distance (Johnson & Wichern, 1988) using Minitab statistical software. Sites with zero species or with incomplete morphology codes description were ignored. Species with less than 5 occurrences per site and species that only occurred at the taxonomic survey sites were also ignored. Unidentified specimens from the Alpheidae, Diogen-

idae and Porcellanidae families were pooled in the respective families. Salinity was not included since it is the only variable that cannot be reviewed on a presence/absence (1–0) scale, and can therefore significantly bias the analysis. All correlation data is based on presence/absence of species and morphological characteristics matrix of 185 sites.

Results

Family and species list

The present Intertidal survey identified 112 decapod species, 90 of which are new records for Socotra Island (Table 1). More than half of the new records include species from the Diogenidae (15), Majidae (15), Xanthidae (14) and Grapsidae (10), amongst other less represented families. These are also the best represented families with Xanthidae having 21 species, followed by the Diogenidae, Majidae and Grapsidae with 17, 15 and 14 species, respectively (Fig. 3). By contrast, the family Ocypodidae is underrepresented with only 8 species. Other families with less than 4% of species were grouped to contribute with 19% of the total number of decapods. These families included the Thalassinidae, Albuneidae, Coenobitidae, Parthenopidae, Goneplacidae, Gecarcinidae, Penaeidae, Callianassidae, Hippidae, Dromiidae, Calappidae, Leucosiidae and Pilumnidae (Table 1).

The abundance and distribution of each family (sum of sites where at least one species belonging to one particular family occurred) presents a different pattern of dominance when compared to the families richness (Figs 3 and 4). From a total of 686 family occurrences at 178 sites, the Grapsidae, Ocypodidae, Coenobitidae and Diogenidae were the most common and evenly distributed families on Socotra, representing 53% of all occurrences and present at 116, 97, 78 and 66 sites, respectively. The Ocypodidae, Coenobitidae, Menippidae and Goneplacidae, for example, are only represented by a reduced or single number of species (9, 1, 1, 1, respectively), although some of these species are abundant and evenly distributed around Socotra Island, present from 38 to 78 sites (Figs 3 and 4). On the other hand, the Xanthidae and Majidae, despite having high species richness (21 and 15, respectively), have species which are rare or clustered together at particular sites (42 and 9, respectively).

The habitat and number of sites where each species was found is also presented (Table 1). From the total number of species found at identified habitats, 55 occur on rocky coasts, 30 on cobble beaches, 30 on sand shores and only 13 occur at areas with mud or mangrove (Table 1). *Grapsus albolineatus* and *Coenobita scaevola* are the two most abundant species occurring at nearly half of the surveyed sites, (83 and 80, respectively). Present at more than 20% of the sites, they were followed by *Ocypode saratan* (69), *Grapsus tenuicrustatus* (58), *Clibanarius virescens* (47), *Eriphia smithii* (42), *Clibanarius signatus* (40) and *Pseudozius caystrus* (38). Other common species included *Cardisoma carnifex* (32), *Uca inversa* (31), *Leptodius exaratus* (29) *Emerita holthuisi* (29), *Metopograpsus messor* (28), and *Ocypode cordimanus* (28) present at more than 15% of the surveyed sites.

Common species (i.e. species present at >5 survey sites) observed during the rapid surveys, are grouped in Figure 5 with cluster membership indicated in Table 2. These results indicate a strong relation between substrate type/structure and the intertidal decapod community that is present at a particular site. The first group of clusters, represented by species that occur at sandy beaches, mud flats and sandy areas with mud (clusters 3, 5 and 2, respectively), is clearly separated from the second group of clusters which represents species present on rock platforms or cliffs and cobble or rock and cobble beaches (clusters 6, 1 and 4, respectively). While this separation between soft sediment and hard rock structures is obvious, the species distribution through clusters 4 and 1 is not so clear as in clusters 3, 5, 2 and 1 due to niche overlap. Species from clusters 4 and 1 predominantly appear in cobble beaches, but are also frequent at rock platforms with pools or boulders. Species associations are further described in the annotated list of habitats.

Site classification and description

The types of substrate observed in the shores of Socotra were classified into hard (granite, limestone, fossil reef, sandstone, conglomerate and coral rubble) and soft (sand, mud). These substrates were found either in stable (platforms, undercut platforms, cliffs, boulder and rock pools) or loose structures (cobbles, shingle and dunes). The 185 surveyed sites are classified according to their shore morphology, and this resulted in 11 categories of shore sites with either 1 or a combination of 2 or 3 basic factors; rock, sand, mud and cobbles. The number of sites in each of these categories representing a certain substrate type and structure are given in Table 3. Note that any one

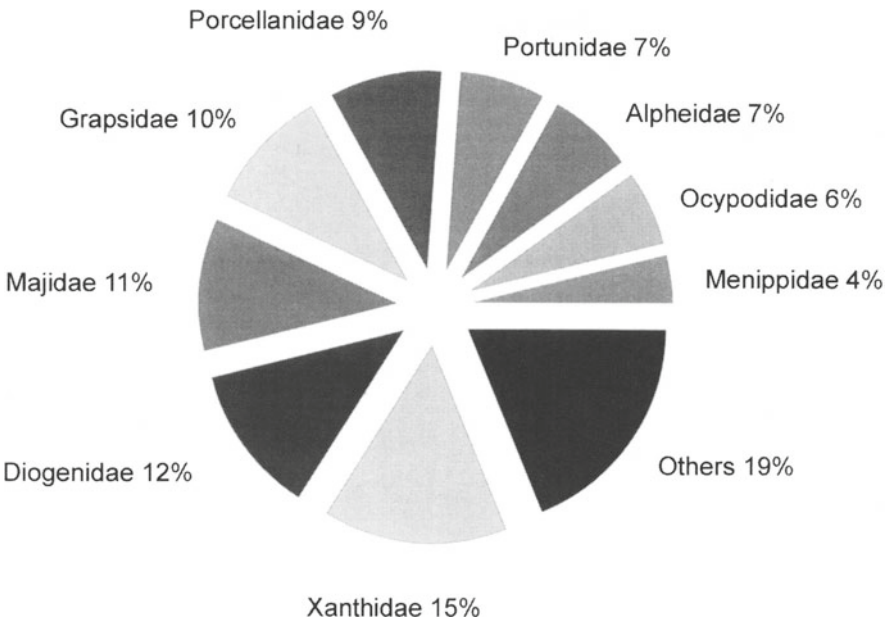

Figure 3. Percentages of the different decapod crustacean families with more than 4% of the total number of species identified for Socotra Island (Table 1). Other families refer to the Thalassinidae, Albuneidae, Coenobitidae, Parthenopidae, Goneplacidae, Gecarcinidae, Penaeidae, Callianassidae, Hippidae, Dromiidae, Calappidae, Leucosiidae and Pilumnidae (Table 1).

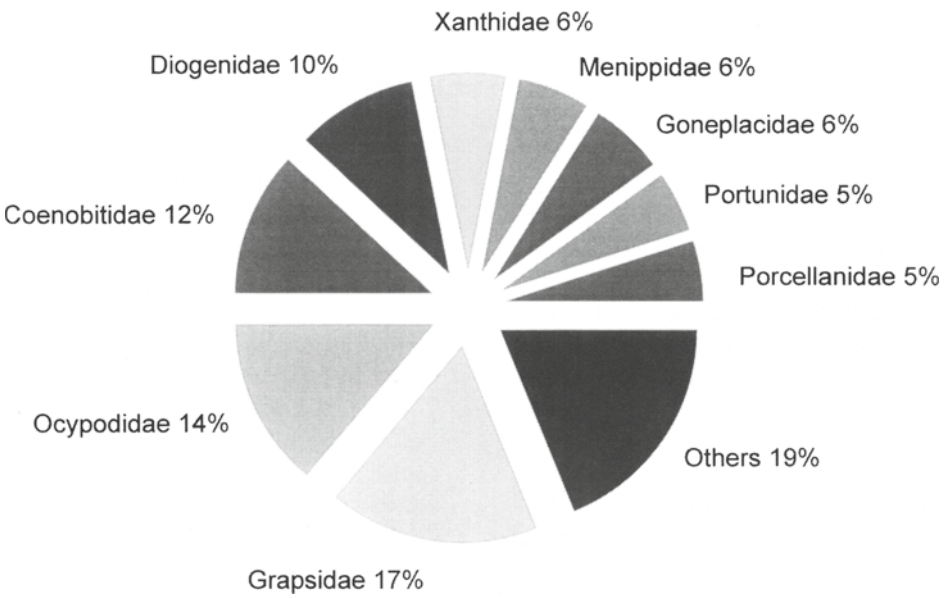

Figure 4. Percentages of decapod families occurrences (total of 686 using 1034 species records) at 178 survey sites. Families with less than 4% were grouped: Alpheidae, Majidae, Thalassinidae, Albuneidae, Parthenopidae, Gecarcinidae, Penaeidae, Callianassidae, Hippidae, Dromiidae, Calappidae, Leucosiidae and Pilumnidae (Table 1).

Table 2. Common species (present at >5 sites) clustered using the presence/absence information of 178 surveyed sites

	Species	Var.		Species Var.	
Cluster 3	*Coenobita scaevola*	3	Cluster 4	*Grapsus granulosus*	13
	Ocypode saratan	25		*Thalamita* cf. *admete*	36
	Ocypode cordimanus	24		*Percnon planissimum*	18
	Emerita holthuisi	21		*Petrolisthes* sp. 1 [aff. *leptocheles*]	33
				Charybdis sp. 1 aff. *orientalis*	34
				Calcinus tropidomanus	7
				Petrolisthes ornatus	31
Cluster 5	*Cardisoma carnifex*	10		*Leptodius sanguineus*	40
	Metopograpsus messor	15		*Pilodius areolatus*	41
	Uca inversa	27			
	Scylla serrata	35	Cluster 1	*Geograpsus crinipes*	11
	Metopograpsus thukuhar	16		*Stomatopoda* (several spp.)	38
	Fenneropenaeus indicus	28		*Petrolisthes rufescens*	32
				Zozymodes cavipes	44
				Calcinus laevimanus	5
				Calcinus latens	6
Cluster 2	*Thalassograpsus harpax*	20		*Clibanarius signatus*	8
	Thalamita crenata	37		*Clibanarius virescens*	9
	Podocallichirus sp. nov.	2		*Eriphia smithii*	22
	Dotilla sulcata	23		*Alpheidae* spp. indet.	1
	Uca annulipes albimana	26		*Pachygrapsus minutus*	17
				Diogenidae (several spp.)	4
				Petrolisthes leptocheles	30
				Porcellanidae (several spp.)	29
Cluster 6	*Grapsus albolineatus*	12		*Leptodius exaratus*	39
	Grapsus tenuicrustatus	14		*Pseudozius caystrus*	42
	Plagusia tuberculata	19		*Xanthias sinensis*	43

Variable (Var.) numbers correspond to Figure 3 where the clustering tree is presented. Sequence of clusters and species of Figure 3 are preserved.

particular site may include a combination of substrate types and structures. The average and maximum number of species recorded for each category is equally presented.

Rock and sand beaches or a combination of both, dominate the surveyed sites adding up to 60% of the total (Table 3). Less common were cobble beaches with a rock or sand landward fringe. Intertidal mud areas were the least represented soft substrate types on Socotra. Although there were only 39 beaches made entirely of sand, sand was present at 108 sites, with nearly half of these sites, occurring in conjunction with cobbles and rock. Hard substrate types were more evenly distributed than soft substrates, with granite forming the commonest (48 sites) followed by coral rubble (35) and limestone (31). Only one shale rock platform was found west of Hadiboh. Coral rubble was particularly common on sand beaches. Stable rock platforms were the most abundant substrate structure, found at 82 survey sites, followed by boulders, cliffs and undercut platforms. Cobbles were present at 51 sites, despite the low number of cobble beaches (9).

The average number of decapod crustacean species per site ranges from 2.8 in exposed sand beaches to 14.7 on cobble beaches with sand and rock (Table 3). Combinations of cobbles with either rock or sand were normally rich in species number (8.38±1.32 and 6.75±0.82, respectively). Furthermore, the ratio between average number of species per site and number of sites for a certain category is highest (4.9) for beaches with a combination of cobbles, sand and rock. All other categories in Table 3 have ratios below 1. Exposed sand beaches, although frequent, have a low diversity of decapod crustacean species, contrasting with the high diversity of cobble beaches with rock platforms and sand dunes which, although only

Table 3. Number of shore sites in each of 11 categories that presented different combinations of substrate

Shore site category		Species/site		Substrate type								Substrate structure							
				Hard						Soft		Stable					Loose		
	N	Mean±SE	Max	Granite	Limestone	Fossil reef	Sandstone	Conglomerate	Coral rubble	Sand	Mud	Platform	Undercut platf.	Cliff	Boulders	Rock pools	Cobbles	Shingle	Dunes
Sandy	39	2.79±0.20	5	–	–	–	2	–	19	39	2	–	–	–	1	–	11	9	15
Sandy/muddy	14	4.3±0.48	8	–	–	–	–	–	–	12	14	–	–	–	–	–	1	–	–
Sandy with cobbles	20	6.75±0.82	13	10	1	–	–	1	3	20	–	–	–	–	1	–	20	11	7
Sandy/rocky	30	4.67±0.60	15	7	8	5	11	3	4	29	–	28	5	3	10	12	–	–	7
Sandy/rocky/muddy	6	5.67±2.29	17	4	–	2	–	1	–	5	5	1	–	4	1	–	–	–	–
Sandy/rocky with cobbles	3	14.67±1.20	17	–	1	–	2	1	1	3	–	3	1	–	1	–	2	3	2
Rocky	40	5.08±0.61	17	13	14	7	8	6	4	–	–	34	13	11	15	25	–	–	–
Rocky/muddy	5	4.2±0.58	5	1	2	4	–	–	–	–	5	4	1	4	2	–	–	–	–
Rocky with cobbles	13	8.38±1.32	15	8	3	–	–	5	1	–	–	12	–	2	3	3	8	2	–
Mud	6	5.84±0.98	10	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–
Cobbles	9	4.44±1.50	15	5	2	–	1	3	3	–	–	–	–	–	3	–	9	6	1
Total	185			48	31	18	24	20	35	108	32	82	20	24	37	40	51	31	32

Shore site categories were classified using four general descriptors (sand, rock, cobbles and mud). Additional information concerning the average number of species found per site for each shore site category is also presented with standard error of the mean and maximum number of species per site. Information from both surveys (rapid and taxonomic) was used. Sites with no species, or with no substrate type and structure information were ignored.

present at three of the surveyed sites, have the highest species diversity (Table 3). A maximum number of 17 species was observed for three categories: rock platforms, cobble beaches with either sand or rock and mud beaches with either sand or rock. Number of observed species per site using rapid survey or taxonomic techniques was not significantly different (W=13258.5 p=0.077 1st N=152 2nd N=26), although the maximum number of species (23) was recorded by the taxonomic team on a sheltered granite rock platform with cobbles.

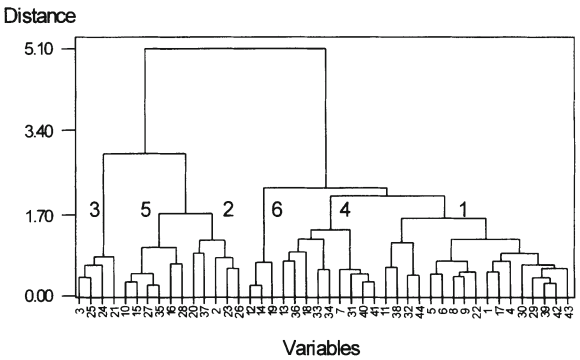

Figure 5. Dendrogram of the most abundant (present at >5 sites) intertidal decapod species grouped in 6 clusters according to presence/absence at 178 survey sites around Socotra Island. Variable clustering (instead of observation clustering) was performed using squared euclidean distance and Ward's linkage method, using Minitab statistical software. Each variable number corresponds to one species or pooled group of species. Species name and cluster affiliation is presented in Table 2.

Annotated checklist of habitats

With tidal amplitude estimated to vary from 1.0 to 1.7 m, Socotra presents a diverse selection of intertidal habitats providing ecological niches for a large number of decapod crustacean species. Seawater temperature during the day averaged 25–26 °C, while salinity was constant at 34%. The following section describes broad intertidal habitats based on their morphology and associated decapod communities. Clear cut differences are not always possible and a certain degree of overlap is frequent for some species with a wide niche range. The distribution and extent of the following habitats is presented in Figure 2.

Exposed sand

Sand beaches are amongst the most common intertidal habitats of Socotra, covering large lengths of coast.

These habitats normally consist of a sand flat with the width depending on degree of exposure, and backed by a dune system (3–4 m high) covered with halophytes (e.g. the North-West and the Net Plains), or a fossil reef platform (e.g. North-East Coast). The lower eulittoral of sandy beach areas is scoured by extreme wave action, sometimes exposing a rock platform substrate or cobbles at the low tide and sublittoral levels (e.g. Net Plain).

The hermit crab *Coenobita scaevola*, which shows a correlation with the presence of coral rubble (r=0.52, N=185, $p<0.0001$), was found scavenging on the supralittoral, and up to 800 m away from the sea. Although more common on sand beaches amongst debris and coral rubble deposited at the base of dunes, this species was equally found on the fossil reef low platforms of the north and west coast, and in sand dunes backing the cobble beaches of the south coast. The ghost crab *Ocypode saratan* has a horizontal range from high tide swash zone to the base of the dunes. The typical male burrow entrance structure (short sand hill) formed wide bands on the supralittoral of sandy beaches running parallel to the coastline. At some sites (Rhiy di Irisal Peninsula) these burrow structures reached densities of 3 m^{-2} and cover large areas. Juvenile *Ocypode saratan* dug burrows further down in the upper eulittoral. Both *Ocypode saratan* and *Coenobita scaevola* are characteristic of the Red Sea/Gulf of Aden regions (Lewinsohn, 1969; Türkay et al., 1996). *Ocypode cordimanus* was found preferentially on the dunes (r=0.86, N=185, $p<0.0001$), amongst halophyte vegetation, sometimes 100's of m away from the sea, and up to 10–16 m above sea level. These 3 species form a very clear community present behind the high tide zone (Cluster 5, Fig. 3, Table 2). On the south coast, a third species of the genus *Ocypode*, *O. ryderi*, was found to coexist at the same site and tidal level with *O. saratan*. The hippid *Emerita holthuisi* was found following the tide level on exposed shores where the waves break. In less exposed sandy shores, the sand crab *Philyra cancella* was commonly found in the lower eulittoral, together with several species of *Diogenes*, including *D. avarus*.

Rock platforms

Rock platforms, together with cobble beaches host the highest diversity of decapod Crustacea. These habitats probably contain the most diverse substrate, structure and exposure range (Table 3) since they include beaches with rock platforms or plateaus that extend from the sublittoral into the supralittoral zone with gentle or moderate slopes. Present all around the island are large rock platform areas, including the north-east coast from Di Lishah to Rhiy di Irisal, the west of the Nojid plain and Rhiy di Shuab, amongst others. The presence of sessile organisms such as algae, barnacles and oysters creates clear zonation bands. Softer rocks, such as limestone, fossil reef and sandstone platforms are generally undercut, forming overhangs of more than 10 m.

Rock pools are also frequent ranging from large pools in the lower eulittoral level, separated from the sea only during low tide, to small shallow ponds which extend the upper distribution limit of littorinid gastropods. Some rock pools are large enough to contain several species of corals (Rhiy di Hamri), while others form deep and perfectly round cylinders such as those in the upper eulittoral of Qalansiyah or at the base of the sheer cliffs near Shuab. Temperature in these pools varied from 26.3 to 32 °C during low tide and salinity ranged from 34 to >140% depending on shore height. Between monsoon seasons, some pools remain cut off from the sea for a long time, resulting in high saline water with red blooms of *Dunaliella salina* (Dunal) Teodoresco, such as observed at Rhiy di Qatanhin.

The supralittoral is dominated by the grapsids *Grapsus albolineatus*, *G. tenuicrustatus* and *Pachygrapsus minutus*. In the eulittoral, *Plagusia tuberculata* can be found close to the water line and the abundant and characteristic menippid *Eriphia smithii* hiding in holes and crevices. The presence of these species was correlated with the presence of solid platforms (r=0.61, 0.53 and 0.45 for *G. albolineatus*, *G. tenuicrustatus* and *E. smithii*, respectively, N=185, $p<0.0001$) and undercut platforms (r=0.42, 0.40 and 0.42 for *G. albolineatus*, *G. tenuicrustatus* and *P. tuberculata*, respectively, N=185, $p<0.0001$). *Clibanarius signatus*, *C. virescens*, *Calcinus laevimanus*, *C. latens* and other Diogenidae are common in shallow rock pools, together with the less frequent alpheids, stomatopods, porcellanids and xanthids such as *Leptodius exaratus*, *L. sanguineus* and *Xanthias sinensis* hiding under stones. A very diverse majid fauna was observed, especially in lower eu- and the shallow sublittoral areas with good macroalgal assemblages.

Cobble beaches

This habitat is formed by loose rocks rounded by wave action. It was one of the most diverse habitats for decapod crustacean species, and shares species commonly found on rocky shores (especially on rock pools). Cobbles were normally semi-buried in sand or

piled upon each other on the top of a rock platform, where, as they tend to accumulate sediment, they form a stable habitat despite strong wave energy. The wave energy generates a gradient of different sizes of stones (shingle, pebbles and cobbles of different sizes reaching 20–50 cm maximum diameter) and algae cover creates a clear zonation pattern. The largest extensions of cobble shores exist on the Nojid plain on the south coast from Mahfirhin to Steroh. Classification of cobble beaches is difficult, since usually cobbles were only dominant on one part of the shore, normally the eulittoral.

The common species mainly found under stones include the diogenids *Clibanarius signatus* and *C. virescens* along with several species of the genus *Calcinus*, porcellanids (mainly *Petrolisthes* spp.), *Grapsus granulosus*, and *Pachygrapsus minutus*, the characteristic goneplacid *Pseudozius caystrus*, the xanthids *Leptodius exaratus*, *L. sanguineus*, *Xanthias sinensis*, the menippid *Eriphia smithii*, and several other species of alpheids, xanthids and pilumnids. Although present on rock platforms under loose stones and in rock pools, most of these species are more abundant on cobble beaches. This community is clearly represented in cluster 1 (Fig. 3, Table 2).

Sheltered and shallow sand beaches

The unique characteristics of Qalansiyah lagoon on the north-western tip of the island separate this area as a distinct category on its own. These characteristics include a permanent and marked intertidal regime, which creates a fully marine habitat. Protected by a narrow sand bar, this shallow soft bottom habitat is extremely sheltered with all zonation height bands compacted within 1 m. The lagoon is composed mainly of sand, although a large area of fine sand/mud on rock with patches of seagrass in the lower eu- and shallow sublittoral occurs close to the eastern shore and mouth of the lagoon. On the supralittoral, extensive sand flats host large and dense populations (450 ± 163.8 burrows m^{-2}) of fiddler and bubbler crabs *Uca inversa*, *U. lactea albimana* and *Dotilla sulcata*, together with the ghost crab *Ocypode saratan*. Portunids such as *Thalamita crenata* and *T. stephensoni* are common in shallow water. In the sand/mud, large numbers of hermit crabs (*Calcinus laevimanus*, *C. latens*, *Clibanarius virescens* and *C. signatus*) were found clustering under infrequent boulders. The porcellanids *Petrolisthes leptocheles* and *P. ornatus* were found under stones together with other less frequent xanthids such as, *Xanthias sinensis*, menippids such as *Eriphia smithii* and unidentified alpheids. The decapod community found in this particular area is described by cluster 2 (Fig. 3, Table 2). However, it also combines species characteristic from other habitats, such as cobble/rock shores, mud flats and exposed sand shores (clusters 1, 5 and 3, respectively, Fig. 3, Table 2).

Sheer rock cliffs

Vertical rock cliffs were classified as a separate habitat due to their high degree of exposure, lack of rock pools, reduced available substrate area, increased shading and differences in the structure of sessile communities which are represented by a high density of barnacles, oysters and incrusting algae.

The cliffs that drop vertically into the sea are usually of limestone, fossil reef, sandstone or conglomerate. At sea level, they are eroded by wave action to create an overhang, but may continue vertically underwater to a sand bottom at 3–12 m depth, or break into large boulders at low tide or subtidally. Rock cliffs are found on long stretches of coast principally between Qalansiyah and Shuab and the Net plain and Rhiy di Qatanhin (Fig. 1), in regions of extreme exposure, where the supralittoral zone extends up to 7 m above sea level.

The characteristic decapod community (closer to cluster 6, Fig. 3, Table 2) found on rock cliffs includes *Grapsus albolineatus* and *G. tenuicrustatus* on the upper and supralittoral and *Plagusia tuberculata* close to the water line. The small *Pachygrapsus minutus* and other small and less common crabs were found in crevices and amongst the large barnacle *Megabalanus* sp. (*tintinnabulum*?) (L.) and *Saccostrea cucullata* (Born) oyster bands. As for other rock platforms, *Percnon planissimum* was found associated with sublittoral boulders. Families such as Alpheidae, Diogenidae, Porcellanidae, Xanthidae, Majidae and some isolated species common on rock platforms were significantly less represented at these sites.

Mud flats

Mud flats are closely associated with Wadis (rivers and streams) and Khawrs (coastal lagoons). Formed mainly of fringing banks of mud or fine sand with mud, some areas have daily tidal influence, while others are separated from the sea for several months during the dry season (January–April). Salinity varied markedly from 0 to >140%, making almost every coastal lagoon or pond different. Sometimes, salinity in lagoons separated by no more than 2–3 km ranged

from 9 to 36%. Within Khawr Qaryih, for example, salinity ranges from 0% in a wadi feeding the Khawr, to 16% close to the sea and 21% in the inland end of the Khawr. Seasonal variations in salinity and water level inside the Khawrs are also clear. For example between November 1998 and February/March 1999, the salinity in one particular site at Qaryih Khawr oscillated from 10 to 28% and the water level dropped 40 cm exposing a vast mud flat, otherwise flooded. Although mud flats are not frequent around Socotra, this habitat can encompass large areas, such as Qaryih, Girmah, Shuab and Dubna Khawrs.

As fishing pressure is low, mud flats bordering wadis and coastal lagoons hosted small, undisturbed populations of *Scylla serrata* and *Fenneropenaeus indicus*. Furthermore, a yet undescribed species of *Podocallichirus* Sakai, 1999 (Callianassidae), the gecarcinid crab *Cardisoma carnifex*, the grapsids *Metopograpsus messor* and *M. thukuhar*, and the ocypodids *Uca inversa*, and at places with more sandy sediments *Uca annulipes albimana* and *Dotilla sulcata,* were the most common decapods at this habitat. However, the ocypodid fauna is surprisingly poor and only one species of the Sesarminae (*Helice leachii*) was observed during the whole survey. The decapod community found in mud flats was represented primarily by cluster 5 (mud) and secondly by cluster 2 (fine sand and mud, Fig. 3, Table 2). Some species such as *Uca inversa*, *Uca annulipes albimana*, *Cardisoma carnifex*, *Metopograpsus messor*, *M. thukuhar* and *Scylla serrata*, are correlated with the presence of mud at the surveyed sites (r=0.84, 0.55, 0.76, 0.76, 0.52 and 0.68, respectively, N=185, p<0.0001). Almost all characteristic species were positively correlated to a low degree of exposure, and to low salinity levels.

Mangroves

A reduced number of trees and areas of mangrove represent this habitat, most of which are already destroyed or under severe human and environmental pressure. The largest and most representative stand is located behind the dune belt at the Net plain. Other smaller fringing mangrove areas mainly around Girmah and Shuab Khawrs, and East of Rhiy di Qadamah are heavily impacted with few remaining trees.

Socotra mangrove areas are monospecific stands of *Avicennia marina* (Forskål) Vierh and almost fully terrestrial habitats. The position of the mangrove is very different from the typical mangrove shore profile facing the open sea. On Socotra, there is no seafringe and the landward fringe is either a salt flat (Sabkha) sparsely vegetated with halophytes, a fossil reef cliff or a sandbar which separates the mangrove from the sea. Biodiversity of decapod Crustacea in these mangroves is extremely low. The penaeid *Fenneropenaeus indicus* was found on the shallow water of Girmah mangrove. The semi-terrestrial gecarcinid *Cardisoma carnifex* was common on the inland edge of most mangrove areas together with the less frequent *Metopograpsus thukuhar*. The fiddler crab *Uca inversa* was common on mud flats bordering mangrove areas. This habitat is closely related to the crab community represented by cluster 5 (Fig. 3, Table 2).

Salt flats (Sabkhas)

Alternating periods of drought and flood and salinity levels frequently reaching above 140% make these environments extremely species poor. When water is not present, the soil retains a salt crust. Apart from the microalgae *Dunaliella salina* in hypersaline ponds and *Ocypode cordimanus* which occasionally burrows on the seaside limits of the sabkhas, no other species were found in these semi-terrestrial habitats. The largest areas occur in the Net and Nojid plains behind the dunes and/or mangrove, where artisanal salt ponds are exploited.

Discussion

Species list

The present survey revealed 112 species of intertidal decapod Crustacea excluding caridean shrimps (mainly Alpheidae) which have not been identified so far. Previous lists from MacAlister et al. (1996) and Wranik (1998) reported 48 and 11 species, respectively, which give a total of 130 intertidal decapod species presently recorded for Socotra Island. There are 25 species reported by MacAlister et al. (1996), which were not observed in the present survey. Nine of these species belong to the family Alpheidae of which many yet unidentified specimens were collected in the present survey. Eleven recorded species are only identified to the genus level. All but one species reported by Wranik (1998) were found in the present survey.

The families Ocypodidae and Grapsidae are quite underrepresented in Socotra (8 and 15 species, respectively) when compared to the nearby coast of Somalia, where 19 species of ocypodids and 28 of grapsids were identified (Vannini & Valmori, 1981a, b). This large difference might be explained by the

reduced area and unusual characteristics of the Socotran mangroves, as most of the lacking species are associated with this habitat.

It must, however, be noted that it was sometimes difficult to classify certain specimens as 'intertidal', especially for many Xanthidae and Majidae collected in very shallow water and tide pools. These are here recorded as 'intertidal', even though their habitat is not necessarily between the tide marks.

Intertidal habitats

Comparing the different habitats, it appears that sand beaches tend to be species poor, but share a very specific decapod community with mud flats. Although diversity is low for these habitats, they normally host a high abundance of decapods. Rock platforms, on the other hand, have a high decapod diversity as they form the most diverse habitat. However, they do not have a very specific decapod community. Except for the common Grapsidae and Diogenidae, rock platforms in general do not have a large abundance of decapods. The highest number of species and also the highest average of species per site recorded during rapid surveys was observed for cobble beaches. This supports the general trend of rich species composition for cobble beaches, which is further enhanced when cobbles are present in combination with sand or rock. Many decapods found on cobble beaches were also found on rock platforms and cliffs, making the definition of a characteristic decapod community for these habitats difficult.

Socotra mangrove are found at only a few sites and the smaller fringing mangrove areas around Girmah and Shuab Khawrs, and East of Rhiy di Qadamah (Fig. 2) and are heavily impacted (MacAlister et al., 1996; Simões & Jones, 1999). The unusual structure of the Net plain mangrove (Fig. 2), protected behind a dune system and completely separated from the sea has also been found in Oman (Hywell-Davies, 1994).

Oceanography and zoogeography

The main effect of reversal in the flow of the Somali Current that affects Socotra is that it creates two distinct upwelling systems: one in the Arabian Sea and the other along the Somali coast. The upwelling process along the Indian Ocean coast of Somalia exchanges cold (13 °C), nutrient-rich deep waters, with the warmer (20–29 °C) coastal surface waters causing distinct changes in productivity (Bryceson et al., 1990). These cyclical events are reported to be short lived and are well documented by Bottero (1969), Currie et al. (1973) and Barrat et al. (1984, 1986).

It is generally thought that both upwelling systems probably have some impact on the marine environment of the Socotran Archipelago (Saeed, 1998). Kemp (1998b) reported seasonal cycles on the production of dense mats of macroalgae *Nizamuddinia zanardinii* (Schiffner) P.C. Silva on the South coast of Socotra that occur at the same time as the Somali coast upwelling. At the time of the survey, these mats just started growing on the lower intertidal rock platforms of the South coast (F. Leliaert, pers. com.). Large and extensive coral beds are only found at the north coasts of Socotra, Darsah, Samha and Abd al-Kuri (Kemp, 1997, Turner et al., 1999), sheltered from the cold water of the Somali current. The present survey observed clear indications of a cyclical colonisation by *Chaetomorpha* sp. of the high supralittoral rocky shores of the Socotra South coast, possibly following the pattern of strong wave action and rich nutrient water of the south-west monsoon. These results further confirm that Socotra is influenced seasonally by the Somali Current and upwelling system, and that these effects are better observed on the more directly exposed South coasts than the north coasts. However, it should be noted that seasonal influences, although discussed here, could not be checked in loco. Consequences of these events on the larval distribution and settlement of decapod species present in Socotra are nevertheless obvious.

Zoogeographically it appears that the intertidal decapod assemblages are more influenced by Red Sea/Gulf of Aden elements than by typical East African ones, which however also contribute to the Socotran fauna. Examples of species that have been recorded at Socotra and are otherwise restricted to the Seas around the Arabian peninsula, but do not occur south of Cap Hafun on the Somali coast are *Clibanarius signatus, Coenobita scaevola, Menaethiops contiguicornis, Menaethiops nodulosa, Stilbognathus erythraeus*, *Heterocrypta petrosa*, *Lydia tenax, Grapsus granulosus*, *Ocypode saratan* and *Uca annulipes albimana*. On the other hand, species known from the East African coast, but so far not from the Gulf of Aden and the Red Sea, are *Clibanarius eurysternus*, *Thalamita stephensoni*, *Percnon guinotae* and *Ocypode ryderi*. Most of the other species found in the intertidal zone of Socotra either have a wide Indian Ocean, Indo-West or even Indo-Pacific distribution or there are taxonomic problems hindering an exact analysis of the distribution of the taxa in question.

It appears that the intertidal decapod fauna of Socotra consists of species from different zoogeographical regions, but is most closely related to that of the Gulf of Aden and the Arabian region. This agrees with observations on the coral fish assemblages of the Socotra archipelago. These are predominantly south Arabian, but while an East African influence is minimal on the Arabian mainland coasts it is evident on Socotra, resulting in previously unrecorded sympatry between Arabian endemic species and their Indian Ocean sister taxa (Kemp, 1998a). This is also the case at least for *Ocypode saratan* and *O. ryderi*, which have for the first time been recorded sympatrically at one site on the South coast.

The species diversity of some groups like ocypodid and sesarmine crabs obviously is lower in comparison to neighbouring regions such as Somalia (Vannini & Valmori, 1981a, b). This may be due to a lack of suitable habitats and to the extreme environmental conditions caused by the upwelling and strong seasonal changes in water temperature around the islands.

This survey confirms Chiffing's (1995) assessment that Socotra has a high conservation priority. The intertidal habitats of the Island are highly varied and are currently in almost pristine condition, except for mangroves. The intertidal habitats of the north coast of Socotra, protected in the lee of the island from the strong effects of the south-west monsoon upwelling, may be isolated habitat islands of possibly great regional significance, extending some decapod species distribution range to their southernmost limit. The same is true for species from East Africa and the Red Sea. For these species, the populations of the Socotra Archipelago may play a significant role in maintaining gene flow between the Red Sea and the Indian Ocean.

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The crab species found on the coasts of Gökçeada (Imbroz) Island in the Aegean Sea

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Key words: Brachyura, Gökçeada (Imbroz) Island, Aegean Sea

Abstract

In this study, 32 crab species belonging to 13 families are recorded from Gökçeada Island, based on bottom material collected at 39 stations around the island at depths between 0 and 70 m, using dredges, drift nets and scoop nets. Ecological properties of the species are provided.

Introduction

The Island Gökçeada (25° 39′ 57″ E and 26° 01′ 00″ E – 40° 05′ 45″ N and 40° 14′ 45″ N) is the largest island of Turkey with an area of 285.5 km^2, it is situated between the Gallipoli Peninsula, Lemnos Island and Samothrace Island.

In the Aegean Sea are three different masses of water, namely surface water, an intermediate layer and deep water. The northern surface waters in the Aegean Sea, where Gökçeada is located, are under the effect of the Black Sea waters which display the characteristics of brackish water (Yüce, 1995).

As far as we know, no scientific investigations on the crab species of the north-eastern part of the Aegean Sea including Gökçeada Island in the Turkish territorial waters have been carried out so far.

The first records of the crab species, *Eriphia verrucosa* and *Pinnotheres pinnotheres*, in the Turkish territorial waters of the Aegean Sea in the Izmir region were by Forskål (1775). Colombo (1885) reported *Pilumnus hirtellus* and *Macropodia rostrata* from the same region. Tortonese (1947) mentioned three crab species from the Aegean Sea. Mater & Kocataş (1967) recorded six crab species, Geldiay & Kocataş (1968) two species, and Kocataş (1971) 41 species in the Izmir Bay and its environs. Kocataş (1981) listed 61 crab species in the Turkish territorial waters, and Koukouras et al. (1992) 99 species in the Greek territorial waters of the Aegean Sea.

The purpose of this study is to determine the crab species living along the coasts of Gökçeada Island and to investigate some of their ecological properties.

Materials and methods

Specimens examined in this study were collected at 39 stations (Fig. 1, Table 1) between 1997 and 1999, from depths of 0 to 70 m, using dredges, drift nets and scoop nets. These specimens were preserved in 4% formaldehyde in sea water.

The following ecological parameters were measured: temperature, by reversing thermometer on the Nansen bottle; salinity, by Mohr-Knudsen method (Ivanoff, 1972); and dissolved oxygen by the Winkler method (Winkler, 1888).

The identifications of the specimens was carried out with the help of Bouvier (1940), Zariquiey Alvarez (1968), Demir (1952), Holthuis (1987) and Ingle (1980, 1983).

Results

A total of our 32 crab species belonging to 13 families were identified and are listed below. Data on maximum carapace length and width, minimum and maximum values of depth, temperature, salinity and dissolved oxygen, as well as bottom structure and the

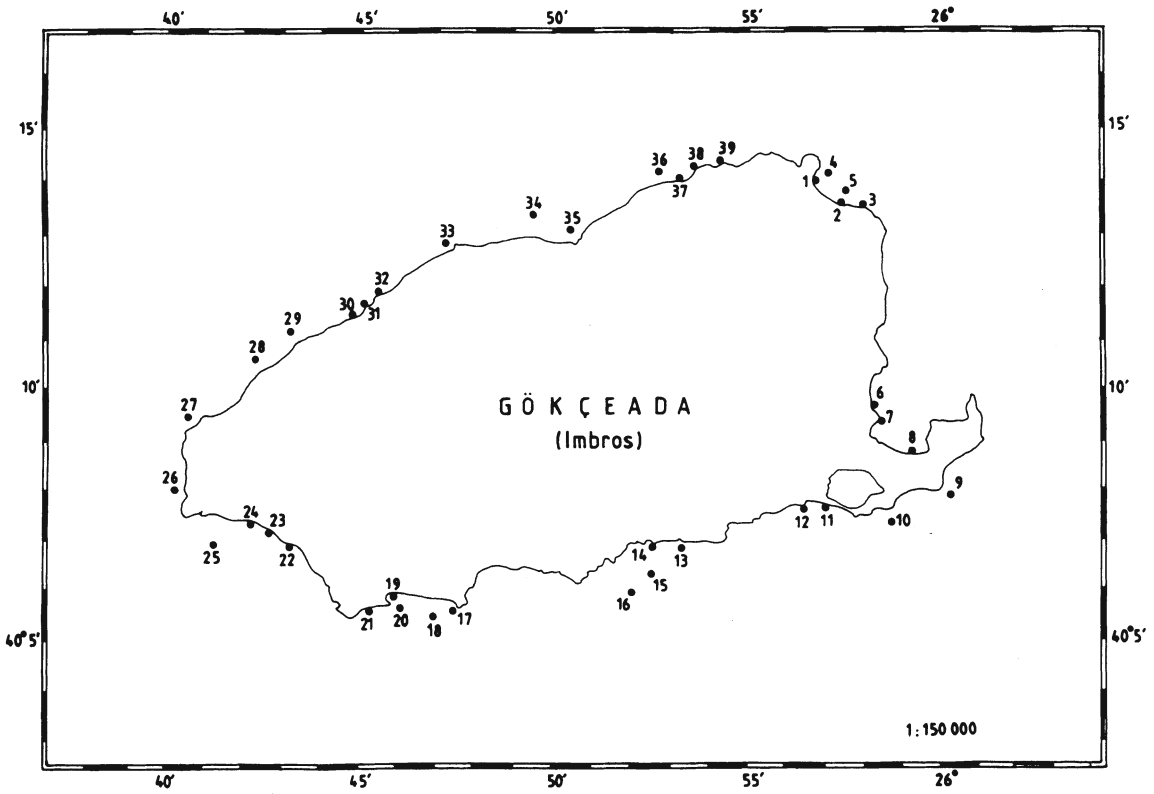

Figure 1. Map of Gökçeada (Imbroz) Island with the sampling stations indicated.

sampling stations on which the species was found, are provided. Nomenclature of the species follows d'Udekem d'Acoz (1999).

Family DROMIIDAE De Haan, 1833
Dromia personata (Linnaeus, 1758). Maximum length of carapace 3.3 cm, width 4.0 cm; ecological properties: 5–9 m, 22.0–23.4 °C, 32.8–33.4 ‰, 4.6–5.9 mg/l; rock bottom; stations: 3, 24, 33.

Family DORIPPIDAE De Haan, 1841
Ethusa mascarone (Herbst, 1785). Maximum length of carapace 1.1 cm, width 0.9 cm; ecological properties: 3–15 m, 22.0–22.8 °C, 33.4–34.2 ‰, 4.9–6.9 mg/l; sand bottom; stations: 9, 20, 36.
Medorippe lanata (Linnaeus, 1767). Maximum length of carapace 2.0 cm, width 2.4 cm; ecological properties: 13–70 m, 16.5–23.0 °C, 34.4–38.0 ‰, 5.6–6.8 mg/l; sand and mud bottom; stations: 25, 35.

Family CALAPPIDAE De Haan, 1833
Calappa granulata (Linnaeus, 1758). Single specimen, length 4.9 cm, width 5.2 cm; ecological properties: 9 m, 22.0 °C, 33.6 ‰, 4.9 mg/l; mud bottom; station: 29.

Family LEUCOSIIDAE Samouelle, 1819
Ilia nucleus (Linnaeus, 1758). Single specimen, length 2.5 cm, width 2.4 cm; ecological properties: 9 m, 22.0 °C, 33.6 ‰, 4.9 mg/l; mud bottom; station: 29.

Family PORTUNIDAE Rafinesque, 1815
Polybius arcuatus (Leach, 1814). Maximum length of carapace 2.7 cm, width 3.4 cm; ecological properties: 4–5 m, 22.0 °C, 33.4 ‰, 3.0–4.7 mg/l; sand and mud bottom; stations: 5, 10, 13.
Polybius (Necora) corrugatus (Pennant, 1777). Maximum length of carapace 3.3 cm, width 4.0 cm; ecological properties: 10–25 m, 21.0–22.0 °C, 33.4–34.1 ‰, 2.9–4.9 mg/l; sand and mud bottom; stations: 4, 28.
Polybius (Polybius) depurator (Linnaeus, 1758). Maximum length of carapace 4.0 cm, width 5.2 cm; ecological properties: 30–70 m, 16.5–19.4 °C, 36.8–38.0 ‰, 4.2–5.6 mg/l; sand and mud bottom; stations: 25, 27, 34.
Carcinus aestuarii Nardo, 1847. Maximum length of

Table 1. Physical characteristics of the sampled stations. DN: Drift net DR: Dredge SN: Scoop Net

Station number	Date	Tool	Depth m	Temperature °C	Salinity ‰	Dissolved oxygen mg/l	Bottom structure
1	26.07.97	SN	0.5	26.0	27.0	2.8	Rock
2	11.06.98	SN	0.5	24.5	29.7	8.3	Rock
3	07.08.99	DN	3	23.4	32.8	4.7	Rock
4	19.09.98	DR	10	22.0	33.4	2.9	Mud
5	19.09.98	DR	5	22.0	33.4	3.0	Mud
6	08.08.99	SN	0.5	24.6	32.2	4.8	Rock
7	09.06.98	SN	0.5	25.0	27.1	5.0	Rock
8	09.06.98	SN	0.5	24.5	29.5	4.5	Sand
9	17.09.98	DR	3	22.0	33.4	4.9	Sand
10	17.09.98	DR	4	22.0	33.4	4.7	Sand
11	28.07.97	SN	0.5	24.8	28.7	5.0	Rock
12	07.08.99	SN	0.5	24.5	32.2	4.9	Rock
13	17.09.98	DR	4	22.0	33.4	4.7	Sand
14	09.06.98	SN	0.5	24.0	29.8	8.5	Rock
15	17.09.98	DR	30	19.0	37.3	4.3	Mud
16	17.09.98	DR	35	22.0	33.5	4.2	Mud
17	17.09.98	DR	3	22.0	33.4	5.2	Sand
18	17.09.98	DR	15	22.0	33.4	4.7	Sand
19	26.07.97	SN	0.5	25.4	27.2	5.4	Rock
20	17.09.98	DR	3	22.0	33.4	4.9	Sand
21	26.07.97	DN	3	24.4	27.4	5.3	Rock
22	10.06.98	SN	0.5	24.8	30.0	7.0	Rock
23	27.07.97	SN	0.5	24.0	27.7	5.3	Rock
24	08.08.99	DN	5	23.2	33.0	4.6	Rock
25	18.09.98	DR	70	16.5	38.0	5.6	Mud
26	18.09.98	DR	30	19.2	36.9	4.2	Sand
27	18.09.98	DR	30	19.4	36.8	4.2	Sand
28	18.09.98	DR	25	21.0	34.1	4.9	Sand
29	18.09.98	DR	9	22.0	33.6	4.9	Mud
30	27.07.97	SN	0.5	24.3	27.3	5.0	Rock
31	10.06.98	SN	0.5	24.5	27.5	7.8	Rock
32	10.06.98	DN	5	23.3	27.8	7.6	Rock
33	18.09.98	DR	9	22.0	33.4	5.9	Rock
34	16.09.98	DR	55	17.0	37.2	5.4	Sand
35	16.09.98	DR	13	23.0	34.4	6.8	Sand
36	16.09.98	DR	15	22.8	34.2	6.9	Sand
37	27.07.97	SN	0.5	25.2	27.0	5.2	Rock
38	11.06.98	SN	0.5	25.0	28.9	8.6	Rock
39	11.06.98	SN	0.5	24.3	29.9	8.4	Rock

carapace 5.1 cm, width 6.5 cm; ecological properties: 0.5–5 m, 23.2–24.8 °C, 27.4–33.0 ‰, 4.6–7.6 mg/l; rock bottom; stations: 3, 11, 12, 21, 24, 32.

Family ATELECYCLIDAE Ortmann, 1893

Atelecyclus rotundatus (Olivi, 1792). Single specimen, length 1.3 cm, width 1.3 cm; ecological properties: 70 m, 16.5 °C, 38.0 ‰, 5.6 mg/l; mud bottom; station: 25.

Family PIRIMELIDAE Alcock, 1899

Pirimela denticulata (Montagu, 1808). Single speci-

men, length 0.9 cm, width 1.0 cm; ecological properties: 0.5 m, 24.8 °C, 28.7 ‰, 5.0 mg/l; rock bottom; station: 11.

Family XANTHIDAE Mac Leay, 1838
Eriphia verrucosa (Forskål, 1775). Maximum length of carapace 6.3 cm, width 9.1 cm; ecological properties: 0.5–5 m, 23.3–24.8 °C, 27.4–32.8 ‰, 4.7–7.6 mg/l; rock bottom; stations: 3, 11, 12, 21, 32.
Xantho pilipes A. Milne-Edwards, 1867. Single specimen, length 1.8 cm, width 2.5 cm; ecological properties: 10 m, 22.0 °C, 33.4 ‰, 2.9 mg/l; mud bottom; station: 4.
Xantho poressa (Olivi, 1792). Maximum length of carapace 2.7 cm, width 4.3 cm; ecological properties: 0.5 m, 24.3–25.2 °C, 27.0–32.2 ‰, 4.9–8.3 mg/l; rock bottom; stations: 2, 11, 12, 30, 37.

Family PILUMNIDAE Samouelle, 1819
Pilumnus hirtellus (Linnaeus, 1761). Maximum length of carapace 1.4 cm, width 2.1 cm; ecological properties: 0.5 m, 24.3–26.0 °C, 27.0–30.0 ‰, 2.8–8.4 mg/l; rock bottom; stations:1, 7, 22, 39.

Family PINNOTHERIDAE De Haan, 1833
Nepinnotheres pinnotheres (Linnaeus, 1758). Single specimen, length 1.4 cm, width 1.5 cm; ecological properties: 9 m, 22.0 °C, 33.6 ‰, 4.9 mg/l; mud bottom; station: 29.

Family GRAPSIDAE Mac Leay, 1838
Pachygrapsus marmoratus (Fabricius, 1787). Maximum length of carapace 2.1 cm, width 2.5 cm; ecological properties: 0.5–5 m, 23.2–26.0 °C, 27.0–33.0 ‰, 2.8–8.6 mg/l; rock bottom; stations: 1, 2, 3, 6, 7, 11, 12, 14, 19, 21, 22, 23, 24, 30, 31,32, 37, 38, 39.
Brachynotus sexdentatus (Risso, 1827). Single specimen, length 0.9 cm, width 1.1 cm; ecological properties: 4 m, 22.0 °C, 33.4 ‰, 4.7 mg/L; sand bottom; station: 10.

Family MAJIDAE Samouelle, 1819
Inachus communissimus Rizza, 1839. Maximum length of carapace 0.7 cm, width 0.8 cm; ecological properties: 9–10 m, 22.0 °C, 33.4–33.6 ‰, 2.9–4.9 mg/l; mud bottom; stations: 4, 29.
Inachus dorsettensis (Pennant, 1777). Maximum length of carapace 2.0 cm, width 1.7 cm; ecological properties: 25–30 m, 19.0–21.0 °C, 34.1–37.3 ‰, 4.3–4.9 mg/l; sand and mud bottom; stations: 15, 28.
Macropodia linaresi Forest & Zariquiey Alvarez, 1964. Maximum length of carapace 0.8 cm, width 0.5 cm; ecological properties: 9–25 m, 21.0–22.8 °C, 33.4–34.2 ‰, 4.9–6.9 mg/l; sand and rock bottom; stations: 28, 33, 36.
Macropodia tenuirostris (Leach, 1814). Single specimen, length 2.0 cm, width 0.8 cm; ecological properties: 10 m, 22.0 °C, 33.4 ‰, 2.9 mg/l; mud bottom; station: 4.
Macropodia longirostris (Fabricius, 1775). Single sample, length 2.5 cm, width 1.3 cm; ecological properties: 25 m, 21.0 °C, 34.1 ‰, 4.9 mg/l; sand bottom; station: 28.
Macropodia rostrata (Linnaeus, 1761). Maximum length of carapace 1.8 cm, width 1.0 cm; ecological properties: 9–25 m, 21.0–22.8 °C, 33.4–34.2 ‰, 2.9–6.9 mg/l; sand, mud and rock bottom; stations: 4, 28, 33, 36.
Eurynome aspera (Pennant, 1777). Single specimen, length 0.8 cm, width 1.0 cm; ecological properties: 35 m, 22.0 °C, 33.5 ‰, 4.2 mg/l; mud bottom; station:16.
Pisa armata (Latreille, 1803). Maximum length of carapace 2.9 cm, width 1.9 cm; ecological properties: 0.5–3 m, 23.4–25.0 °C, 27.1–32.8 ‰, 4.7–5.3 mg/l; rock bottom; stations: 3, 7, 21.
Pisa nodipes (Leach, 1815). Maximum length of carapace 5.2 cm, width 3.4 cm; ecological properties: 0.5 m, 24.5–24.6 °C, 29.5–32.2 ‰, 4.5–4.8 mg/l; rock and sand bottom; stations: 6, 8.
Pisa tetraodon (Pennant, 1777). Maximum length of carapace 2.7 cm, width 2.0 cm; ecological properties: 0.5 m, 24.5–25.2 °C, 27.0–32.2 ‰, 4.9–5.2 mg/l; rock bottom; stations: 7, 12, 37.
Maja crispata Risso, 1827. Maximum length of carapace 5.7 cm, width 4.7 cm; ecological properties: 0.5–3 m, 22.0–25.2 °C, 27.0–33.4 ‰, 4.7–8.6 mg/l; rock and sand bottom; stations: 3, 11, 12, 17, 37, 38.
Maja squinado (Herbst, 1788). Maximum length of carapace 9.2 cm, width 6.9 cm; ecological properties: 15–30 m, 19.2–22.0 °C, 33.4–36.9 ‰, 4.2–4.7 mg/l; sand bottom; stations: 18, 26, 27.

Family PARTHENOPIDAE Macleay, 1838
Parthenope angulifrons Latreille, 1825. Single specimen, length 2.4 cm, width 2.3 cm; ecological properties: 15 m, 22.8 °C, 34.2 ‰, 6.9 mg/l; sand bottom; station: 36.
Parthenope massena (P. Roux, 1830). Single specimen, length 0.5 cm, width 0.5 cm; ecological properties: 15 m, 22.8 °C, 34.2 ‰, 6.9 mg/l; sand bottom; station: 36.

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Evidence of paraphyly in the neotropical Porcellanid genus *Neopisosoma* (Crustacea: Anomura: Porcellanidae) based on molecular characters

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Key words: Porcellanid crabs, *Neopisosoma*, *Pachycheles*, comparative morphology, molecular systematics, mitochondrial DNA

Abstract

Molecular data were used to evaluate the validity of the genus *Neopisosoma* Haig, 1960. Comparisons of morphological features within *Neopisosoma* suggest the existence of two lineages, represented among others, by *N. angustifrons* (Benedict, 1901) and *N. neglectum* Werding (1986). Both lineages of *Neopisosoma* are more similar to two morphologically different species groups of the genus *Pachycheles*, than to congeners of the other group. Comparative morphology of larvae from *N. angustifrons*, *N. neglectum* and species of *Pachycheles* shows that *N. angustifrons* closely resembles *Pachycheles* species, whilst *N. neglectum* is set apart. Sequences of a 465 bp segment of the mitochondrial gene cytochrome oxidase I (COI) were obtained and used to infer phylogenetic relationships among *N. angustifrons*, *N. neglectum*, one species of *Pachycheles* and seven other species of porcellanids, representing three other genera. Results of the molecular analysis were congruent to results of comparative morphological studies of larvae: *N. angustifrons* grouped with the *Pachycheles* species, whereas *N. neglectum* is placed apart. This led us to the conclusion that the genus *Neopisosoma* is probably paraphyletic and that the criterion used by Haig (1960) is not reliable to define the genus. A revision on a world-wide basis of the genera included, and additional sequence information will be necessary to satisfactorily resolve relationships within the Porcellanidae.

Introduction

Porcellanids are small, crab-like anomurans, typically littoral or sublittoral, distributed throughout all tropical faunal regions and in a lesser degree in the neighbouring temperate regions (Haig, 1960). Roughly 90 species belonging to 12 genera are found on the American Pacific coasts and approximately 40 species, grouped in 10 genera, on the Atlantic American coasts (Werding, 1992).

Species of the genus *Neopisosoma* Haig (1960) occur along tropical coasts on both sides of the Americas, with 3 species in the Pacific and 4 species in the Atlantic. They are predominantly found in the wash zone of rocky shores.

The genus *Neopisosoma* was established by Haig (1960) to separate from the genus *Pachycheles* Stimpson, all species which differ in the structure of the lateral walls. The lateral walls in *Pachycheles* species consist of a large anterior piece and a posterior portion composed of one or more fragments separated by membranous interspaces (Fig. 1a–c). In all *Neopisosoma* species, the posterior portion is occupied only by a membrane (Fig. 1d). Haig (1960) herself questioned the status of the new genus *Neopisosoma*, suggesting that it might prove to be a subgenus of *Pachycheles*. Werding (1986) questioned the validity of Haig's criterion to separate the two genera, since form and number of epimeral fragments behind the frontal piece vary widely among different *Pachycheles* species. For example, in *P. susanae* Gore & Abele there is only one large piece (Fig. 1a), in *P. riisei* (Stimpson) one large plate appears surrounded by smaller fragments (Fig. 1b) and in *P. rugimanus* A.

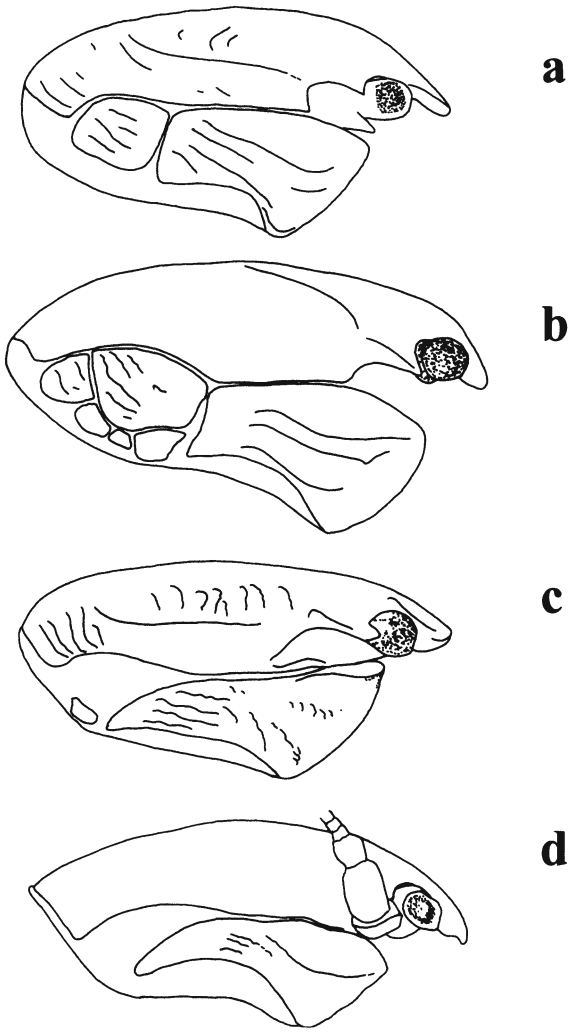

Figure 1. Variation in form and number of epimeral fragments behind the frontal piece in different *Neopisosoma* and *Pachycheles* species: (a) *P. susanae*, (b) *P. riisei*, (c) *P. rugimanus*, (d) *N. neglectum*.

Milne Edwards only a very small plate occurs over a large membranous portion (Fig. 1c). Comparisons of other morphological features within *Neopisosoma* suggest the existence of two different morphotypes that are equally represented in the eastern Pacific and in the Caribbean Sea. Both morphologically coherent groups are represented on the Caribbean coast, among others, by *Neopisosoma angustifrons* (Benedict, 1901) and *N. neglectum* Werding (1986).

Both lines of *Neopisosoma* species show a marked affinity with two morphologically different groups of *Pachycheles,* which are more similar (and subsequently more related?) among themselves than to their congeners of the other assemblage. Table 1 shows the two groups formed by members of both genera and the morphological features that characterize each assemblage.

Larval features of *N. angustifrons*, like the number of spinules in the antennal exopod and the arrangement of the hook-like spines in the tail fan setae in zoea I (Table 2), resemble those of several *Pachycheles* species (Gore, 1977; Konishi, 1987), and are different from larval features of *N. neglectum* (Werding & Müller, 1990).

Molecular data have contributed most significantly in areas where morphological data are inconclusive, deficient, non-existent or poorly analysed (Patterson et al., 1993). Mitochondrial DNA (mtDNA) has shown to be a useful marker to estimate phylogenetic relationships of animals at different taxonomic levels. The attraction of mtDNA derives, in part, from the relative ease with which homologous sequences can be isolated, aligned and analysed (Harrison, 1989). The mitochondrial gene cytochrome oxidase I (COI) has been used to infer phylogenetic relationships of some crustaceans belonging to the families Penaeidae (Palumbi & Benzi, 1991), Alpheidae (Knowlton et al., 1993), Hippidae (Tam et al., 1996), Gammaridae (Meyran et al., 1997) and Palinuridae (Sarver et al., 1998).

We present results of a preliminary molecular phylogenetic study using partial sequences of the COI gene from 9 neotropical species of porcellanids, representing 4 genera, including *Neopisosoma* and *Pachycheles*. One European species, from a fifth genus, was included in the study, from which specimens of two biogeographically separated populations were sampled to have estimates of sequence divergence at the population level.

Materials and methods

Sampling

Caribbean species were collected in shallow water on the coast of Colombia, near Santa Marta. Specimens of the European species, *Porcellana platycheles*, were sampled on the French Atlantic coast, near Saint Maló, and on the Spanish Mediterranean coast, Costa Brava. They are designated along this paper as *P. platycheles* I and II, respectively. Specimens collected for this study and their respective substrates and locations are listed in Table 3. Specimens were identified using morphological traits and stored in a buffer consisting of 10%

Table 1. Groups of *Neopisosoma* and *Pachycheles* species represented by *N. angustifrons* and *N. neglectum*, and morphological features that characterize each group

angustifrons - Group	*N. neglectum* - Group
N. angustifrons (Benedict) *N. dohenyi* Haig *P. cristobalensis* Gore *P. greelei* (Rathbun) *P. serratus* (Benedict) *P. chacei* Haig *P. calculosus* Haig *P. setimanus* Lockington	*N. neglectum* Werding *N. orientale* Werding *N. curacaoense* (Schmitt) *N. bicapillatum* Haig *N. mexicanum* (Streets) *P. susanae* Gore & Abele *P. vicarius* Nobili
• Carapace rounded	• Carapace subquadrate
• Front rounded or only slightly trilobate	• Front trilobate with pronounced frontal and lateral lobes
• Chelipeds extremely different sized, very robust with granulated surface	• Chelipeds less different sized, less robust with smooth surface
• Carpus short, no longer than broad with irregular denticulation on inner border	• Carpus distinctly larger than broad, with regular triangular teeth on inner border, decreasing in size from proximal to distal end
• Surface of carpus without crests and grooves	• Surface of carpus with strong longitudinal crests defined by grooves

Table 2. Comparison of some larval features of some *Pachycheles* species, *N. angustifrons* and *N. neglectum*

Pachycheles (7 spec.) - *N. angustifrons*	*N. neglectum*
Zoea I antennal exopod	
3–4 spinules	1 subterminal spinule
Zoea I tail fan setae	
hook-like spines on both exterior setae in two opposed files	hook-like spines on inner side of all setae

EDTA, 0.5% NaF, 0.5% Thymol and 1% Tris–HCl. In the laboratory, crabs were stored at −20 °C and only thawn once for DNA extraction.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the chelipeds of each porcellain crab. Removed musculature was minced in a CTAB isolation buffer (Doyle & Doyle, 1989) at 60 °C, incubated for 30 min and purified once by chloroform-isoamyl alcohol (24:1; v:v) extraction. DNA was precipitated by the addition of 0.6 vol of cold isopropanol and pelleted. The pellet was washed with 70% ethanol, dried at 37 °C and then resuspended in 40 μl of double-distilled water.

A 625-bp region of the COI gene was amplified by the polymerase chain reaction (PCR). Two COI primers: 5′ CCTGCAGGAGGAGGAGAYCC

Table 3. Species collected and substrates where specimens were found

Species	Habitat
Caribbean species, Santa Marta, Colombia	
Neopisosoma angustifrons (Benedict, 1901)	Incrustation of barnacles - intertidal
Neopisosoma neglectum Werding, 1986	Incrustation of barnacles - intertidal
Pachycheles serratus (Benedict, 1901)	Interstices of coralline rocks - intertidal
Clastotoechus nodosus (Streets, 1872)	Incrustation of barnacles - intertidal
Clastotoechus vanderhorsti (Schmitt, 1924)	In boreholes of *Echinometra* - sea urchin - intertidal
Petrolisthes magdalenensis Werding, 1978	Under boulders - 2 m
Petrolisthes jugosus (Streets, 1872)	Under sponge-covered stones - 3 m
Petrolisthes galathinus (Bosc, 1801)	Coral debris - 2 m
Petrolisthes tonsorius Haig, 1960	Under stones, in the surf
European species	
Porcellana platycheles I	Atlantic, Saint Maló, France: Under stones in the surf
Porcellana platycheles II	Mediterranean, Costa Brava, Spain: Under stones in the surf

3′, known as COIf (Palumbi & Benzi, 1991) and 5′AGARTATCGTCGIGGTATTCC 3′, specifically designed for porcellanid material and designated as COIa1, were used. PCR products were amplified in a total reaction volume of 50 μl, containing 5 μl of 10 × PCR Reaction Buffer (Goldstar: Eurogentec), 1.5 mm MgCl2, 1.25 mm of each dNTP (Pharmacia), 1.5 U of *Taq* DNA Polymerase (Goldstar: Eurogentec), 0.02 mm of each primer and 1 μl DNA extract. PCR amplification was performed using 30 cycles of 94 °C for 40 s, 50 °C for 45 s, 72 °C for 40 s, an initial denaturation step at 94 °C for 4 min and a final extension step at 72 °C for 5 min.

PCR products were purified using the PCR product pre-sequencing kit (US 70995: Amersham) and used directly in cycle sequencing using the GATC-Bio Cycle Sequencing Kit. Direct blotting electrophoresis was performed using the GATC 1500-System. Pre-sequencing and sequencing reactions and direct blotting electrophoresis followed the manufacturer's protocol. Only one individual from each species or population was sequenced. Sequences have been deposited in GenBank (Accession Numbers AF 222720–21, 222723–28, 222730–31 and 296178)

Sequence comparison and phylogenetic analysis

Sequences were aligned using CLUSTAL (Higgins & Sharp, 1989). The homologous sequences from *Petrolisthes cinctipes* (accession number AF060776), and *Pagurus longicarpus* (AF150756) were obtained from GenBank and included in the analysis. The sequence of the latter species, representing a different family (Paguridae) was used as outgroup in the phylogenetic analysis.

A Maximum Parsimony (MP) reconstruction was carried out using the branch and bound search option in PAUP* Version 4.3a (Swofford, 1999). The robustness of reconstructions was assessed by performing 1000 bootstrap replicates (Felsenstein, 1985). Bootstrapping was conducted on parsimony informative characters, with random addition of taxa for each of the 1000 replicates, and TBR branch swapping each replicate. Finally, a Kishino–Hasegawa test (Kishino & Hasegawa, 1989) was conducted, in which the shortest trees under the constraint of the monophyly of the two *Neopisosoma* species included in this study was compared with the shortest tree of the data set.

Results

Analysis of nucleotide sequences

From the amplified 629 bp segment of the COI gene, about 465–500 bp could be sequenced. For data analysis, 465 bp were examined from which 139 positions were found to be parsimony informative. The sequences show a considerable bias to thymine (average 37.5%) and adenine (average 28.6%), also found in the mtDNA of other crustaceans (Palumbi & Benzi, 1991; Machado et al., 1993; France & Kocher, 1996; Meyran et al., 1997; Kitaura et al., 1998; Sarver et al., 1998) and other arthropods (Clary & Wolstenholme, 1985;

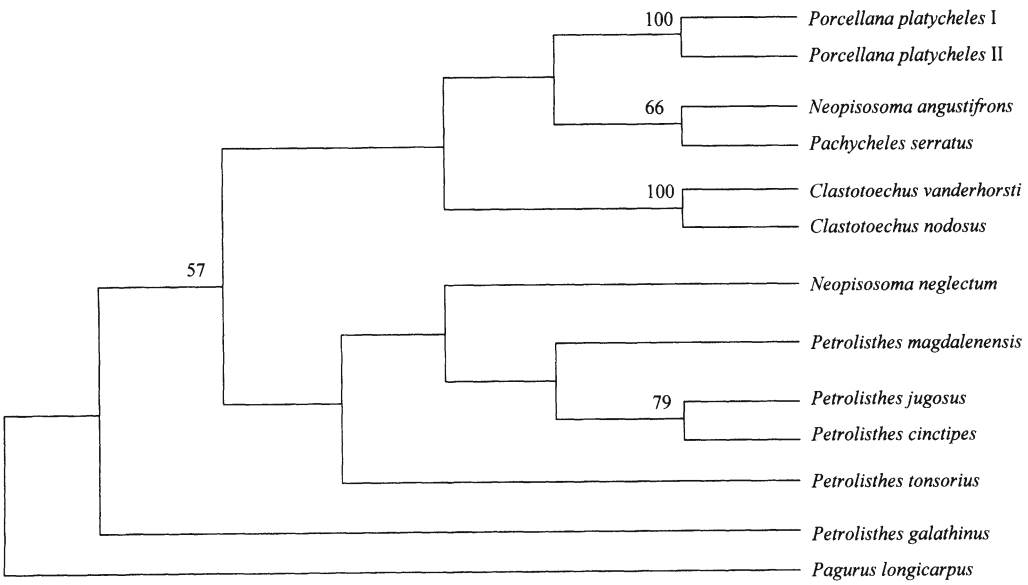

Figure 2. Bootstrap 50% majority-rule consensus tree produced by the maximum parsimony method (MP) using the branch and bound search option and with *Pagurus longicarpus* as outgroup. The consensus tree is 452 steps long with consistency index (CI) 0.480 and retention index (RI) 0.453. Bootstrap values for 1000 replicates are given at each node. Nodes without numbers indicate that bootstrap support values are lower than 50%.

Croizer & Croizer, 1993; Brown et al., 1994). A higher percentage of transitions (57.3%) than transversions (42.7%) was found.

In the MP analysis, characters were treated unordered and transversions were given the same weight as transitions. Figure 2 shows the bootstrap 50% majority rule consensus tree obtained by the MP method.

The *P. platycheles* populations and the *C. nodosus/C. vanderhorsti* clades are well supported (both with 100% support). *N. angustifrons* and *N. neglectum* are not nested together. The *N. angustifrons/P. serratus* clade has 66% bootstrap support. *P. jugosus* and *P. cinctipes* are nested together with a 79% bootstrap support. *N. neglectum*, *P. magdalenensis* and *P. tonsorius* are nested apart with low bootstrap values.

In the Kishino–Hasegawa Test ($p<0.005$), a monophyly of the two *Neopisosoma* species can be significantly rejected

Analysis of aminoacid sequences

The nucleotide sequences were translated to amino acid sequences using the genetic code of *Drosophila* mtDNA. They are 155 residues long and show 35 (22.6%) variable sites. About 98% of changes at the third position represent silent substitutions. Most of the interspecific differences at the nucleotide level are not detectable at the aminoacid level.

Discussion

The paraphyly of the genus *Neopisosoma* Haig (1960) was tested using molecular evidence. Despite a limited number of taxa included in this study, the paraphyly of the genus*Neopisosoma* and of the genus *Pachycheles* as well, can be confirmed, based on the MP reconstruction, in which the *Neopisosoma* species are not nested together, and the Kishino-Hasegawa Test, in which a monophyly of both *Neopisosoma* species is significantly rejected.

These results are consistent with morphology-based studies of larvae; in which *N. angustifrons* groups together with all *Pachycheles* species, while *N. neglectum* is separated from that group. Therefore, the lack of epimeral fragments behind the frontal piece should not be considered a decisive character to separate the genus *Neopisosoma* from the genus *Pachycheles*.

A revision of both genera on a world-wide basis and a molecular study, that includes as many species as possible belonging to each of the morphology-based assemblages formed by *Neopisosoma* and *Pachycheles*, remains to be done in order to confirm the presented results. The genus *Petrolisthes* Stimpson (1858) proves to be taxonomically problematic, as attested in Haig's monograph (1960), where five different morphotypes were distinguished. Werding (1992)

mentioned that within this genus, with nearly 100 species, several morphologically distinct groups would justify the establishment of separate genera. In our MP reconstruction, the *Petrolisthes* species do not form a monophyletic clade, except *P. jugosus* and *P. cinctipes*. As in the case of *Neopisosoma* and *Pachycheles*, a revision of the genus remains to be done.

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Growth in Crustacea – twenty years on

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Key words: Crustacea, growth, hormones, age determination

Abstract

Developments during the past 20 years are reviewed for four aspects of crustacean growth. These are the hormonal control of moulting, the effects of external factors on growth rate, the patterns of growth and the determination of age. *Hormonal control.* The nature and structure of Moult Inhibiting Hormone has been determined, though the mechanism by which it inhibits crustecdysone production is still unclear. A role in moult control by Crustacean Hyperglycaemic Hormone has been demonstrated, but needs clarification. Methyl farnesoate, a juvenile hormone like substance, occurs in Crustacea: however, a clear function as a juvenile hormone has yet to be shown. *External factors.* The effect of increased temperature in reducing moult increments is supported by further data. Reduced food supply causes smaller moult increments and longer intermoult periods: the latter effect is generally proportionately greater. A role for CHH in this process is hypothesised. *Patterns of growth.* Little advance has occurred in understanding the rationale for the diversity of growth patterns. Computer modelling offers promise, but is constrained by lack of data on natural mortality for validation. *Determination of age.* The basic methods available remain size frequency analysis and tagging programmes. There have been advances in technology and methods of analysis, but no major breakthrough. Novel methods include radionuclide ratios (expensive, complex and give only duration of current intermoult), lipofuschin pigment assay (promising, but needs further validation), and annular structures in the infra-cerebral organ (still very speculative).

Introduction

Some 20 years ago I wrote a review on growth in Crustacea (Hartnoll, 1982), which covered both absolute and relative growth. At the end of that review, I summarised it thus: "At first sight, this body of knowledge... is quite impressive. However, it masks an ignorance of the underlying mechanisms which control growth....Virtually nothing is known of the physiological mechanisms which mediate the expression of inherited growth patterns".

Twenty years on, I am taking this opportunity to review the current situation. Have major advances been made, or would I today still draw essentially the same conclusions? I limit this analysis to absolute growth. I do not feel that our understanding of relative growth has developed much over the intervening period, though there are increasingly complex and sophisticated ways of analysing it. The computer has largely replaced the laboratory in that field.

The following topics will be considered in relation to absolute growth:

1. The hormonal control of moulting.
2. The effects of external factors.
3. The patterns of growth.
4. The determination of age.

The coverage of each topic is deliberately selective. I have not attempted to comprehensively cover the literature since 1980.

The hormonal control of moulting

There are several aspects of moulting where hormonal control is important: determining when a moult will occur; controlling the onset of the terminal anecdysis; determining when the puberty moult will be initiated.

The occurrence of moulting

The basics of the hormonal control of moult initiation by two antagonistic hormones were understood well before 1980.

A Moult Inhibiting Hormone (MIH) is produced by neurosecretory cells in the medulla terminalis in the eyestalk, and stored in the sinus gland. This understanding was based on selective ablation (Pasteur-Humbert, 1962). Whilst this was accepted as a general phenomenon in Crustacea (for reviews see Vernet-Cornubert, 1961; Sochasky, 1973), MIH had not then been reliably isolated nor categorised (though see Freeman & Bartell, 1976; Soyez & Kleinholz, 1977).

The second hormone was a moulting hormone produced mainly by the Y-organs or moult glands (Gabe, 1953). The removal and re-implantation of the moult glands had been shown to, respectively, prevent and permit resumption of moulting in a range of crustaceans (see Vernet, 1976 for review). The moulting hormone was first isolated and characterised by Hampshire & Horn (1966) who named it crustecdysone. It is a steroid, variously known as β-ecdysone, ecdysterone and 20-hydroxyecdysone. In the 1970s moulting hormone levels were being directly measured in individual animals and tissues using radioimmunoassay and chromatography (e.g. Bebbington & Morgan, 1977; Chang et al., 1976; Stevenson et al., 1979). Injection of crustecdysone had been shown to trigger precocious moulting (e.g. Dall & Barclay, 1977). Lachaise et al. (1993) provides a very comprehensive review of the structure and activity of the moult glands.

A major advance since 1980 has been the isolation and characterisation of MIH, which is now known to be a neuropeptide. This proved difficult because of the production by the eyestalks of a family of structurally very similar neuropeptides with different functions. The initial advance was the development of a bioassay which measured the effect *in vitro* of MIH in reducing ecdysteroid synthesis by y-organs (Soumoff & O'Connor, 1982). This led to the characterisation of MIH from the shore crab *Carcinus maenas* (Linnaeus) (Webster, 1986; Webster & Keller, 1986), followed by elucidation of its complete amino acid sequence (Webster, 1991). The sequence has also been determined for several other crabs (see Webster, 1998 for review). They are all quite similar (about 80% identity) with 78 residue peptides with free N- and C-termini, and three intra-chain disulphide bridges. Although so much is now known of the chemistry of MIH, there is still uncertainty regarding the exact mechanism by which it inhibits crustecdysone production (Lachaise et al., 1993).

MIH activity and the secretory structures involved have now been identified in larval *Carcinus maenas* by the use of immunocytochemical methods (Webster & Dircksen, 1991).This indicates that the mechanism of moult control is similar in both larval and post larval stages.

Another major development since 1980 has been an appreciation of the role of crustacean hyperglycaemic hormone (CHH) in moult regulation. CHH is a neuropeptide with a very similar structure to MIH (see Webster, 1998 for details), and Webster & Keller (1986) found that it inhibited ecdysteroid synthesis in *Carcinus maenas* Y-organs, though much less so than MIH. This moult inhibiting effect of CHH has now been shown to be widespread in decapods (Van Herp, 1998; Webster, 1998). The effects of MIH and CHH appear to be synergistic (Webster, 1998). Although MIH and CHH are structurally distinct in brachyurans, the distinction is not necessarily clear in other decapods (see Van Herp, 1998 and Webster, 1998 for discussion). Levels of CHH can rise in response to stress. This has been observed following emersion stress in *Cancer pagurus* Linnaeus (Webster, 1996) and various stresses including starvation in the crayfish *Orconectes limosus* (Rafinesque) (Keller & Orth, 1990). Raised CHH levels have been suggested as a possible factor in extending the intermoult period beyond its normal length with reduction in food supply (Oh & Hartnoll, 1999).

There is also evidence that methyl farnesoate (see below) can stimulate moulting by increasing ecdysteroid production. This was demonstrated in vitro for the Y-organs of *Cancer magister* Dana (Tamone & Chang, 1993). There is clearly more to moult control than the simplistic MIH/crustecdysone interaction.

We now know a great deal regarding the proximate control of moulting. The structure, production and interactions of the various hormones involved are understood in substantial detail. However, this does not really help very much in understanding why the intermoult period and the moult increment are so varied both within and between species – there is still a long way to go.

The onset of terminal anecdysis

In 1980, it was recognised that moulting ceased definitively in a variety of crustaceans (Hartnoll, 1982,

Table 1. The effect of temperature on the moult increment (additional to references cited in Hartnoll, 1982)

Species	Reference	Temperature range	Effect of temperature increase
Larvae			
Carcinus maenas (Linnaeus)	Dawirs et al. (1986)	12–18 °C	no change
Carcinus maenas	Mohamedeen & Hartnoll (1989b)	15–20 °C	decrease
Inachus dorsettensis (Pennant)	Hartnoll & Mohamedeen (1987)	15–20°C	decrease
Pilumnus hirtellus (Pennant)	Hartnoll & Mohamedeen (1987)	15–20 °C	decrease
Postlarvae			
Calanus pacificus Brodsky	Vidal (1980)	8–15 °C	decrease
Carcinus maenas	Mohamedeen & Hartnoll (1989a)	15–20 °C	decrease
Corophium insidiosum (Crawford)	Nair & Anger (1979)	10–20 °C	decrease
Hyale barbicornis	Hiwatari & Kujihara (1988)	16–20 °C	decrease
Hyas araneus (Linnaeus)	Kunisch & Anger (1984)	2–12 °C	decrease
Hyas coarctatus Leach	Anger (1984)	6–9 °C	increase
Hyas coarctatus	Anger (1984)	9–18 °C	decrease
Hyas coarctatus	Bryant (1991)	8–14 °C	decrease
Mytilocypris henricae (Chapman)	Martens (1985)	10–15 °C	increase
Mytilocypris henricae	Martens (1985)	15–25 °C	decrease
Neomysis integer (Leach)	Astthorsson & Ralph (1984)	9–16 °C	no change
Palaemon serratus (Pennant)	Yagi & Ceccaldi (1983)	13–29 °C	decrease

Table 1). A more extensive list was produced later (Hartnoll, 1985). The mechanism which controlled this termination of moulting had been examined some time earlier by Carlisle (1957), who coined the phrase 'terminal anecdysis' for the stage after the final moult. Carlisle (*op. cit.*) studied the hormonal basis of terminal anecdysis in two crabs, *Carcinus maenas* and *Maja squinado* (Herbst), by a bioassay using the prawn *Palaemon serratus* (Pennant). Extracts of the eyestalks and the moult glands from crabs before and after the terminal moult were injected into the prawns, and changes in the incidence of moult initiation were determined. The mechanism maintaining terminal anecdysis in the two crabs was quite different. In *Carcinus* there was a continued high production of MIH after the terminal moult which suppressed moulting: however bilateral eyestalk ablation removed this inhibition and could initiate further moulting. In *Maja*, there was a low MIH production, but the moult glands atrophied: eyestalk ablation had no effect. It was presumed, on the basis of eyestalk ablation, that the burrowing crab *Corystes cassivelaunus* (Pennant) had a mechanism similar to *Carcinus* (Hartnoll, 1972).

Since that time, there have been few developments, and the new techniques of direct hormonal assay have been little used to examine the question of terminal anecdysis. In the blue crab *Callinectes sapidus* Rathbun terminal anecdysis females will moult after eyestalk ablation, indicating a '*Carcinus* type' control (Havens & McConaugha, 1990) – not surprising as they are in the same family. On the other hand, the Y-organs have been found to degenerate after the terminal moult in the spider crab *Acanthonyx lunulatus* Risso (Chaix & De Reggi, 1982) and in males of the isopod *Sphaeroma serratum* (Fabricius) (Charmantier, 1980). Low levels of ecdysteroids have been measured in terminal anecdysis specimens of *Callinectes sapidus* (Soumoff & Skinner, 1983), *Acanthonyx lunulatus* (Chaix & De Reggi, 1982) and *Sphaeroma serratum* (Charmantier, 1980).

The initiation of the puberty moult

In many crustaceans, the general pattern of growth involves a series of immature instars of generally similar morphology, terminated by a moult at which there are distinct morphological changes. The crustaceans become sexually mature in the following instar, and this distinctive moult has been termed the 'puberty moult' (Perez, 1928). The occurrence of the puberty moult has been recognised in many crustacean groups (see

Hartnoll, 1985 for review), and is particularly well developed in the spider crabs (Hartnoll, 1963). The puberty moult may, or may not, be also the terminal moult (see Hartnoll, 1985).

This pattern of growth in crustaceans is analogous to that in insects, where there is a clear-cut moult or metamorphosis between the juvenile and adult instars. In insects, this change is hormonally regulated by Juvenile Hormones (JH), sesquiterpenoid compounds secreted by the corpora allata. High titres of JH maintain the insect in the juvenile phase. In 1980, no comparable hormones were known in crustaceans, though there was some indirect evidence for their existence (see Laufer et al., 1986, for discussion). Costlow (1968) demonstrated that in *Callinectes sapidus* zoea eyestalk removal hindered moulting to the megalopa. Hinsch (1972) had shown that in the spider crab *Libinia emarginata* (Leach) removal of the eyestalks prevented a puberty moult and produced abnormally large immature females. These observations indicated a probable hormonal influence on these moults, with the eyestalks inhibiting the production of a putative JH at critical moults.

It has now been demonstrated that a JH-like substance, methyl farnesoate, is produced by the mandibular organ in crustaceans, the initial identification being in the spider crab *Libinia emarginata* (Laufer et al., 1987a). It has subsequently been confirmed as secreted by the mandibular organs of other crustaceans tested (Borst et al., 1987). Methyl farnesoate is structurally related to JH III, which has JH bioactivity. The mandibular organ is possibly homologous to the insect corpus allatum (Byard et al., 1975). It has also been clearly shown that production of methyl farnesoate by the mandibular organ is inhibited by an eyestalk factor (MOIH) (Laufer et al., 1987b; Wainwright et al., 1996).

In insects, JH not only maintains the juvenile phase, but influences gametogenesis in the adults, stimulating vitellogenesis (Engelmann, 1983). The latter is also a function of methyl farnesoate in crustaceans, and most of the observations of its activity in crustaceans relate to that aspect of its physiological role (Hinsch, 1980; Borst et al., 1987; Laufer et al., 1987a; Laufer et al., 1993). In male *Libinia emarginata*, for example, reproductively active males with large reproductive tracts have high levels of methyl farnesoate (Laufer & Ahl, 1995).

However, observations of its action on development are scant – thus in lobster larvae, the presence of methyl farnesoate in the seawater causes a small but significant delay in their metamorphosis (Borst et al., 1987). So despite the initial promise of a JH analogue in crustaceans, this has not at present provided a control mechanism for the puberty moult – effects of methyl farnesoate on the puberty moult have not been shown. There do appear to be effects on larval metamorphosis: is this larval–juvenile transition the effective analogue of insect metamorphosis, and is the puberty moult in fact under some quite different control?

The effects of external factors

Various external factors were found to have a substantial influence upon growth (Hartnoll, 1982), and of these temperature and food supply are perhaps the most widespread in their importance. Both have been shown to affect growth under laboratory conditions, and both can vary substantially in the field both spatially and temporally.

The effect of temperature

An increase in temperature almost universally increases the growth rate of crustaceans, usually without any evidence of an optimum temperature. Growth rate continues to rise with temperature, though at the highest temperatures mortality may increase substantially. Growth rate may decline at the highest temperatures, as in *Palaemon serratus* at 30 °C (Reeve, 1969), but generally with very high mortality so that the decline has little biological significance.

Increased temperature could accelerate growth by shortening the intermoult period, or increasing the moult increment, or both. The effect on the intermoult is a consistent and substantial shortening with increasing temperature (see Hartnoll, 1982 for listing of examples). A very good example is the ostracod *Cyprinotus* where the intermoult shortens consistently from 9 °C to 31 °C (Kurata, 1962). Another is the larvae of *Hyas araneus* (Linnaeus) with consistent shortening from 2 °C to 18 °C (Anger, 1983). There are a relatively few exceptions where the intermoult lengthens again above an 'optimum' temperature (examples in Hartnoll, 1982). Studies since 1980 have generally confirmed this temperature effect, which is not unexpected. Metabolic processes in poikilotherms increase at higher temperatures, and all of the processes involved in the accumulation of the reserves needed for moulting, and their mobilisation and the

preparatory stages for moulting, will follow this trend. Nevertheless, the relationship is neither simple nor consistent, and very great differences in the Q_{10} value occur both within and between species. Generally, the Q_{10} decreases with temperature and with body size.

The effect of increased temperature on the moult increment is both more variable and intrinsically more interesting, since it is not what might be intuitively expected. A series of examples were listed in Hartnoll (1982), of which four showed no effect of temperature, eight showed a reduction in increment with increased temperature, and only two showed an increase with raised temperature. A random selection of more recent examples, with no pretence to be comprehensive, is listed in Table 1. These indicate no change in two cases, a reduction in increment with increasing temperature in nine cases, and a reduction over most of the temperature range studied in the other two. So it would be fair to generalise that an increase in temperature reduces the moult increment.

Temperature increase, then, has an antagonistic effect on the rate of growth by reducing the time between moults on one hand, but reducing the size increase at each moult on the other. However, the former effect is proportionately much greater, so that the almost universal outcome is an increase in growth rate (see above). The nature of most data makes comparable calculations of the level of effect difficult, but the amphipod *Corophium insidiosum* can provide an example. For a rise in temperature from 10 °C to 20 °C, there was a 51% shortening of the intermoult period, but only a 21% reduction in the moult increment (Nair & Anger, 1979). In most other cases, the disparity is greater. The net result is a substantially increased growth rate.

The consequence of temperature decrease on growth is that the time to reach a particular stage of the life cycle (e.g. the end of larval development, or the onset of sexual maturity) is increased, but the size on attaining that stage is also increased. In simpler terms, the life span is extended and the body size is increased. This is a tendency which can be observed in different populations of the same species occurring in seasonal or geographical temperature gradients, or in different species within a taxon distributed over such a gradient. There is the general trend of smaller species in the tropics and larger species in higher latitudes. This is not a trend confined to crustaceans, but it is interesting to be able to present at least a proximate mechanism to account for it in the Crustacea. It is not clear exactly how it operates, but presumably the processes which determine the onset of the next moult, and those which determine the level of accumulated reserves, are not precisely coupled and respond differently to an increase in temperature.

The effect of food

If food supply is reduced below the optimum level, there is invariably a reduction in growth rate (Hartnoll, 1982), a reduction resulting from the combination of an extended intermoult period and a reduced moult increment. The main point of interest identified was which of these two effects had the greatest influence on growth rate. Available studies attributed the major impact to reduced increment (Knowlton, 1974), or to extended intermoult (Chittleborough, 1975), or in most cases to a combination of both (see discussion in Hartnoll, 1982). A reliable evaluation requires data on both intermoult and increment under controlled conditions, of which there are relatively few. Most growth studies look only at the increase in size with time. However, there are now several more recent studies. These include larval stages – *Ranina ranina* (Linnaeus) (Minagawa & Murano, 1993), and post larval stages – *Carcinus maenas* (Mohamedeen & Hartnoll, 1989a), *Palaemon elegans* Rathke (Salama & Hartnoll, 1992) and *Crangon crangon* (Linnaeus) (Oh & Hartnoll, 1999). A comparison of the effects of reduced food on intermoult and increment in terms of percentage change from a full diet is provided in Table 2.

In larval development, a reduction in food leads more or less universally to an extended duration for each larval stage, of which there are plentiful records (see discussion in McConaugha, 1985). The lack of effect in *Palaemonetes vulgaris* (see Table 2) is misleading in that the animals on reduced diets passed through additional larval stages, which has the same effect as prolonging the intermoult. In carideans, which generally show variation in larval stage number, low food supply generally both increases the number of larval stages and the intermoult period (see McConaugha, 1985). Clear data on changes in moult increment in crustaceans where the number of larval stages is not variable are relatively scarce. However, available data indicate reductions in increment.

There is rather more data for postlarval development (Table 2), and generally there is both a lengthened intermoult and a reduced moult increment. The relative importance of the two effects varies between species, and within species between different

Table 2. Effects of reduced food supply on the intermoult period and moult increment. Values are expressed as the percentage change from a full diet to when given a reduced diet

Species	Reference	Variants	Intermoult period	Moult increment
Larvae				
Palaemonetes vulgaris Say	Knowlton (1974)		±0	−23
Ranina ranina	Minogawa & Murano (1993)		+35	−16
Postlarvae				
Carcinus maenas	Adelung (1971)		+65	−11
Carcinus maenas	Klein Breteler (1975)	15 °C	+44	−28
		20 °C	+64	−27
Carcinus maenas	Mohamedeen & Hartnoll (1989a)	15 °C	+32	−48
		20 °C	+51	−47
Crangon crangon	Meixner (1969)		+54	*
Crangon crangon	Oh & Hartnoll (2000)	males	+10	−19
		females	+18	−32
Palaemon elegans	Salama & Hartnoll (1992)	natural food	+79	−39
		Artificial food	+47	−21
Panulirus longipes (A. Milne Edw.)	Chittleborough (1975)	small reduction	+25	+2
		high reduction	+86	−27
Phronima sedentaria (Forskål)	Laval (1975)	12 °C	+46	−12
		19 °C	+59	−21

*Moulted with negative increments on a reduced diet.

experimental protocols. However, there is a marked preponderance of cases where the increase in intermoult period is relatively greater than the decrease in increment.

There is currently insufficient known concerning the factors initiating moulting to empirically expect one effect to predominate over the other. However, intuitively the major effect might be expected on the intermoult period, on the presumption that a certain reserve of energy must be accumulated before a moult can occur (Adelung, 1971). But whilst reduced food does almost invariably prolong the intermoult, the following moult almost always displays a substantially reduced increment. It follows that moulting can, therefore, occur before some absolute threshold of reserves has accumulated. We can only speculate on the mechanisms involved, but a possible hormonal mechanism was discussed above. It is accepted that ecdysis is initiated when levels of MIH fall, but little is known of what triggers that fall. Perhaps that fall is not triggered solely by accumulated resource level, but happens after a certain time for a specimen of a given size under given physical conditions. There is a second hormone, CHH, which also has a moult-inhibiting activity (Van Herp, 1998; Webster, 1998), and which is produced under various stresses including starvation (Keller & Orth, 1990). If food reduction generally induces increased CHH production this could prolong the intermoult beyond its normal length, though without doing so sufficiently to enable the accumulation of the usual level of reserves. Further investigation requires the assay of MIH and CHH levels in well fed and underfed crustaceans over the course of the moult cycle.

The patterns of growth

The diversity in the patterns of growth (or 'growth format') within the Crustacea has been clear for a long time (see Hartnoll, 1982, Table I). The main variables which generate this diversity are:

1. Whether or not there is a terminal anecdysis. If there is not, growth is indeterminate and continues indefinitely. If there is, then the terminal anecdysis follows the last moult, and growth is determinate.
2. The position of the onset of sexual maturity or puberty moult in the total sequence of moults. Whether there is more than one mature instar.

3. In cases of determinate growth, whether the number of instars is the same in all specimens, or variable.
4. Whether every mature instar is ovigerous, or whether there is an alternation of ovigerous and non-ovigerous instars.
5. The number of batches of eggs produced in each ovigerous instar.

This variety has been discussed in more detail (Hartnoll, 1985; Hartnoll & Gould, 1988). Correlation between growth format and phylogenetic position was also examined there in some depth, a correlation which could arise because the evolution of a taxon did not provide the potential for alternatives, or because a particular format is the most adaptive throughout that taxon. It was concluded that there was little correlation between growth format and phylogenetic level. Thus, among the more primitive taxa, the Branchiopoda have indefinite growth, and produce a single egg batch in each of a sequence of mature instars. In contrast, the Ostracoda and Copepoda have definitive growth with a defined number of instars, and produce multiple egg batches in the single mature instar. In the most advanced taxon, the Decapoda, a similar diversity is still to be found. Thus, the lobster *Homarus* has a growth format which parallels the Branchiopoda, and the spider crabs such as *Maja* have one similar to the Ostracods and Copepods. Gross ecological habit similarly failed to produce any general correlation with growth format. Thus the similarly pelagic Cladocera and Copepoda have totally different formats, as do the similarly benthic crabs *Cancer* and *Maja*. There is some correlation with latitude, in that species in higher latitudes produce fewer batches of eggs, but this is due to longer incubation time and extreme seasonality rather that differences in the basic format.

So despite more detailed analysis, no adequate explanation for the scattered distribution of growth formats has been derived. Nevertheless, since it is more or less an article of faith that life history patterns are adaptive, attempts have been made to examine possible grounds for this adaptiveness. One approach has been to examine through modelling the effect of different growth formats on lifetime egg production under different levels of mortality.

In spider crabs, there is a consistent and simple growth format of a puberty moult followed by a single mature instar: the puberty moult and the terminal moult are one and the same. The major variable is the carapace length at the puberty moult, which can vary from 10–12 mm in small species (e.g. *Eurynome, Pelia*) up to 400 mm in the giant Japanese *Macrocheira*. A very simple computer model (Hartnoll, 1985) demonstrates that as mortality level increases, the size of sexual maturity for optimal lifetime egg production falls from 145 mm to 15 mm. This is a gross over-simplification, since in the wild mortality will be size-related in different ways in each species. There is also the problem that the mortality data from natural populations needed to validate the model are not available.

More complex models have been developed, based upon the growth format of *Carcinus maenas* which has a puberty moult, and then a number of mature instars before a final moult leading to a terminal anecdysis. Experimental data were used to parameterise the model as far as possible. The model was initially run at a fixed mortality level, examining the effect of varying the position of the terminal moult for given positions of the puberty moult. When these were integrated an optimal format emerged with puberty at the 12th moult, and the final moult at the 23rd. There was no benefit in extending the instar sequence. In fact, *Carcinus maenas* becomes mature at the 13th instar (Mohamedeen & Hartnoll, 1989b), and probably stops moulting after the 15th, providing an encouraging agreement with the model. The model has been further developed to examine different mortality levels, and has also been applied to other basic growth formats characteristic of indeterminate growth (e.g. *Cancer pagurus*), and of determinate growth with one mature instar (e.g. spider crabs) (Bryant, 1991).

We are still far from understanding the rationale of the diversity of crustacean growth patterns, despite a much increased body of data. The modelling approach has shown promise, but until the models can be progressively tested and validated, they will remain speculative.

The determination of age

The accurate determination of the age of crustaceans in wild populations has always been a major problem for academic and fisheries scientists alike. The basic cause of this problem is the loss of all integumental structures at each moult. This means that there are no persistent skeletal structures to retain a record of the age and growth of an individual, as can do the scales and otoliths of fish, the genital plates of sea urchins and the shell rings on bivalve molluscs. A fur-

ther problem is that conventionally attached external tags are lost at moulting, though there are ways of circumventing that problem.

The basic techniques then available for measuring crustacean age were summarised by Burkenroad (1951), and re-iterated in Hartnoll (1982):

i. Observations of captive individuals.
ii. Synthesis of data on moult increment and intermoult period.
iii. Tagging or marking of specimens.
iv. Size frequency distribution analysis.

The first two methods are of limited use: where the second has been employed it has been based essentially on data from tagging programmes – for example, the study on *Homarus americanus* H. Milne Edwards by Campbell (1983). This section will first consider any recent developments in the third and fourth methods, and then evaluate any radical ideas which have developed since 1980.

Methods of attaching external tags so that they were retained at moulting were well developed in 1980, and have developed subsequently only in detail. The main advances have been in the use of internal tags, which can be implanted in structures such as the musculature which are not lost at moulting. Coded ferromagnetic wire tags have been widely used, which can be detected using a coil detector. The disadvantage is that the specimen must be sacrificed to recover and decode the tag, though this is not necessarily a problem where the specimens are sourced from commercial catches.

A more recent development has been the use of microchips, or passive integrated responders (PIT tags). These can be injected into the specimen, and recognised externally and non-invasively using an electronic reader: since they respond passively, they have an effectively unlimited lifespan. They have been used extensively for the routine identification of domestic and farm animals, and for large mammal studies. They are now being used for aquatic organisms, including fish (AFMA, 1994) and turtles (Van Dam & Diez, 1997). In crustaceans, they have been employed in freshwater crayfish (Wiles & Guan, 1993) and in the coconut crab *Birgus latro* (Linnaeus) (Hartnoll, unpublished). There are currently two constraints to their use. One is size, since the smallest microchips measure 12 mm by 2 mm, so they cannot be used in small organisms. The other is the detection range, which is a maximum of 25 cm even with the more powerful trackers. This effectively limits their use to specimens which can be recaptured or which are highly territorial.

Another quite different development in tagging methods has been the use of 'living tags'. The principle is to implant a piece of living tegument from a donor animal into the abdominal haemocoel of the host. Once implanted, the donor cuticle becomes encapsulated by its associated epidermis to form a cyst: every time the host moults, the epidermis of the implant secretes a layer of cuticle to the inside of the cyst. The cysts are retained at moulting, and the number of cuticular layers provides a record of the moult history since implantation. This method was first laboratory tested on *Homarus gammarus* (Linnaeus) and *Nephrops norvegicus* (Linnaeus) (Shelton & Chapman, 1986, 1987), and subsequently used successfully in the field for *Nephrops norvegicus* (Shelton & Chapman, 1995). The field study showed that specimens had undergone up to three moults, and confirmed that females moulted less frequently and with smaller moult increments. The method is technically complex, and tagged specimens must have an external tag to enable them to be recognised and individually identified in addition to the 'living' tag. The cyst must be removed and sectioned to determine the number of moults.

Size frequency analysis depends upon the identification of modes in the distribution, which can be equated with year classes or with recruitment cohorts. A single sample may be analysed to detect a series of year classes, or serial samples may be collected to follow the progression of modes with time. Where the modes are clear, analysis and interpretation are straightforward. However, this is rarely the case, and efforts have been made to develop means to analyse less well defined distributions.

For the analysis of a single size frequency distribution, the early graphical methods of Harding (1949) and Cassie (1954) have been superseded by computer-based approaches (Macdonald & Pitcher, 1979; Pauly & Caddy, 1985) which were coming into use in the eighties. Macdonald & Pitcher's (1979) work led to the 'MIX' program. All methods are based on the assumption that the distribution pattern is comprised of a series of normal distributions. The same approach can be developed to allow modal progression analysis (Sparre, 1987). A comprehensive description and discussion of these methods is presented in Sparre et al. (1989). The ELEFAN programme is a standard package for executing these analyses (Gayanilo et al., 1995). However, whilst these methods are powerful and flexible, they do have to be used and interpreted with caution (Grant et al., 1987). To run the programs

requires input of additional data or assumptions, and depending upon these alternative outcomes are possible. The caution by Macdonald & Pitcher (1979), that "interpretation of the graphs still leaves much to the imagination of the user, especially when the original size frequency distributions are not clearly polymodal", is still very valid. Certainly none of these approaches will provide a simple answer to ageing long-lived species, especially those lacking a well defined recruitment season.

Three novel radical methods have been proposed for ageing crustaceans in recent years – the use of radionuclide ratios, the concentration of lipofuscin pigments in the brain and the structure of the infra-cerebral organ.

The ratios of various uranium and thorium decay chain nuclides change by decay processes, so the duration since biological tissues were deposited can be determined. The use of the radionuclides ^{226}Ra, ^{210}Pb, ^{210}Po, ^{228}Ra and ^{228}Th in various combinations can provide measurements over different time scales. The method was originally applied to molluscs (e.g. Turekian & Cochran, 1981), and gave an estimate of the actual age. It has subsequently been applied to crustaceans where, because of the moulting of the integument, it can only provide an estimate of the time since the last moult. Bennett & Turekian (1984) studied the hydrothermal vent crab *Bythograea thermydron* Williams, and determined a maximum duration of 4 years between moults. Le Foll et al. (1989) applied the method to specimens of *Maja squinado* and *Homarus gammarus* which had moulted at a known time in order to validate the method. The results were generally good, giving a slight underestimate since mineralisation of the integument continues for some time after the moult. The moulting frequency in the Norway lobster *Nephrops norvegicus* has been investigated in the same way (Latrouite et al., 1991; Talidec & Reyss, 1993), showing two moults per year in males and immature females, and only one per year in mature females. The method has potential in appropriate circumstances, but it is expensive and needs specialist facilities and will hardly become a routine procedure.

Lipofuscin is a pigment which accumulates in the post-mitotic tissues, such as the brain, of older animals, raising the possibility that its concentration may provide an index of age. This would be especially valuable in crustaceans where no hard tissues are retained, and Ettershank (1983) first proposed its use for determining age in Antarctic krill, using a solvent extraction and spectrofluorimetric method. However, a number of studies using this method failed to demonstrate links between extraction levels and age (see discussion in Sheehy, 1990a, b). An alternate method was developed which involved sectioning the brain and measuring lipofuscin directly by histological fluorescence (Sheehy, 1990a). The method was shown to be applicable to a wide range of crustaceans (Sheehy, 1990b). Studies on the crayfish *Cherax quadricarinatus* (Von Martens) showed that lipofuscin levels were much better correlated with age than with size (Sheehy, 1990a). However, this was based on laboratory reared material, and the variability of a field environment might disturb the relationship. In the wider range of species examined (Sheehy, 1990b), there was a less consistent correlation with chronological age. Studies on *Nephrops norvegicus* and *Homarus gammarus* have demonstrated that lipofuscin levels were correlated with size, but the relationship with age was not tested (Tully, 1993). Later age-related studies have included *Nephrops norvegicus* (Belchier et al., 1994), *Homarus americanus* (Wahle et al., 1996) and *Panulirus cygnus* George (Sheehy et al., 1998). The results are promising, with some good correlations between lipofuscin concentration and age. In *Panulirus cygnus* lipofuscin concentration/frequency analysis revealed the presence of age classes not revealed by size/frequency analysis (Sheehy et al., 1998). The method is showing clear promise, but the sectioning and analysis of material is a skilled and labour-intensive task. Furthermore, the level of lipofuscin accumulation with age is not constant across species, nor necessarily within species under different conditions. It would seem to "reflect physiological age, the accumulated metabolism of individuals, rather than simply the passage of time" (Wahle et al., 1996). Consequently, the method will need fresh calibration for each new study.

A very tentative approach to crustacean ageing involves the infra-cerebral organ or ovoid body which has so far been observed only in clawed lobsters (Nephropsidae). It was first described by Bazin (1970) in *Nephrops norvegicus*. It lies beneath the brain at the insertion of the antennal nerves: it is calcified, and is made up of a number of concentric layers giving it an onion-like structure. Bazin described it as consisting of 6–8 layers, but recent studies on *Nephrops norvegicus* have shown it to consist of between 2 and 10 layers, with some of these possibly consisting of a number of finer layers (M. Belchier & C. Pescod, unpublished). It has no known function. The existence of

a layered calcified structure which is not lost at moulting raises the possibility that it could contain some record of the moult or growth history of the specimen. Could the number of layers be related to the number of previous moults in the individual? The number of layers does increase with body size in *Nephrops norvegicus*, but is well below the expected number of post larval moults for a given size. The organ merits further study, especially the examination of its structure in specimens of known age and moult history.

General conclusions

Clearly, much valuable knowledge has been accumulated in the last 20 years, and our understanding of the proximate mechanisms controlling growth processes, such as the hormonal systems, has been substantially extended. Nevertheless, there are still major questions in need of resolution. What mechanism controls the puberty moult – does methyl farnesoate really function as a juvenile hormone analogue? What triggers moulting when no absolute threshold of resources appears to be a prerequisite?

In relation to broader questions, progress has been more limited. It is not known what determines the wide intrinsic variation in moult increment and intermoult period both within and between species. The adaptive value of the great diversity of growth patterns in the Crustacea can still not be explained. Much of our work on crustacean growth has still to progress substantially beyond the descriptive phase.

The determination of age in crustaceans is still effectively dependent on the analysis of size frequency distribution, or on tagging programmes. Despite some advances, both these methods are either restricted in their applicability, expensive in time and resources, or both. Novel techniques either provide a restricted range of information, or are still unproven.

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Sex-related variability of rostrum morphometry of *Aristeus antennatus* (Decapoda: Aristeidae) from the Ionian Sea (Eastern Mediterranean, Greece)

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Key words: A. antennatus, rostrum relative growth, Ionian Sea, Mediterranean, Decapoda, Aristeidae

Abstract

Sex-related rostral variability was studied in the aristeid shrimp *Aristeus antennatus* from the Eastern Ionian Sea (Mediterranean). Shrimps were collected on a monthly basis from December 1996 to November 1997 using a commercial bottom trawl in a depth range of 446–728 m. Female relative growth of rostrum proved to be negative allometric both seasonally and in the pooled annual data set. Males on the other hand, showed no or negative correlation of rostrum length with size. Mature males with short rostra dominated in the male population all year around. The appearance of males with long and intermediate rostra during winter, which disappear thereafter in favour of those with short rostra, indicates that rostrum shortening takes place during the end of winter. The increase of mated females during spring supports the hypothesis already addressed by other authors on the function of the male short rostrum in this species mating behaviour. Nevertheless, the paucity, in comparison to other Mediterranean populations, of males with long or intermediate rostra could indicate that for the bulk of the male population, the process of rostrum shortening in the Eastern Ionian Sea occurs outside the geographical locality or depth range sampled.

Introduction

The shrimp *Aristeus antennatus* (Risso, 1816) is a common species of the upper and middle slope in the Western Mediterranean and adjacent seas (Sardà et al., 1998; Carbonell et al. 1999). It is a target species of the deep-water demersal trawl fishery in the Western and Central Mediterranean. In the Italian Ionian Sea, *A. antennatus*, together with the other aristeid shrimp *Aristaeomoprha foliacea*, represents the most valuable species of trawl fishing (Matarrese et al., 1994). Despite the abundant information on the biology and population dynamics available for Western Mediterranean (see Bas & Sardà, 1998 for references) and Western Ionian Sea stocks (in Bianchini & Ragonese, 1994), only scanty data exist on *A. antennatus* distribution and biology in the Eastern Mediterranean (Thessalou-Legaki, 1994; NCMR, 1999; Kapiris et al., 2000).

The study of the functional morphometry, carried out in a number of *A. antennatus* body parts from Western Mediterranean populations, has revealed adaptive differences between size groups and sexes. These differences have been related to differences in swimming and/or feeding behaviour (Sardà et al., 1995). Furthermore, morphometric analyses showed significant differences between populations from a number of localities along the Mediterranean and the adjacent Atlantic waters, while genetic analyses of 27 enzyme systems gave a rather weak evidence for genetic differentiation (Sardà et al., 1998). Finally, long-term changes over a 30-year period of the relative growth of some female body parts have been detected by Bas & Sardà (1998), being attributed to increasing fishing pressure.

Rostral variability is commonly found in aristeid shrimps. It is known to be related to sex, sexual maturity and size (Burukovsky & Romensky, 1972). Burukovsky (1972) related rostral function with habitat of several shrimp species, while Palombi (1939) stated that short rostrum in mature males is used in mating behaviour. Nevertheless, other rostral func-

tions in species belonging to the Aristeidae have been proposed, such as swimming behaviour (Burukovsky & Romensky, 1972), sexual segregation (Sardà & Gordoa, 1986) and feeding (Cartes & Sardà, 1989). The male rostral shortening of *A. antennatus* has been studied in the Western (Sardà & Demestre, 1989) and Central Mediterranean (D'Onghia & Maiorano, 1996). Within the framework of obtaining information on the biology of this species along the Mediterranean, the present study deals with rostral variability in males and females of *A. antennatus* further eastwards.

Materials and methods

Shrimps were collected on a monthly basis from December 1996 to November 1997 in a depth range from 446 to 728 m. The trawl surveys were carried out in the area between Zakynthos island and Peloponissos in the Southeast Ionian Sea (E. Mediterranean Sea), using a commercial bottom trawl with a mesh size of 14 mm at the cod end. In total, 618 females and 296 males were examined.

Linear rostral length (RL) from the distal tip to the eye orbit and carapace length (CL) (as defined in Sardà & Demestre, 1989) were measured to the nearest 0.1 mm using calipers. Male sexual maturity was determined on the presence of sperm in the terminal ampulla. Joined hemipetasmata and the presence of the appendix masculina have also been examined. Female functional maturity was determined on the presence of spermatophores in the thelycum (Demestre & Fortuño, 1992).

For both sexes, Analysis of Variance (ANOVA) was used for testing the significance of temporal and bathymetric differences of mean CL and RL values and Spearman's correlation coefficients (r_s) between mean CL and RL values of sampling months were calculated.

The relationship between RL vs. CL was investigated using the linearized allometric model,

$$\ln Y = \ln a + b \ln X,$$

where Y and X are the morphological dimensions and $\ln a$ and b are the regression constants. The type of allometry was determined by testing the slope (b) of the obtained regression equations against the isometric slope of 1 by Student's t-test. The regression equations of RL on CL were estimated for each season and sex separately.

Table 1. Descriptive statistics of the pooled sample of males ($N = 296$) and females ($N = 618$) of *A. antennatus* used in the analyses (in mm)

Variable	Min	Max	Mean	SD
Males				
CL	10.95	35.59	26.76	2.38
RL	3.21	21.45	8.06	2.75
Females				
CL	13.93	58.66	36.97	7.66
RL	3.65	50.66	31.63	6.48

Table 2. ANOVA results (F statistic) for testing the effects of depth and sampling month on carapace (CL) and rostrum length (RL) for both sexes of *A. antennatus* (* = $P < 0.05$)

Variable	Month		Depth	
	Males	Females	Males	Females
CL	3.20*	3.94*	5.31*	3.79*
RL	2.63*	2.74*	2.69*	3.61*

In order to check for difference in relative rostrum growth between females with ($N = 151$) and without ($N = 120$) spermatophores, the comparison of regression equation parameters (slopes and intercepts) was performed by Analysis of Covariance (ANCOVA).

For the sake of comparison with previous studies (Sardà & Demestre, 1987, 1989), three types of rostra have been defined in males: short (RL < 12 mm), intermediate (12 < RL < 17 mm) and long (RL > 17 mm). In addition, the seasonal percentage of specimens presenting each of the above rostral types was tallied for six 3-mm size classes as follows: I: CL $\leq$ 21.4 mm; II: 21.5 $\leq$ CL $\leq$ 24.4 mm; III: 24.5 $\leq$ CL $\leq$ 27.4 mm; IV: 27.5 $\leq$ CL $\leq$ 30.4 mm; V: 30.5 $\leq$ CL $\leq$ 33.4 mm and VI: CL $\geq$ 33.5 mm.

Results

Summary statistics of the measurements for the two sexes of *A. antennatus* of the pooled sample from all surveys are given in Table 1. Mean values of female CL and RL were greater than in males. The effect of time and depth on mean CL and RL proved to be statistically significant in both sexes (one-way ANOVA results in Table 2). Temporal and bathymetric

variations of the examined variables are presented in Figure 1. Male rostrum size (means of each sampling month) showed no correlation to respective mean shrimp size ($r_S = -1.293$, $P > 0.05$), while positive correlation existed in females ($r_S = 0.857$, $P < 0.05$). In particular, a constant decrease in male mean RL is prominent from January to August.

Seasonal and pooled sample equation parameters representing the relative growth of the rostrum of both sexes are given in Table 3. In the same table, the correlation coefficient (r) and the type of allometry are included. In all seasons and the pooled sample, female regressions were statistically significant (regression ANOVA, $P < 0.05$) and the allometric growth of rostrum proved to be negative in all cases (t-test results, $b < 1$). In males, significant regressions occurred in only two occasions: a positive relationship (in which isometry could not be rejected) in summer and a negative relationship or eniantometry according to Teissier (1960), with decreasing rostrum length as size increases, in winter. In all other seasons, as well as in the pooled sample from the whole sampling period, the regressions in males were not significant (regression ANOVA, $P > 0.05$). It should be noticed, however, that due to the variability in the number of individuals caught in each season, the sample size is not similar in all equations. The highest abundance of males was observed in spring followed by autumn, while during summer and winter only a small number of individuals was caught. On the contrary, female presence was significant in all seasons.

The sampled male population of *A. antennatus* consisted mainly of mature individuals, since over the whole sampling period almost all males presented hemipetasmata (97.8%), appendix masculina (88.2%) and sperm in the terminal ampullae (86.9%). The carapace length of the smallest mature male, based on the presence of sperm in the terminal ampullae, was 19.54 mm.

The seasonal occurrence of each type of rostrum in males of *A. antennatus* by size class is shown in Figure 3. In all seasons, the great majority of the individuals had short rostra (RL < 12 mm) which is in agreement with the observations on maturity already mentioned. Only in winter all three types of rostra coexisted, with short and intermediate rostra being present mainly in the smaller size classes. A few intermediate rostra still occurred in spring, while during summer and autumn, only the shortest type was present. It is suggested, therefore, that rostrum shortening of males took place during the end of winter–beginning of spring.

The seasonal occurrence of mated females bearing spermatophores showed that there was a shift starting from winter with low percentage of mated individuals (16.0%) to summer, when almost all females had already mated (99.7%). In autumn, the abundance of mated females started to decrease again (29.2%). Smaller individuals (CL < 20 mm), not yet mated, appeared in the female population mainly during winter and spring. ANCOVA revealed no differentiation in the CL-RL relationship between females with and without spermatophore (comparison of slopes: $P = 0.578$ and of intercepts: $P = 0.083$).

Discussion

A. antennatus represents an economically important resource for the West Mediterranean fisheries. The knowledge of the biology of the species in the Hellenic Ionian Sea is important for the sustainable future exploitation of the stock. The morphometric analysis and the study of the relative growth of the rostrum are studied in the present paper in a first attempt to obtain data on the reproductive biology of the species as it has already been made for feeding aspects (Kapiris et al., 2000).

Rostrum shortening of male *A. antennatus* has been described as a gradual process but marked both with seasonality and coexistence of three different (long, short, intermediate) types (Sardà & Demestre, 1989). The results of the present study indicate that a seasonal rostral shortening occurred in males during the end of winter. Mature males represented the major part of the male population in all seasons. During summer and autumn, the low number of individuals caught were all mature. Rostral shortening and the coexistence of long, intermediate and short rostra in mature males during winter has also been reported from other areas, e.g. Western Mediterranean (Sardà & Demestre, 1989), Sardenia Channel (Mura & Cau, 1989) and Western Ionian Sea (D' Onghia & Maiorano, 1996).

Although no direct evidence exists on the function of the rostrum, it is suggested that mature males require the short type of rostrum for courtship prior to the act of mating (Palombi, 1939; Bliss, 1982; Sardà & Demestre, 1989). The findings of the present study support this hypothesis, as male rostral shortening precedes gonadal ripening of females which starts in May (NCMR, 1999). The highest abundance of males was

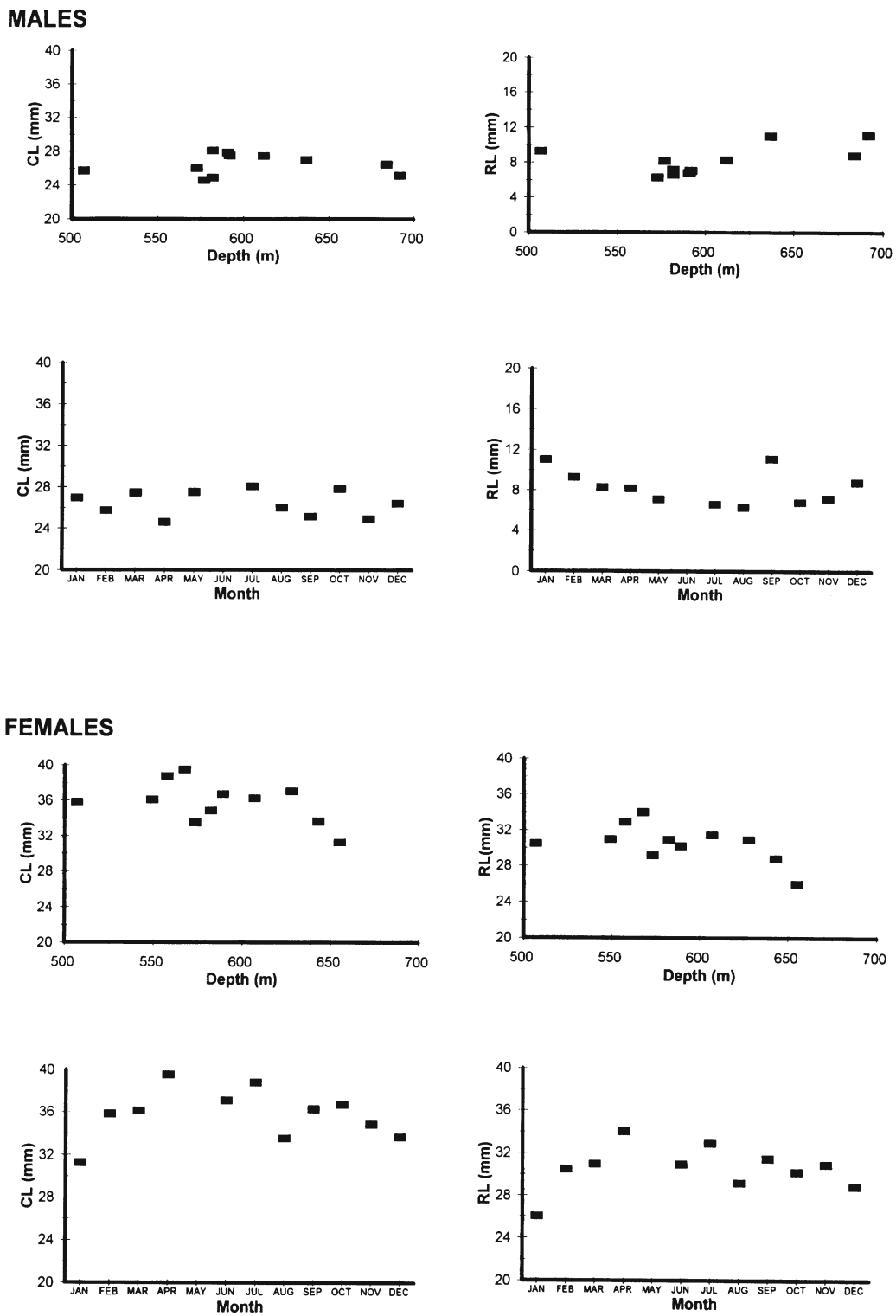

Figure 1. A. antennatus: Depth and time variations of carapace length (CL) and rostrum length (RL) in males and females.

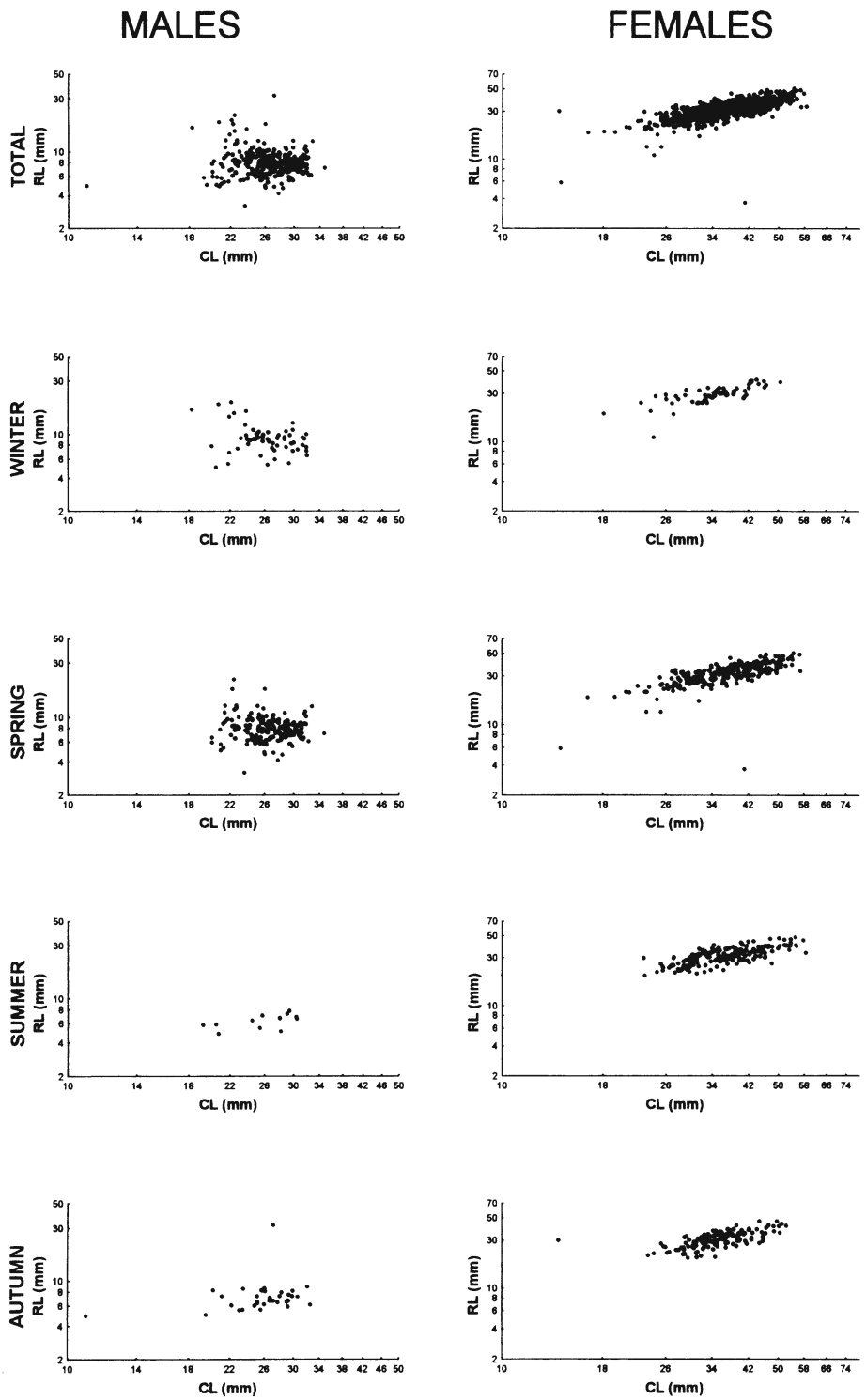

Figure 2. A. antennatus: Seasonal and pooled sample carapace (CL) vs. rostrum length (RL) relationships for males and females.

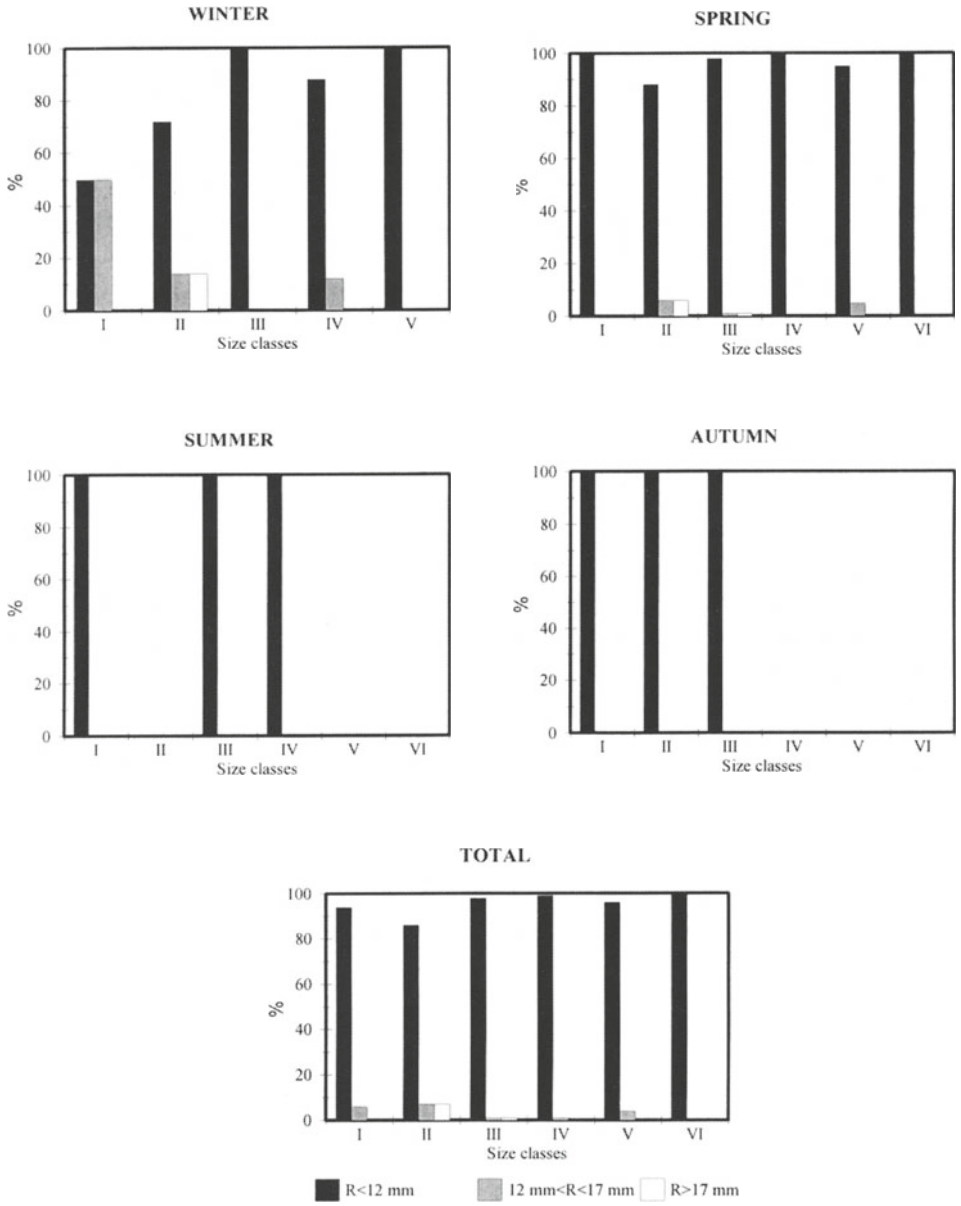

Figure 3. A. antennatus: Seasonal and pooled-sample distribution of rostrum types according to male size; I: CL ≤ 21.4 mm; II: 21.5 ≤ CL < 24.4 mm; III: 24.5 ≤ CL ≤ 27.4 mm; IV: 27.5 ≤ CL ≤ 30.4 mm; V: 30.5 ≤ CL ≤ 33.4 mm and VI: CL ≥ 33.5 mm.

found during spring when mated females increased substantially. As already found in other localities in the Mediterranean Sea, mating occurs during this season (Relini & Tunesi, 1987; Mura et al., 1992).

Rostrum shortening did not always coincide with other maturity features and it was not exclusive in a certain size range. As summarized by Sardà & Demestre (1989), many factors could play an important role on growth and maturation processes such as the irregular and relatively asynchronous molting, the extended spawning season, the number of spawnings, the prolonged period of recruitment and the duration of larval stages in the pelagic habitat.

Regarding relative growth of rostrum, females exhibited a positive relationship irrespectively of season or reproductive status (presence of spermatophore). Negative allometric growth of rostrum was found for females in all seasons. In the Western Mediterranean, the same type of allometry was observed for a pooled sample combining all seasons (Sardà & Demestre,

Table 3. Allometry of rostrum in *A. antennatus*. Values of regression parameters for the linearized allometric model $\ln Y = \ln a + b \ln X$ applied for males and females according to seasonal and pooled data; Student's t-tests for testing the type of allometry (H_o: $b = 1$)

Season	N	$\ln a$	b	SE	r	$\|t\|$	Allometry
Females							
Spring	251	1.6705	0.5601	0.0387	0.67	11.3668	negative
Summer	178	0.8866	0.7882	0.0553	0.73	3.8275	negative
Autumn	124	1.3224	0.6568	0.0700	0.64	4.9031	negative
Winter	62	1.2335	0.6825	0.0787	0.74	4.0347	negative
Pooled	615	1.4246	0.6302	0.0268	0.68	13.7961	negative
Males							
Summer	13	2.0773	0.6415	0.2721	0.57	p1.3117	isometry
Winter	66	3.5361	−0.1264	0.0566	−0.26		eniantometry

1989). This allometric relationship was proved significant in males only in two cases in the present study: a negative RL-CL relationship in winter which can be attributed to the above mentioned rostrum shortening, and a positive relationship (with isometry) in summer, which should be treated with caution because of the small sample size ($N = 13$).

In contrast to the findings from the Western Mediterranean (Sardà & Demestre, 1989) and the Western Ionian Sea (D' Onghia & Maiorano, 1996), the population sampled in the present study exhibited a shortage in males with long or intermediate rostra during the whole year. In those two localities, in a depth range similar to the present study, mature and immature individuals with long and intermediate rostra were present in high percentages in all but the summer months. The reason for this difference is not clear. Possible causes could include differences in abundance, depth distribution, migration patterns, size at maturation of males or even the use of different sampling methods. Further studies with comparable gear and in a wider depth range are needed in order to obtain more information on the male component of the population of *A. antennatus* in the Eastern Ionian Sea and test the hypothesis that the bulk of the male population undergoes rostral shortening outside the geographical locality or the depth range sampled.

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Influence of diet on sex differentiation of *Hippolyte inermis* Leach (Decapoda: Natantia) in the field

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Key words: Hippolyte inermis, shrimp, food, sex reversal, development, *Posidonia oceanica*

Abstract

The gut contents of the shrimp *Hippolyte inermis* were investigated for 1 year along a depth transect through a seagrass bed. Besides size, sex and weight of all individuals were recorded. The diets of immature and adult individuals were compared to detect any influence of food on sex development, since previous investigations indicated a correlation of the life cycle of this protandric species with the abundance of algal food in the environment, and laboratory experiments demonstrated the effect of diatoms of the genus *Cocconeis* on the direct development of females. Results indicated that the shrimp is an opportunistic herbivore, able to feed on both plant and animal items, with a preference for macroalgae and diatoms present on the leaves of *Posidonia oceanica*. Small females, deriving from direct differentiation, had a diet significantly different from that of males. The difference was due to a larger abundance of microalgae in the guts of young females. The influence of microalgal food on the sex reversal mechanism of this species, previously detected through laboratory experiments, was demonstrated to control the life cycle of *H. inermis* in the field.

Introduction

Hippolyte inermis Leach, 1815 is a small shrimp (maximum length is 25 mm) living in shallow waters of the Mediterranean Sea and along the Atlantic coast of Spain (Zariquiey Alvarez, 1968). It forms stable populations in seagrass meadows (Gambi et al., 1992), mainly in *Posidonia oceanica* and *Cystoseira nodosa,* but it is also present in other coastal environments (Guillen Nieto, 1990). Most individuals exhibit a green mimic colour, although they can shift to grey, brown or white, according to the environmental conditions (Bedini et al., 1997).

Investigations by Reverberi (1950) and Veillet et al. (1963) demonstrated individuals experiencing a male stage prior to switching to female (i.e. protandric sex reversal; Gherardi & Calloni, 1993). It is also well known (Le Roux, 1963; Regnault, 1969a) that juvenile diet is based on microalgae, but there is a lack of information about the feeding ecology of postlarvae in the field. Sex differentiation occurs at a size of 5–7 mm of total length (Veillet et al., 1963); sex reversal is observed in individuals of 10–13 mm, corresponding to the age of 7–12 months. Not all individuals exhibit sex reversal; in fact, young females of 5–6 mm size were present in natural populations (Zupo, 1994). They are smaller than any male and supposedly derive from direct differentiation. Large females, deriving from sexual inversion, were designated as *alpha* females, while small females, directly developed, were designated as *beta* females (Zupo, 1994).

Two main periods of recruitment, in spring and fall, were detected in the life cycle of *H. inermis*. Individuals born in spring grow quickly and develop as both females or males, while individuals born in fall grow slowly and develop as males, reverting sex in the next spring. A significant relationship between epiphyte abundance in *Posidonia oceanica* meadows and the frequency of ovigerous females was demonstrated (Zupo, 1994). Moreover, the period of maximum abundance of *beta* females in natural populations corresponds to a massive epiphytic production in the leaf stratum of *P. oceanica* (Mazzella & Buia, 1989). Laboratory experiments (Zupo, 2000) demonstrated that diatoms of the genus *Cocconeis* allow the direct development of *beta* females. Therefore, mi-

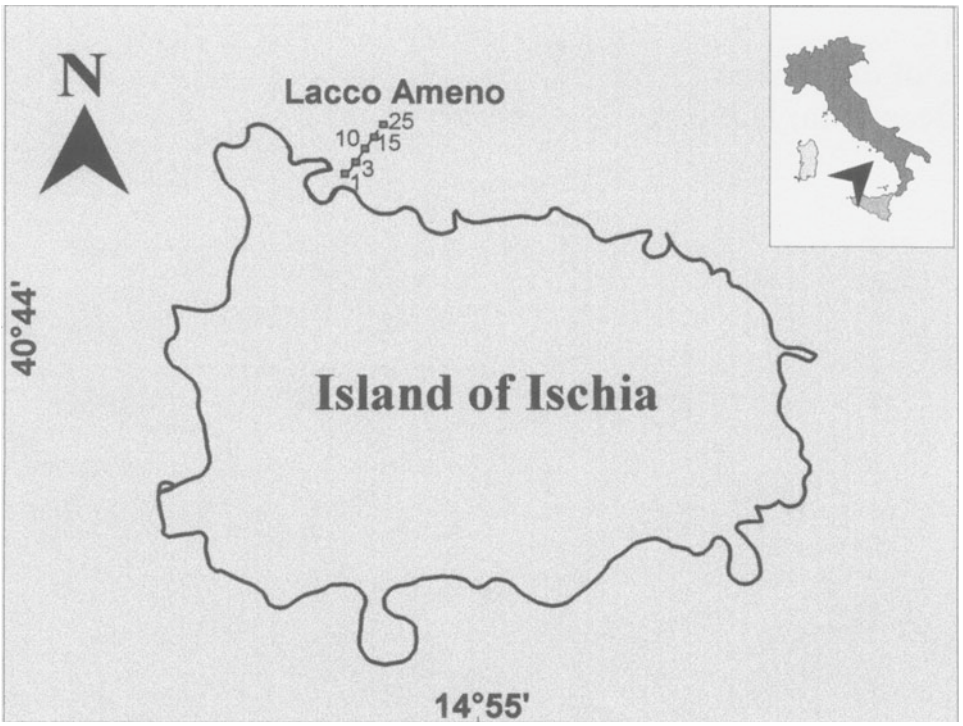

Figure 1. The sampling area in Lacco Ameno d'Ischia (Gulf of Naples, Italy). Small squares in the Lacco Ameno area represent the sampling points, along a depth transect, at 1, 3, 10, 15 and 25 m.

croalgal food influences the life cycle of *H. inermis* in the laboratory and it may play a role, also in the field, in the sex differentiation of this species. Therefore, a field investigation was devised to study the relationships existing between the diet of shrimps during the phases of sex development and the pattern of abundance of *alpha* and *beta* females.

Materials and methods

Two replicate samples of *Hippolyte inermis* were monthly collected, during 1 year, by a hand-towed net (0.4 mm mesh size) in Lacco Ameno d'Ischia (Gulf of Naples, Italy) along a depth transect at 1, 3, 10, 15 and 25 m depth (Fig. 1). This method (Ledoyer, 1962; Russo & Vinci, 1991; Gambi et al., 1992) is considered to be best suited to collect vagile organisms associated with the leaf stratum. It is semi-quantitative and allows to collect a large number of individuals to submit to further analyses. After collection, animals were deep frozen to prevent digestion and successively fixed in 70% alcohol (Zupo & Fresi, 1985). After identification, the total length of all individuals (except damaged specimens in which total length or sex could not be determined) and their sex were recorded. The total length was measured from the tip of the rostrum to the posterior medial notch of the telson, pressing each specimen against a metal ruler, to obtain a measure of bodies at the maximum extension. Sex was determined based on the form and the presence of setae on pleopod II (Fernandez-Muñoz & Garcia-Raso, 1987). Intra- and inter-sample similarity was evaluated by performing a mean linkage cluster analysis on the central scalar product matrix obtained from the 'size *vs.* stations' matrix (Orloci, 1978).

All shrimps belonging to the 'critical' sizes for the study of sex reversal (Zupo, 1994) were selected: 3 mm (all juveniles); 7 mm (juveniles, males and females); 17 mm (all females). The selected individuals were dissected and their guts were analysed using an optical microscope, to identify all prey up to the lowest taxonomic level. The abundance of each food item was evaluated using an ocular reticule. The gut contents were homogeneously dispersed on a microscope slide and the percentage of squares containing at least one fragment of each item was recorded. A matrix 'individuals *vs.* gut contents' was obtained and analysed

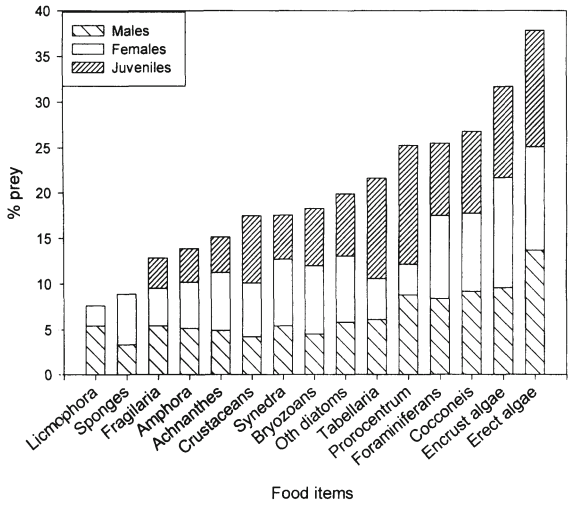

Figure 2. Percent abundance of food items found in the gut contents of juveniles, females and males, after pooling all yearly data.

by multivariate statistical analyses. Average linkage cluster analysis (Orloci, 1973) and Correspondence Analysis (Benzecri, 1980) were performed on the data matrix, to investigate the relationships existing among diet, sex and size of individuals. The results, expressed for each of the five depths, were used to investigate the correlations between the average abundance of food items in the gut contents and the spatial and temporal distribution of *alpha* and *beta* females. Kruskal-Wallis one-way analysis of variance was performed to test differences among gut contents assemblages obtained for different months, depths and sizes. Chi-square tests were performed on proportions of microalgae, macroalgae and animal items found in the gut contents, to test the significance of differences among gut contents found in different months.

Moreover, the dry weight of the gut contents, grouped by sex, size and sample, was measured after washing in distilled water and filtering the suspension through pre-weighed, dried (90 °C) *Millipore* membranes (0.45 μm). These data were used to compare the weight of gut contents and the sex of individuals of different sizes.

Results

Cluster analysis revealed high levels of similarity of the replicates for each station; therefore the replicates of each station, for each month, were pooled. In total, 213 individuals (60 immatures, 76 males, 77 females) belonging to the 'critical' sizes for the study of sex reversal were selected, and their gut contents were analysed. The analysis of gut contents revealed a similar pattern in the abundance of food items among the diet of males, females and immature individuals (Fig. 2). Kruskal-Wallis test indicated no significant differences among the gut content assemblages observed at different depths or in individuals of different size. Also the differences among the diets of sexually immature individuals, males and females were not significant. Diatoms of the genus *Licmophora* and sponge tissues, however, were present only in the diet of adult individuals (Fig. 2).

Microalgae were consumed by all individuals, but immature shrimps fed preferentially on some dynoflagellates, such as *Prorocentrum*. Erect and encrusting algae represented common and abundant items for all the individuals, although animal prey, represented by sessile species living on the seagrass leaves or small animals living in the epiphytic layer, were present in most guts. When the total abundance of all items was considered (Fig. 2), macroalgae and diatoms of the genus *Cocconeis* (two common items in the leaf stratum) were the most consumed items, followed by animal prey (foraminiferans), and other microorganisms. Sponges and such diatoms as *Licmophora* and *Achnanthes* were the least preferred items. Prey easy to detach, such as erect algae and foraminiferans, were preferred to motile prey, such as small crustaceans (mainly copepods).

The abundance of microalgae in the guts was higher at shallowest depth (1 m), where a higher abundance of dynoflagellates of the genus *Prorocentrum* and diatoms of the genus *Amphora* were found (Fig. 3a). The highest abundance of macroalgae was found in guts collected at 10 m depth (Fig. 3b), coinciding with a decrease in the gut abundance of microalgae. Erect algae were constantly preferred to encrusting algae. Animal prey, mainly represented by foraminiferans and bryozoans, were consumed at all depths (Fig. 3c).

Taking into account the seasonal trend of the appearance of each item in the guts (Fig. 4), diatoms of the genus *Cocconeis* were consistently the most common microalgal prey, with maximum abundance in April–May, the period of higher occurrence of *beta* females. Macroalgae followed a trend similar to that of the plant life cycle (Fig. 4b); however, erect algae were consistently preferred to crustose ones. Among animal prey, foraminiferans were the preferred item in several months, although bryozoans were abundant in the gut contents all over the year (Fig. 4c). Crusta-

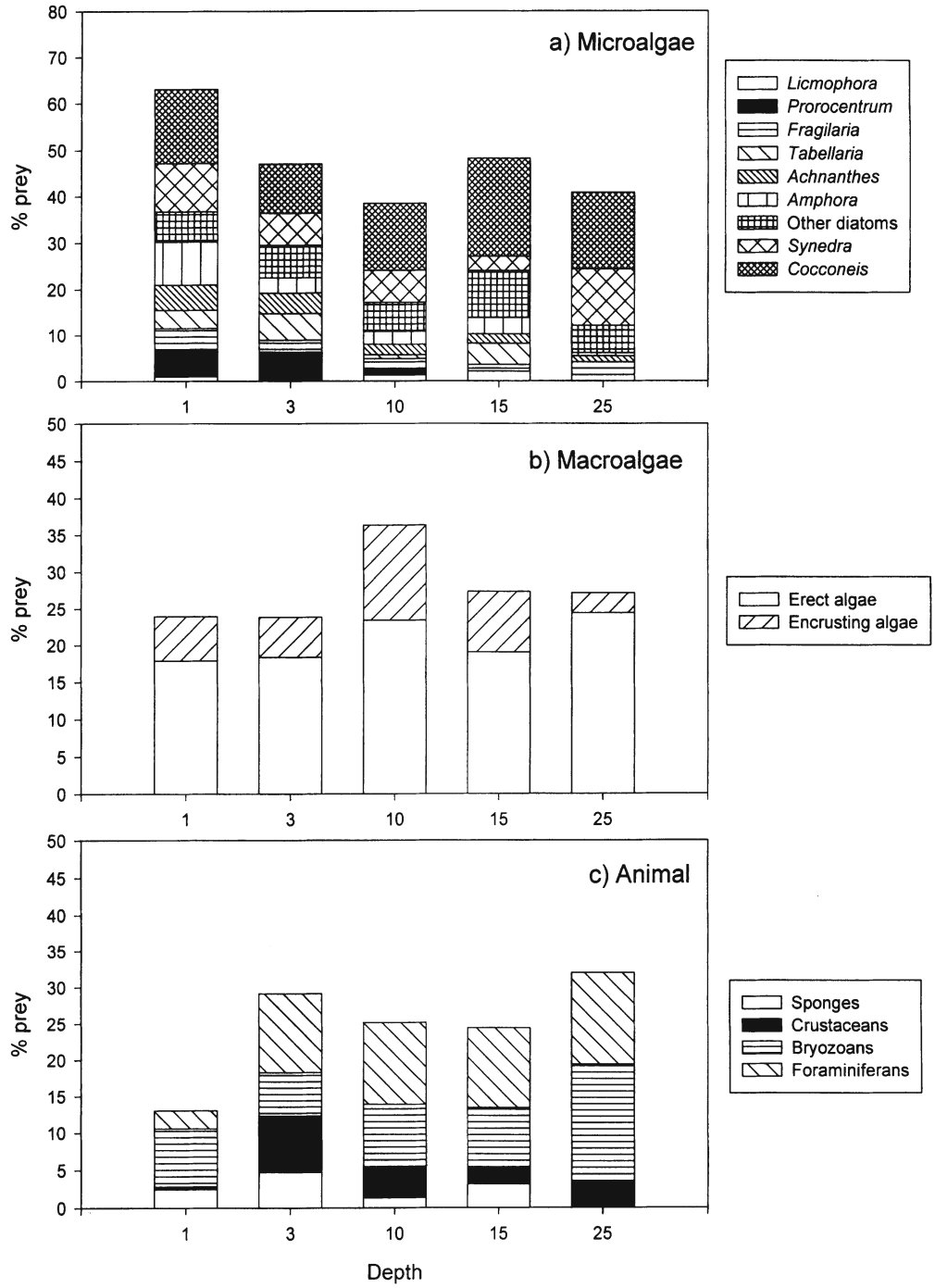

Figure 3. Percent abundance of food items found in the gut contents, along the depth transect, separated in the main food categories: (a) microalgae; (b) macroalgae; (c) animal prey. Data were pooled for all individuals sampled.

ceans were particularly abundant in summer and fall, coinciding with the reproductive burst of some species of harpacticoid copepods. Chi-square tests performed on proportion of microalgae *vs.* other prey indicated that in April and May the proportion of microalgae in the diet was significantly different ($p<0.001$) from all the other months (Figs 5a, b). Similar tests, performed for all other months, indicated no significant differences. Also the differences between the proportions of

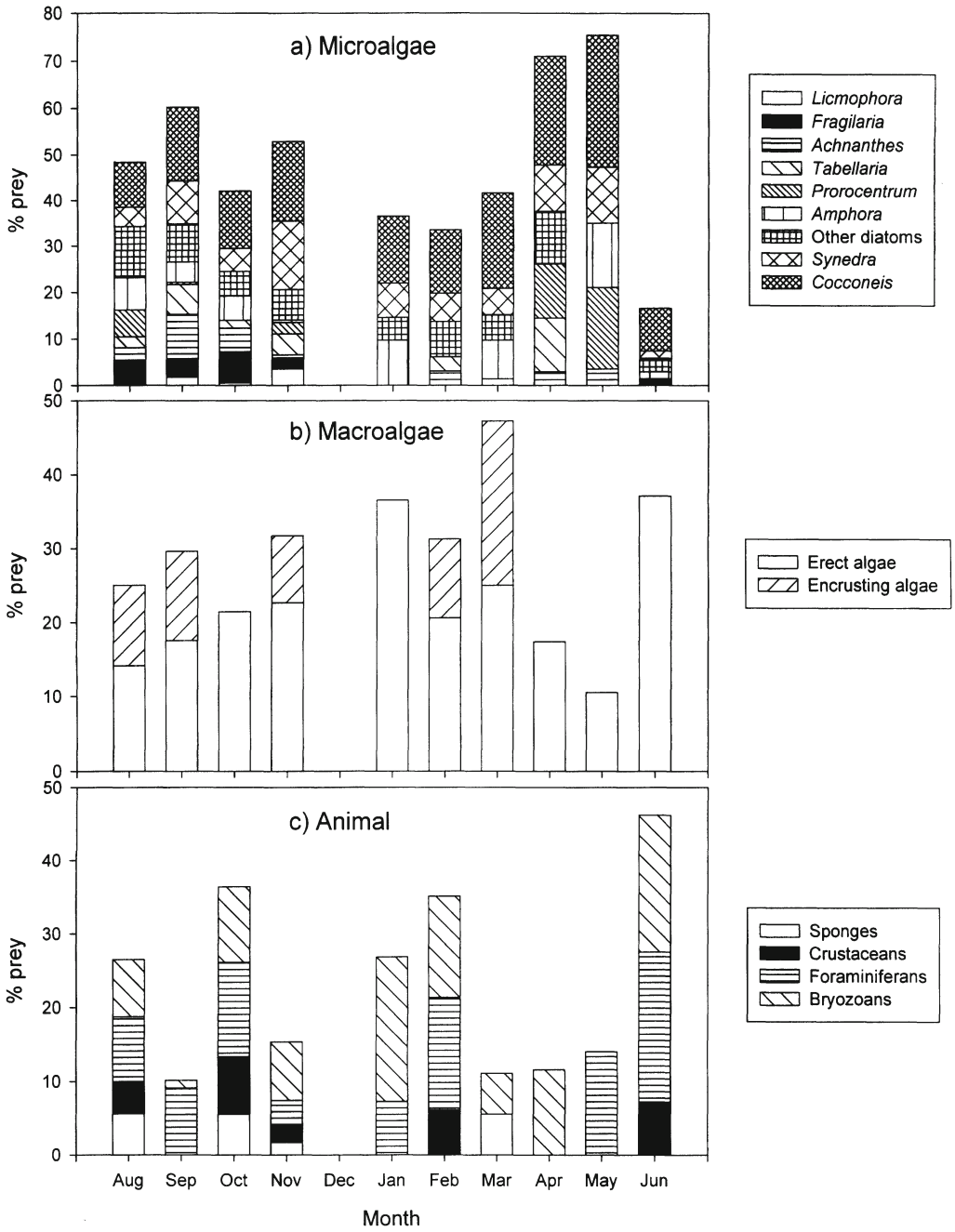

Figure 4. Monthly percent abundance of microalgae (a) macroalgae (b) and animal prey (c) found in the gut contents. Data were pooled for all individuals sampled.

microalgae and other prey, performed among different depths, were not significant.

A correspondence analysis performed on the matrix individuals *vs.* food items indicated two important poles (Fig. 6), defined by the first two factors. In the first quadrant, the carnivore pole, identified by all animal prey; in the second quadrant, the diatom pole; macrophytes were ordered centrally and linked to small males. Small females (*beta* females) were linked to the diatom pole, while large females (*alpha* females) were linked to the animal pole. Immatures were ordered in the third and fourth quadrant, dragged down by the item '*Prorocentrum*' and linked to diatoms such as *Tabellaria*. Diatoms of the genus *Coc-*

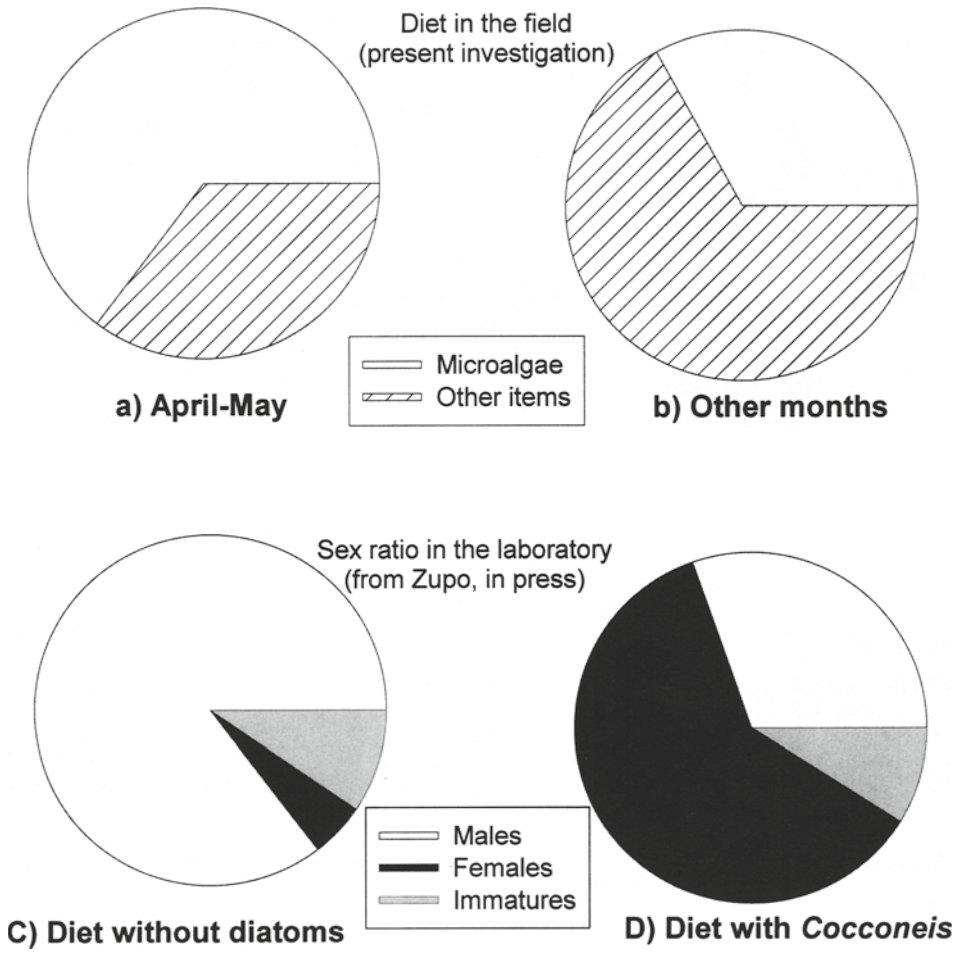

Figure 5. Percent abundance of microalgae vs. all other items in the gut contents of individuals sampled in the period of development of *beta* famales (a) and in all other months of sampling (b), compared to the results obtained in the laboratory (45 days after hatching), in a previous investigation (from Zupo, 2000) in which *H. inermis* was fed with diets not containing diatoms (c) and with the addition of diatoms of the genus *Cocconeis* (d).

coneis and *Tabellaria* were ordered centrally among immature individuals (J3) and young shrimps at the age of differentiation belonging to both sexes (F7, M7), although young males were closer to macroalgae and young females were closer to the diatom pole. A correspondence analysis performed on the matrix gut contents *vs.* depth confirmed the importance of *Cocconeis* and macroalgae, consumed at all depths. Whenever possible, plant materials were the preferred prey. The analysis, however, showed that the abundance of food items in the guts was also in accordance to the depth distribution of prey in the field: the abundance of some animal prey in the guts increased with depth, in accordance with decrease of plant prey.

Results of cluster analysis performed on the matrix individuals *vs.* food items indicated three main groups of observations (Fig. 7). The first group contained only large females (17 mm), separated at a high level of variance. The second group was composed of individuals of 3 and 7 mm, which were further split into two clusters. The first sub-cluster contained juveniles and small females; the second contained juveniles and males.

There was a significant relationship between size of individuals and weight of their guts (r=0.98). Dry weight of guts, in the period of sex maturation, ranged from 0.030 mg ±0.01 (males), to 0.035 mg ±0.01 (juveniles) to 0.045 mg ±0.02 (females). Females exhibited the largest variability in gut weight, reaching 0.1mg ± 0.04 at a size of 17 mm. The smallest juveniles (size 3 mm) exhibited the lightest guts (0.02 mg ± 0.01). No significant differences were detected among

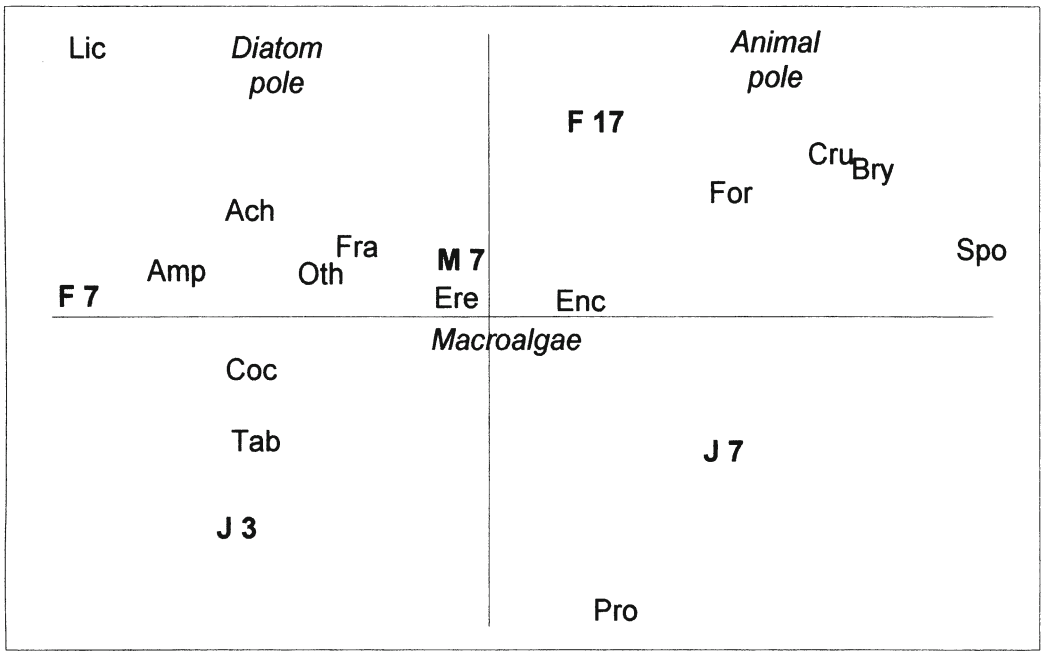

Figure 6. Results of the Correspondence Analysis performed on the matrix 'individuals vs. gut contents'. Observation and variable points are represented in the space defined by the first two significant axes, F1 and F2. Individuals of different size and sex are represented in bold (J3= juveniles of 3 mm size; J7=juveniles of 7 mm size; M7= males of 7 mm size; F7= females of 7 mm size; F17= females of 17 mm size). Acronyms refer to food items: Lic= *Licmophora*; Amp= *Amphora*; Ach= *Achnanthes*; Fra= *Fragilaria*; Coc= *Cocconeis*; Tab= *Tabellaria*; Pro= *Prorocentrum*; Oth= Other diatoms; Ere= Erect algae; Enc= Encrusting algae; For= Foraminiferans; Cru= Crustaceans; Bry= Bryozoans; Spo= Sponges. The three main diet poles in the plot are indicated in italics.

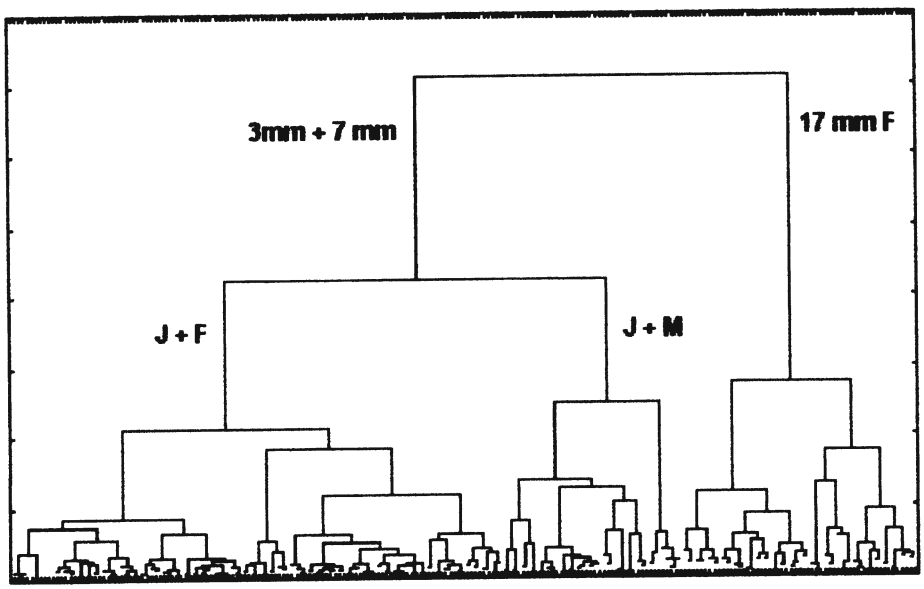

Figure 7. Results of the cluster analysis performed on the matrix 'individuals vs. gut contents'. Individuals are grouped on the basis of their gut contents. 3 mm, 7 mm and 17 mm indicate groups of individuals with total length of 3, 7 and 17 mm, respectively. M= males; F= females; J= immatures.

the gut weights of females, males and juveniles of the same size.

Discussion

The data obtained establish that *Hippolyte inermis* feeds on both plant and animal items. Besides slight differences in the diet between adults and juveniles or individuals of recent maturation, the patterns of abundance of food items in the guts appear consistent, when all yearly data are pooled. Erect and encrusting algae are an important dietary component and they are present, at the same abundance, in guts of individuals of different ages. A slight difference in the consumption of macroalgae was observed along the depth transect. The trend coincides with the pattern of abundance of erect and encrusting algae in the shallow meadow (Mazzella & Ott, 1984). An increase in the consumption of erect macroalgae was observed at the deep meadow (25 m), where a lower abundance of this item was recorded in the field (Mazzella & Ott, 1984). This finding may be explained observing, at the same depth, a higher consumption of animal prey (mainly bryozoans and foraminiferans) and a lower presence of diatoms in the gut contents: at higher depths, *H. inermis* integrates the lack of microalgae in the field (Buia et al., 1992) with a higher consumption of macroalgae and animals. It is notable that the abundance of macroalgae and animal prey increased consistently with the decrease in the abundance of microalgae. Microalgae appear to be the preferred item, but they may be replaced by other prey when their abundance in the field decreases.

Due to this opportunistic strategy, the gut weight of individuals collected at different depths is consistent, according to size. In fact, the amount of food in the guts is not related with sex changes nor depth, as it was demonstrated that no significant difference exists among the different experimental groups. The amount of food ingested is only related to the size of shrimps and animal prey are a diet integration, as they were present in small abundance, coinciding with seasonal burst of animal communities (Mazzella et al., 1989) and the depth distribution of fauna in the leaf stratum of *P. oceanica*. This diet pattern is common in several species of natantian decapods (Nelson, 1981; Zupo & Fresi, 1985; Gutierrez-Yurrita et al., 1998). This also explains the consistency in the abundance of different items in the gut contents of immatures, males and females, when the yearly data are pooled. In fact, the diet pattern of *H. inermis*, averaged over 1 year, indicates that this shrimp is a polytrophic species.

The diet of males and females during the period of sex maturation of *beta* females, however, is different, as demonstrated by the cluster analysis performed on prey items. The first separation in the cluster three, between large females (*alpha* females) and all the other individuals, is probably due to the shrimp size; larger individuals feed on larger prey. The second separation pools all the smaller individuals (3 + 7 mm), and segregates immatures and young males from immatures and young females (*beta* females) on the basis of their diets. The difference may be due to seasonal variations in the abundance of some items in the field, as most *beta* females are present only in spring, while males are produced in both of the reproductive bursts (in April–May and September). Therefore, there are items in the meadow of *P. oceanica*, more abundant in spring, having an influence on the sex determination of *H. inermis*. These items may be identified among the microalgal prey, as a significantly higher abundance of microalgae was found in the gut contents of individuals sampled in April and May (when *beta* females appear in natural populations). These results are in accordance with the sex reversal mechanism described through laboratory experiments (Zupo, 2000), demonstrating that diatoms of the genus *Cocconeis* influence the direct development of *beta* females (Fig. 5c, d).

H. inermis, however, was demonstrated to be sensitive to several environmental cues, at least during larval development (Regnault, 1969 b), and the influence of other factors (Aréchiga & Rodriguez-Sosa, 1997), variable according to seasons, may not be excluded (Edgar, 1983, 1990), although similar light and temperature values were found along the depth transect, at the same site, in April and September (Zupo et al., 1997). Le Roux (1963) also hypothesised that the 'transitory stages' between the larval stage IV and the last zoea before the postlarva, were a consequence of type and abundance of food.

The Correspondence Analysis supports previous findings. In fact, *alpha* females (F17) are linked to animal prey and macroalgae; young males (M7) lie in a central position, in the factorial space defined by F1 and F2: they fed on all possible items, mainly on macroalgae; in contrast, young females (*beta* females) are positioned in the microalgae pole. This pattern indicates that the diet of *beta* females is based on microalgae, although the species is able to feed on different items. There are factors, in the group of diatoms contained in the microalgae pole (*Licmophora*,

Achnanthes, *Fragilaria*, *Cocconeis*), that trigger the direct development of *beta* females.

Diatoms have been demonstrated to influence the ecology and the life cycle of other crustaceans (Miralto et al., 1995). Egg production and hatching success in individuals of the copepod *Centropages typicus* decreased when diatoms were fed to adult females (Ianora et al., 1995). Moreover, the harmful impact of a diatom (*Thalassiosira rotula*) on the reproductive biology of the copepod *Calanus helgolandicus* was demonstrated (Poulet et al., 1994). The production, by several species of diatoms, of compounds detrimental to the development and survival of grazers, may have major implications on secondary production (Miralto et al., 1996). However, in this species, other effects could be possible (Adiyodi & Adiyodi, 1970), according to co-evolutionary processes (e.g. apoptotic disruption of the male gonadic bud, allowing a direct development as female). In fact, *H. inermis* is largely adapted to the life in *P. oceanica* (d'Udekem d'Acoz, 1996) and the toxic effect of diatoms could be translated, in this species, in a spring signal for the development of *beta* females. It was demonstrated that the presence of *beta* females is a crucial factor in maintaining a constant sex ratio, allowing the September reproductive burst (Zupo, 1994). Therefore, the presence of *beta* females in the spring population, triggered by the feeding on species of diatoms abundant in this season, may be an adaptive strategy to provide a sufficient number of females for the next mating period in September.

The results of the present investigation reinforce previous findings, suggesting a seasonal correlation of the life cycle of *Hippolyte inermis* with the pattern of abundance of epiphytic algae in *Posidonia oceanica* meadows. They are in accordance with results obtained in the laboratory (Zupo, 2000), indicating a direct effect of diatoms on the development of *beta* females in this species. Maximum breeding intensity was observed in spring (Zupo, 1994), when the abundance of epiphytes in the leaf stratum is high (Mazzella et al., 1989). In the same season the largest burst of *beta* females was found and data of the present investigation confirm that some microalgae, very abundant in the field, are actively selected in the diet of young shrimps and correlated to the production of *beta* females undergoing a mechanism of direct development.

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Combined effects of temperature and salinity on the larval development of the estuarine mud prawn *Upogebia africana* (Crustacea, Thalassinidea)

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Key words: temperature, salinity, larval development, estuaries, *Upogebia africana*

Abstract

The larval stages of the mud prawn *Upogebia africana* were reared in the laboratory, from hatchings of females collected in the Mgazana estuary, South Africa. The larvae were tested for the combined effects of temperature and salinity in a factorial designed experiment, using 3 females and 2 replicates of 10 larvae per combination. Combinations were made from 5 temperatures (15, 20, 25, 30 and 35 °C) and 4 salinities (15, 25, 35 and 45). Results were tested by ANOVA and multiple regression was applyed to generate contour models by polynomial equation. Results showed that *U. africana* develops optimally in near to sea water salinity at around 25 °C, with slightly wider tolerance to low salinity in zoeal stage I, and with increased moult rate at lower salinity in late stages. A comparison with similar experimental results for other species is made, namely in view of the life cycle strategies for dispersal and return migration.

Introduction

The larval stages of mangrove crustacean species, and estuaries in general, undergo development under a range of environmental conditions which affect differentially their survival and growth. Several mechanisms account for these stress factors, and throughout development species usually exhibit migratory patterns that position larvae in optimal water masses for feeding, growth and osmoregulatory needs. As osmoregulatory capacities develop through the sequence of larval stages, most estuarine species migrate rapidly after hatching to neritic areas, thus avoiding the changing estuarine environment at an early stage (e.g. *Carcinus maenas*: Queiroga, 1996). Some will undergo complete or partial larval development within the estuarine water masses (e.g. *Palaemon longirostris*, *Crangon crangon*: Paula, 1998), though early and late incoming stages often experience a degree of temperature and salinity fluctuation.

Decapod species are much diversified in the range of habitats in which they live, from marine to freshwater, although only a few (e.g. *Macrobrachium australiense*: Lee & Fielder, 1981; *Palaemon paucidens*: Fidhiary et al., 1991) can reach juvenile stages when reared in freshwater. Other freshwater species either migrate to brackish water to release larvae (e.g. *Palaemon longirostris*: Cartaxana, 1994; *Armases roberti*: Diesel & Schuh, 1998) or hatch as juveniles, when osmoregulatory capacities are more effective (e.g. Read, 1986). Estuarine releasing species, even if the whole series of stages is retained within the estuarine boundaries, have optimal development in high salinity and generally present an initial wider tolerance range (e.g. *Palaemon longirostris*: Paula, 1993). However tolerance varies between species, each with its more or less narrow optimal range. Species which present a pattern of exporting newly-hatched stages from the estuarine environment undergo most development in sea water, but up to a certain degree may tolerate lower salinity.

Among the Thalassinidea, a few works have been published relating to temperature and salinity tolerance of larval stages, such as *Callianassa kraussi* (Forbes, 1978), *C. tyrrhena* (Thessalou-Legaki, 1990) and *C. japonica* (Miyabe et al., 1998).

Upogebia africana is a common thalassinid in estuaries of South Africa and Mozambique, forming locally dense populations on the edge of salt marshes and mangrove channels. Emmerson (1983), Wooldridge (1994) and Wooldridge & Loubser (1996) studied the larval dynamics of *U. africana*, showing that newly-hatched stages are flushed to the ocean during nocturnal ebbing tides, and that return migration is accomplished by the megalopal stage. Emmerson (1983) states that the larval sequence of stages was yet to be described at that time.

The objective of this work was to study the temperature and salinity effects on the larval development of *Upogebia africana*, in relation to survival and duration of larval stages.

Materials and methods

Female collection

Ovigerous females of *Upogebia africana* were collected at Mgazana estuary, Transkei coast in South Africa. *Upogebia africana* was sampled at the edge of the mangrove creeks, by hand digging in the mud. Ovigerous females were transported to the marine laboratory of University of Transkei, Umtata. The prawns were maintained individually and unfed at 25 °C and at a salinity of 35 until larvae hatched.

Experimental design

The experiment followed a factorial design, and combinations were made from 5 temperatures (15, 20, 25, 30 and 35 °C) and 4 salinities (15, 25, 35 and 45). In each combination, 3 random females were used from a set of 6 hatchings, each with 2 replicates of 10 larvae in separate bowls. Larvae were chosen among the most active of the respective batch. Each replicate was cultured in a 400 ml glass bowl. Water was periodically brought from the shore, and filtered in a series of Millipore filters down to 0.5 μm. Dilution used deionised water and concentration was obtained by evaporation. Temperatures were maintained in enclosed chambers with 0.5 °C precision.

Larvae were transferred to new media each day, and fed on newly-hatched *Artemia* nauplii. While transferring, larvae were checked for mortality and sorted by developmental stage. At high temperatures (30 and 35 °C), the cultures were covered with adherent plastic to prevent evaporation and consequent salinity increase. Experiments lasted until juvenile stage was reached.

Statistical methods

Temperature and salinity effects were tested by 2-way ANOVA based on replicate cultures. A multiple regression was performed using average values by temperature and salinity combination (linear, quadratic and intersection effects). Percent survival values were transformed with the arcsin transformation (arcsin v proportion). Regression coefficients were used in the polynomial expression to generate a surface response contour (Box & Youle, 1955):

$$Z = b_0 + b_1 T + b_2 S + b_{11} T + b_{22} S + b_{12} T S,$$

where Z is the surface response, T is temperature, S is salinity, b_0 is multiple regression constant, b_1 and b_2 are the linear effects of temperature and salinity, b_{11} and b_{22} are the quadratic effects, and b_{12} is the intersection effect. Contour plot is shown for zoeal stage I survival only, as contraction of experimental universe over the larval sequence, due to total mortality in extreme temperature and salinity combinations, makes the presentation as mainly extrapolative.

Results

The cumulative sequence of larval stages obtained in the various temperature and salinity combinations is presented in Figure 1. The larval series of *Upogebia africana* consists of 3 or 4 zoeal plus 1 megalopal stages. In view of the small numbers of zoeal stage IV in the experiment and its facultative nature, results for zoeal stage III combine this third moult.

For the ranges of temperature and salinity tested, the former mainly affects the survival of the larval stages. The lowest (15 °C) and highest (35 °C) temperatures at all salinities showed complete mortality for stage I, with the larvae surviving longer at 15 °C (up to 30 days), while at 35 °C total mortality occurred during the first day. The lower salinity combinations also reveal a strong negative effect on survival, though at 25 °C megalopal stages were obtained. The combinations which produced juvenile stages were between

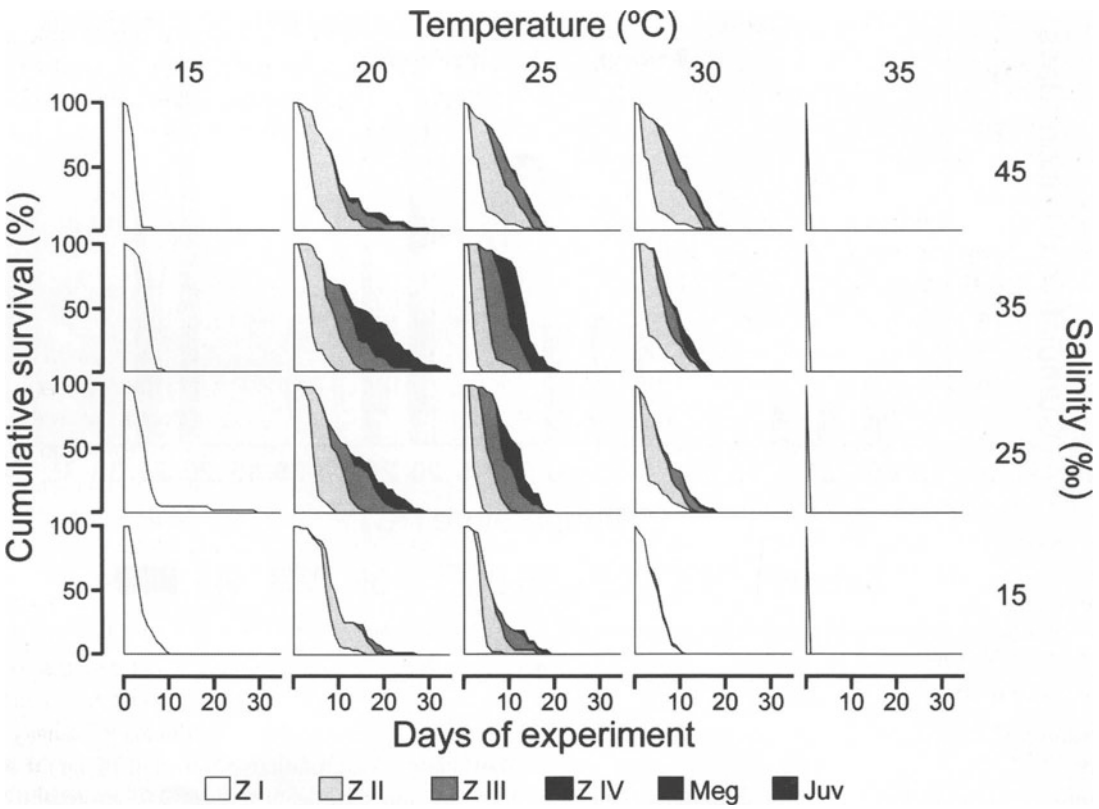

Figure 1. Cumulative survival of sequence of developmental stages of *Upogebia africana* obtained in the temperature and salinity combinations. ZI – ZIV – zoeal stages I – IV, Meg – megalopa, Juv – first juvenile.

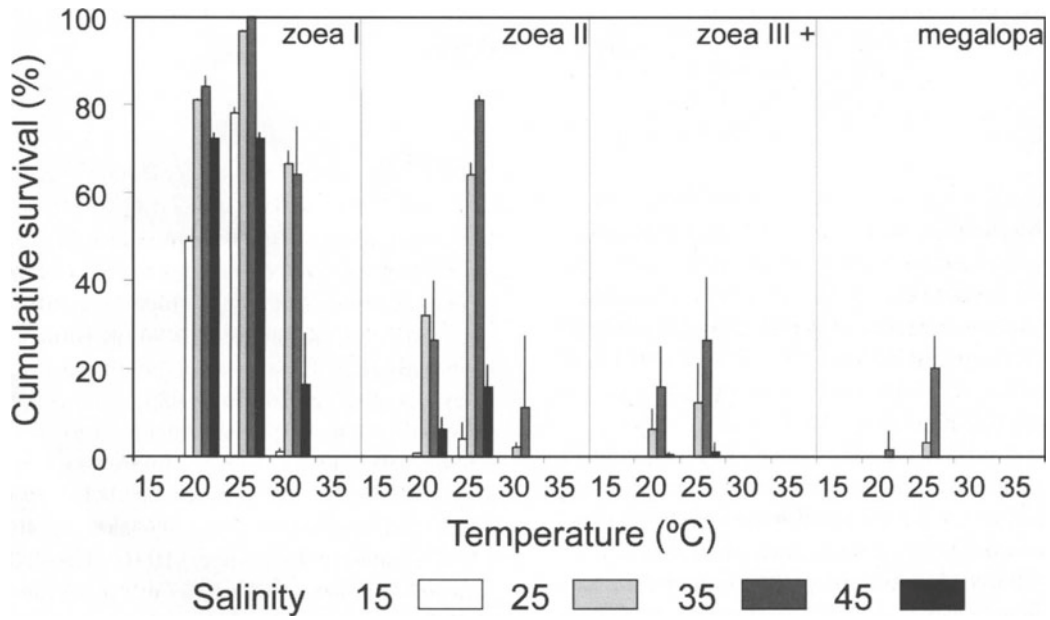

Figure 2. Average cumulative survival of sequence of larval stages of *Upogebia africana* obtained in the temperature and salinity combinations. Error bars refer to standard error.

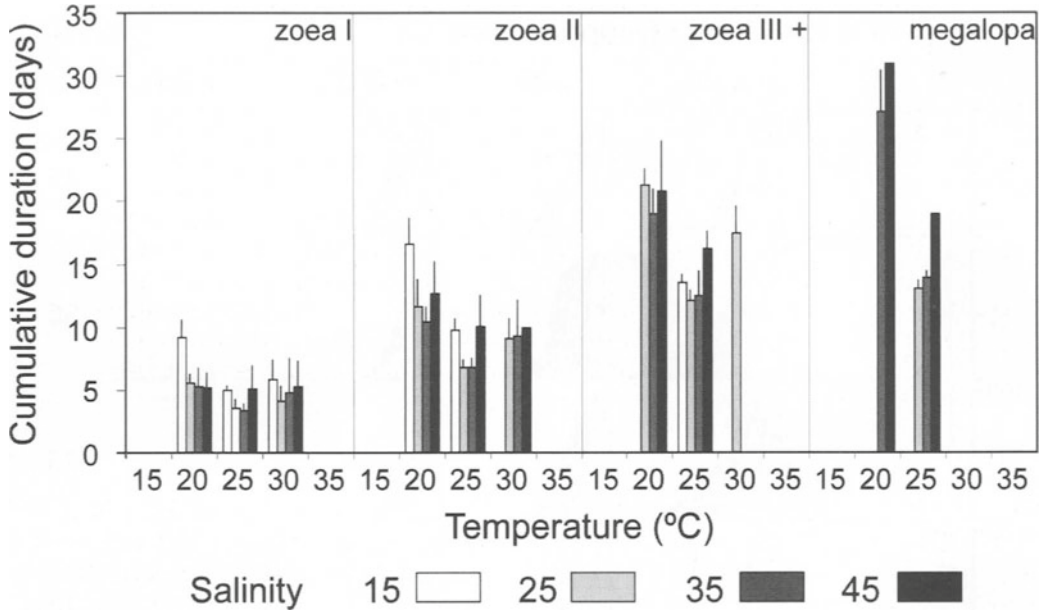

Figure 3. Average cumulative duration of sequence of larval stages of *Upogebia africana* obtained in the temperature and salinity combinations. Error bars refer to standard error.

temperature of 20 and 30 °C and between 25 and 35 salinity.

Figure 2 presents the results of cumulative survival in the temperature and salinity combinations for each larval stage. In all zoeal stages, the highest survival was at 20 and 25 °C (with maximum at 25 °C), with decreased towards the salinity extremes tested. At 30 °C, survival was significantly lower, and larvae seem to be very sensitive to salinity extremes. This pattern is consistent to all larval stages. At 15 and 35 °C, mortality for the newly-hatched stage was 100%.

The results of cumulative duration of larval stages are consistent with the results obtained for survival, i.e. the optimal points for survival are coincident with the highest developmental rate up to a certain degree (Fig. 3). Again, extremes of salinity are the most adverse with increased developmental rates, but lower (20 °C) rather than higher (30 °C) temperature seems to influence the moulting process most.

Figure 4 presents the contour plot for cumulative survival obtained by the polynomial expression based on multiple regression for zoeal stage I. Optimal central area is around 25 °C temperature and between 25 and 35 salinity. Most of the regression coefficients are significant (Table 1). The intersection effect is always non significant at 0.05 probability level, as are all effects involving salinity at megalopa stage.

Table 2 presents the significance of regression coefficients for cumulative duration of larval stages, the only non significant term at 0.05 probability level is the intersection effect in zoeal stage II. All other terms are significant. A slight shift in the optimal point at late zoeal development towards lower salinity can be observed. It was impossible to test for the megalopal stage because of limited survival.

Discussion

Variability in larval stage number is a relatively common feature among certain groups of decapod crustaceans. Carideans show the highest number of documented cases (Knowlton, 1974), as for instance palaemonids (e.g. Broad, 1957; Fincham, 1977, 1979; Antonopoulou & Emson, 1989; Paula, 1993) and crangonids (e.g. Criales & Anger, 1986; Villamar & Brusca, 1988; Paula, 1993), although we can find examples from the anomurans (e.g. Boyd & Johnson, 1963) and brachyurans (e.g. Costlow & Bookhout, 1959; Costlow, 1965; Anger, 1991). The thalassinids *Naushonia crangonoides* (Goy & Provenzano, 1978), *Callichirus major* (Strasser & Felder, 1999) and *C. islagrande* (Strasser & Felder, 2000) may develop an extra stage, and *Upogebia africana* has shown to de-

Table 1. Results of multiple regression and associated statistics for cumulative survival of larval stages of *Upogebia africana* T = temperature, S = salinity

	Zoea I	Zoea II	Zoea III	Megalopa
R^2	0.9248	0.7882	0.5976	0.3850
F	34.4450	10.4210	4.1570	1.7530
p	0.0001	0.0002	0.0159	0.1873
intercept	−7.731 (p=0.0001)	− 5.102 (p=0.0001)	− 2.981 (p=0.0033)	− 1.829 (p=0.0319)
T	0.659 (p=0.0001)	0.373 (p=0.0001)	0.198 (p=0.0034)	0.113 (p=0.0434)
S	0.067 (p=0.0424)	0.092 (p=0.0168)	0.070 (p=0.0372)	0.046 (p=0.1227)
T^2	− 0.013 (p=0.0001)	− 0.008 (p=0.0001)	− 0.004 (p=0.0023)	− 0.002 (p=0.0372)
S^2	− 0.001 (p=0.0246)	− 0.002 (p=0.0104)	− 0.001 (p=0.0365)	− 0.001 (p=0.1326)
$T \times S$	0.000 (p=0.8577)	0.000 (p=0.9974)	− 0.000 (p=0.6949)	− 0.000 (p=0.7702)

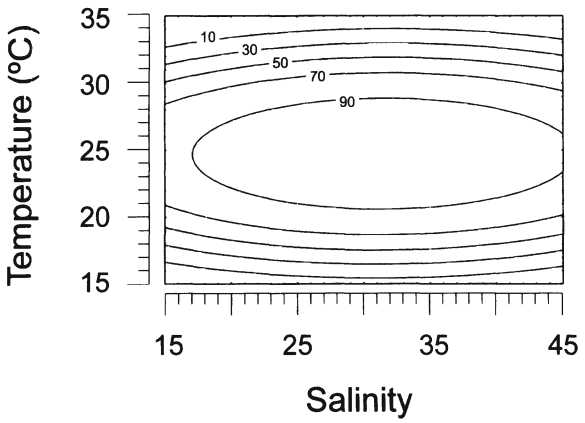

Figure 4. Contour plot for cumulative survival of first larval stage of *Upogebia africana*. Contour line numbers refer to percent survival.

velop successfully to megalopal stage both with 3 or 4 zoeal stages.

The presence of an eventual third zoeal moult in the present experiment was regarded initially as an additional stage induced by the experimental environment. However, after analysis of full results, it is clear that this fourth zoeal stage only occurs around the optimal combinations of temperature and salinity, namely those that produced higher survival of the total larval series. This suggests that this stage could be a normal instar in the sequence of stages occurring in the natural environment, though whether it is obligatory or facultative remains unclear at this stage. There are, however, in the literature indications of genetic and environmental induced changes in the number of developmental stages among thalassinids. Stresser & Felder (2000) reported 4 or 5 zoeal stages for *Callichirus islagrande*, in different proportion according to parental female. In *Callichirus major*, however, if zoeal stage IV is provided with a combination of appropriate settlement cues will moult directly to decapodid stage (Stasser & Felder, 1999). Also, those larvae which moulted to zoea V had higher incidence of mortality and deformity at decapodid stage.

Caridean shrimps showed that variability may occur in the natural environment (Paula, 1993), presumably as responses to adverse conditions, allowing species to respond to the diversity of coastal waters with a potential diversity of growth rates, natatory and predatory capacities. The results obtained for *U. africana* indicate that the additional fourth stage occurs in combinations of temperature and salinity with maximum overall survival, and not in particularly adverse conditions.

Temperature affects the duration of larval stages more strongly than salinity. It is interesting however to note a decrease in late larval duration with decreased salinity, as observed for other decapod crustaceans (e.g. *Sesarma reticulatum*: Paula et al., 1992). The larval stages of estuarine species, especially the megalopa, will encounter a changing estuarine environment in terms of salinity when migrating to parental areas, and although their survival can physiologically be similar to the previous stages, the acceleration of the moulting process with a lowering in salinity can account for increasing survival. Several studies refer to plasticity in the timing of moult for late larval stages (e.g. Sulkin & Van Heukelem, 1986; O'Connor, 1991; Strasser & Felder, 1999), which can be an adaptation to the variability of coastal waters when undertaking the return migration. These results thus corroborate those of Emmerson (1983) which report *Upogebia africana* as exporting newly-hatched stages from the estuary and a return migration by late larval stages.

Table 2. Results of multiple regression and associated statistics for cumulative duration of larval stages of *Upogebia africana* T = temperature, S = salinity

	Zoea I	Zoea II	Zoea III
R^2	0.8890	0.9241	0.9952
F	9.6120	12.1800	83.3740
p	0.0079	0.0079	0.0119
intercept	57.889 (p=0.0015)	110.538 (p=0.0013)	251.305 (p=0.0047)
T	− 3.265 (p=0.0073)	− 6.652 (p=0.0043)	− 16.741 (p=0.0045)
S	− 0.765 (p=0.0027)	− 1.095 (p=0.0074)	− 2.013 (p=0.0223)
T^2	0.056 (p=0.0134)	0.123 (p=0.0070)	0.303 (p=0.0041)
S^2	0.007 (p=0.0082)	0.014 (p=0.0103)	0.014 (p=0.0232)
$T \times S$	0.011 (p=0.0377)	0.008 (p=0.4032)	0.050 (p=0.0313)

Survival of larval stages of *Upogebia africana* in response to salinity is similar throughout development, with the optimal point close to sea water. The first zoeal stage is more tolerant to salinity variation, which suggests adaptation to the salinity gradient that can affect newly-hatched stages prior to estuarine exportation.

Strictly marine species develop preferentially at normal salinity, with little tolerance to low salinity (e.g. *Lithodes antarcticus*: Vinuesa et al., 1985; *Palaemon serratus*: Antonopoulou & Emson, 1989). Freshwater species which release their larvae in brackish water usually demonstrate a wider tolerance to salinity, narrowing through development to higher salinity (e.g. *Palaemon longirostris*: Antonopoulou & Emson, 1989; Paula, 1993; *Macrobrachium vollenhovenii*: Wilfuhr-Nast et al., 1993), with maximum larval survival in medium salinities (e.g. *Macrobrachiun japonicum*: Yagi & Uno, 1983; *Sesarma angustipes*: Anger et al., 1990; among many others). Another life cycle adaptation is obviously complete reduction of the larval phase, or reduction of the instar sequence to a few advanced larval stages with hyperregulatory capacity (e.g. *Callianassa jamaicence*: Felder et al., 1986).

Some species inhabiting mangroves, generally characterised by strong thermo-saline fluctuations at different temporal scales, show a wide range of tolerance, such as *Sesarma curacaoense* (Schuh & Diesel, 1995b). Other specific adaptations to harsh environments, such as those observed for larvae of *Armases miersii* living in rock pools (Schuh & Diesel, 1995a), also include a wider tolerance to temperature and salinity combinations.

Some estuarine species will maintain a wide tolerance throughout development (e.g. *Palaemonetes varians*: Antonopoulou & Emson, 1989), showing capacity of retention in the upper part of estuaries. Some other species however will develop optimally at low salinity, such as the palaemonids *Palaemon paucidens* (Fidhiary et al., 1991) and *Macrobrachium acanthurus* (Figueiroa & Gamino, 1992), at 9 and 12 respectively. However many estuarine species show maximum survival in medium salinity (e.g. *Panopeus herbstii*: Costlow et al., 1962; *Cardisoma guanhumi*: Costlow & Bookhout, 1968; *Macrobrachium petersi*: Read, 1986; *Aratus pisonii*: Diaz & Bevilacqua, 1986; *Eurytium limosum*: Messerknecht et al., 1991; *Crangon crangon*: Paula, 1993; among others). In estuarine species, which show an obvious strategy of larval exportation from the estuarine boundaries, salinity tolerance is narrow, reaching optimal development in near to sea water salinity (e.g. *Sesarma reticulatum*: Paula et al., 1992; *Carcinus maenas*: Nagaraj, 1993). *Upogebia africana* fits in this scheme, further supporting the universal pattern of temperature and salinity tolerance and consequent life cycle strategies among estuarine species world-wide.

The developmental pattern of tolerance to temperature and salinity is not dependent on phylogeny, but follows the particular adult environment and associated life cycle strategy. Good examples are the genera *Palaemon* and *Macrobrachium* within the Caridea, and the sesarmid brachyurans. These taxa have species with different degrees of adaptation to brackish and freshwater. Evolution of different life cycle strategies is related to the capacity for hyper-hypo-regulation through ontogenic development (Charmantier, 1998; Charmantier et al., 1998), demonstrated by progress-

ively stronger tolerance to low salinity with evolutionary occupation of freshwater/terrestrial environments.

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Mitotic and meiotic chromosomes of the American lobster *Homarus americanus* (Nephropidae, Decapoda)

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Key words: Crustacea Decapoda, *Homarus americanus*, B chromosomes, chromosome banding

Abstract

The chromosomes of *H. americanus* have been characterised by C-banding, fluorochrome banding and restriction endonuclease banding. Thanks to these techniques, it has been possible to identify mitotic and meiotic figures clearly and to study the distribution and structure of heterochromatic regions. Moreover, we have identified small supernumerary chromosomes, variable in number and often asynaptic in first meiotic metaphase.

Introduction

Homarus americanus H. Milne Edwards is one of the main objectives of North American commercial fishery. Despite the numerous studies on its biology and molecular genetics (reviewed in Tam & Kornfield, 1996), information about the karyology of this species is very scanty and mostly concerns an estimation of the chromosome number. The reported data, unsupported by chromosome banding techniques, point out the presence of a remarkable intraspecific numerical variability (Roberts, 1969; Hughes, 1982).

In this paper, thanks to improvements in the air-drying technique, it has been possible to apply C-, restriction endonuclease- and fluorochrome- banding and to characterise the mitotic and meiotic chromosomes of *H. americanus.* These techniques have revealed the presence of small supernumerary chromosomes, variable in number and often asynaptic in first meiotic metaphase.

Materials and methods

Chromosome preparations were obtained from the testicular tissue of 10 males from three different commercial stocks using the air-drying technique according to Doussau De Bazignan & Ozouf-Costaz (1985). The slides were stained for 8 min in 25% Wright's stain (in 0.06 M phosphate buffer, pH 6.8).

Testicular histological sections were performed and stained as described by Cau et al. (1988a).

C-banding was obtained according to Deiana et al. (1996) and *MspI* digestion (restriction target: C↓CGG) according to Cau et al. (1988b).

CMA_3 and DAPI stainings were as described by Schweizer (1976) and Schweizer et al. (1978). Slides were observed using a Zeiss Axiophot fluorescence microscopy with a filter set 02 for DAPI and a filter set 06 for CMA_3. The photos were taken with a Kodak T-MAX 400 ASA film.

Results

The analysis of spermatogonial mitosis and of second meiotic metaphases showed that the chromosome complement of *H. americanus* is made up of metacentric, submetacentric and acrocentric chromosomes (Fig. 1a, c). Late prophases and metaphases I showed chromosomal pairing and different meiotic configurations: ring-, cross- and dumbell; the dumbell figure is typical of Crustaceans (Niiyama, 1959) and is derived from a chromosomal end by end pairing (Fig. 1b). Asynaptic, small chromosomes were visible, they were variable in number and generally isolated (Fig. 1b).

With fluorochrome banding, it was possible to detect the occurrence of GC- and AT- rich chromosomal

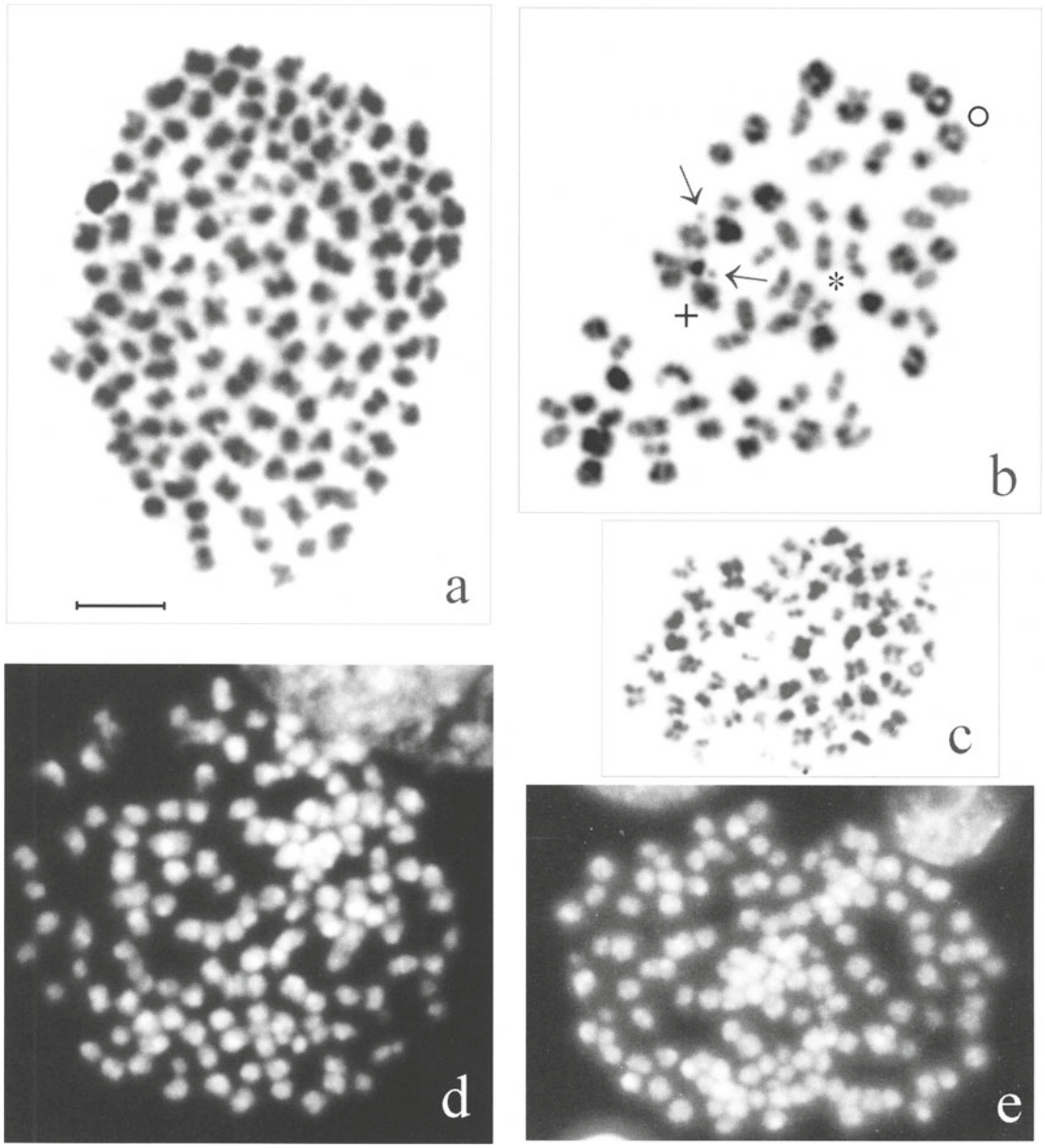

Figure 1. (a) spermatogonial metaphase, (b) meiotic metaphase I and (c) meiotic metaphase II after Wright's staining; in b) cross(+), ring (°), dumbell (∗) figures and supernumerary chromosomes (→) are shown; (d) spermatogonial metaphase after CMA_3-banding; CG-rich centromeric/paracentromeric clusters are evident, (e) DAPI staining, AT-rich bands are mostly localised in centromeric regions. The bar in (a) represents 5 μm.

regions. Chromomycin A_3 produced a bright fluorescence, corresponding to GC- rich heterochromatic clusters, prevalently in centromeric/parecentromeric regions (Fig. 1d). Different centromeric regions were fluorescent after staining with the AT-specific fluorochrome, DAPI (Fig. 1e).

C- and restriction enzyme-induced banding allowed the study of the distribution and structure of heterochromatic regions. In particular by C-banding, it was possible to locate the constitutive heterochromatin in most centromeric regions and in some large paracentromeric bands (Fig. 2a); in metaphase I, the meiotic figures were sharply identifiable and the small asynaptic chromosomes were clearly visible (Fig. 2b). After *MspI*-induced banding some regions, mostly centromeric, were undigested and dark stained; the

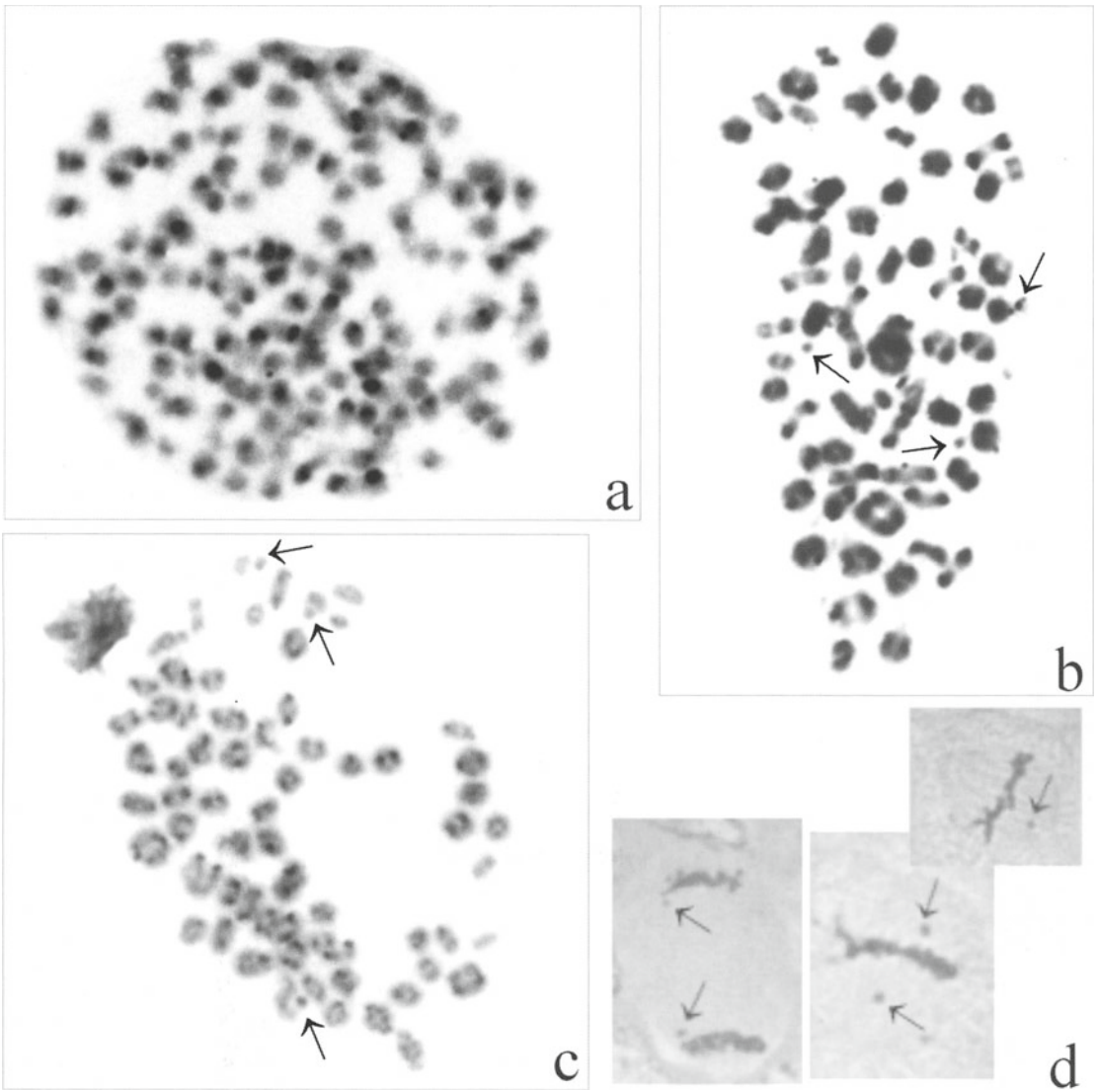

Figure 2. (a) spermatogonial mitosis and (b) meiotic metaphase I after C- banding, most centromeres and some paracentromeric regions are C-positive; (c) meiotic metaphase I after *MspI*-induced banding, in (b) and (c) asynaptic B chromosomes are recognisable (→); (d) testicular histological sections show metaphase and anaphase delay of B chromosomes (→).

meiotic bivalents as well as the asynaptic chromosomes were clearly distinguishable (Fig. 2c).

Moreover, the analysis of testicular histological sections pointed out the presence of some chromosomes showing metaphase and anaphase delays (Fig. 2d).

The count of 20 spermatogonial mitoses showed a diploid number comprised between 130 and 142 with a predominance of 135–136 values. Variability was observed among individuals as well as among cells of the same individual. In metaphase I, out of 15 plates, the bivalents were between 64 and 68; unpaired B chromosomes were counted separately, they were between 2 and 4.

Discussion

Five out of 43 species of the Nephropidae family have been studied from a karyological viewpoint and most of them before 1985 (Corni et al., 1989). Like other Decapods, most of the studied species show more than a hundred chromosomes (Nakamura et al.,

1988; Lécher et al., 1995). Numerical variability is reported in *Homarus americanus*, *H. gammarus* and *Nephrops norvegicus* (see Corni et al., loc. cit). Furthermore, DNA content, reported in *Homarus americanus*, *H. gammarus* and *Nephrops norvegicus*, shows a relatively low variation (Deiana et al., 1999).

In this work, the use of banding techniques, especially C- and *MspI* induced-banding, allowed a sharp differentiation of mitotic chromosomes and meiotic figures of *H. americanus* and the identification of supernumerary chromosomes. B chromosomes, that are generally additional to the normal chromosome complement and highly polymorphic in number and morphology within populations and species (Jones & Rees, 1982), have already been hypothesised in this species by Roberts (1969) and Hughes (1982). In crustaceans, they have been already pointed out in many species (Jones & Rees, loc. cit) and, among Nephropidae, the presence of very large B chromosomes have been reported in *Nephrops norvegicus* (Deiana et al., 1996). In *H. americanus* the supernumerary chromosomes are smaller than the other members of the complement, in meiotic metaphase I they are recognisable because asynaptic and heterochromatic and their number is variable within and among individuals.

As regards the $2n$ and n values, the mitotic chromosome counts (135–136) were closer to the modal number ($2n$=138) published by Roberts (1969) than to the modal number (110) published by Hughes (1982); while the meiotic counts agree with both the modal number published by the two authors (n=69 and n=66.5–68, respectively). In this work, the wide range of numerical variability previously reported has been greatly reduced.

The identification of B chromosomes in *H. americanus* genome provides a new datum on the cytogenetic characterization of this species and of the family Nephropidae.

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Larval abundance and recruitment of *Carcinus maenas* L. close to its southern geographic limit: a case of match and mismatch

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Key words: Carcinus maenas, larvae, recruitment, *Zostera*, lagoon, Ria Formosa

Abstract

In the Ria Formosa lagoon (S. Portugal), *Carcinus maenas* larvae have been observed only during the cold part of the year (October–May), when the water temperature stays below 23 °C. Larvae suffer high mortality during day time (about 80% during 6 h). Hence, they will only develop outside the lagoon, as proved by the observation of zoea I stages exclusively. Main recruitment starts in April, i.e. much later than the first larvae appear. Small crabs prefer *Zostera noltii* patches and colonize other sites when the carapace width exceeds about 5 mm. A reserve of some small crabs is found in *Zostera noltii* patches nearly all year round. It is postulated that one of the key factors for successful propagation of the species is a match between physiological reactions (here in particular temperature effect on reproductive cycle and larval release), and the ecological conditions for larval survival and recruitment, such as predator impact, food availability or currents.

Introduction

Although today the shore crab *Carcinus maenas* can be looked upon as a nearly cosmopolitan species of the temperate regions of the world, the original distribution was limited to the European Atlantic coast, with its southern expansion close to Mauretania (Almaça, 1962).

Most data on the biology have been collected in Central Europe. From these sites, it is reported that *C. maenas* larvae may be the dominant decapod species during summer months in the North Sea plankton (Rees, 1952, 1955; Steiff, 1989). The pelagic period lasts for 3–4 weeks, during which 4 zoea stages are passed (Dawirs, 1985; Mohamedeen & Hartnoll, 1990). The larvae are ready to metamorphose to a benthic organism at the megalopa stage. Consequently, the recruitment is in summer (Beukema, 1991).

Temperature is probably the most important environmental factor which affects the reproductive cycle of marine animals (Giese, 1959). Due to elevated temperatures at the southern geographic limit, larval release and recruitment should consequently shift from summer to winter months. However, this means that reproduction and recruitment take place under different ecological conditions, particularly with a different quality and quantity of food organisms and another spectrum of predators.

Larval abundance and recruitment have been followed during the seasonal cycle in the Ria Formosa lagoon (S. Portugal). This site is close to the southern geographic limit of the species. Under which aspect will reproduction and recruitment differ from that in more temperate zones?

Area description, method and material studied

The Ria Formosa lagoon extends for about 55 km on the south coast of Portugal (Fig. 1). A seaward belt of dunes protects a system of tidal flats and salt marshes of up to 3 km width. The lagoon is mesotidal with a tidal amplitude of about 1.30 m at neap tide and 2.80 m at spring tide. A strongly ramified system of creeks and channels is connected with the Atlantic Ocean by only a few inlets.

Plankton samples have been taken during the whole year cycle of 1995 at every spring and neap tide. In practical terms, 2 samples were collected weekly during day time, one 3 h before, the other 3 h after high tide at approximately the same water level. When

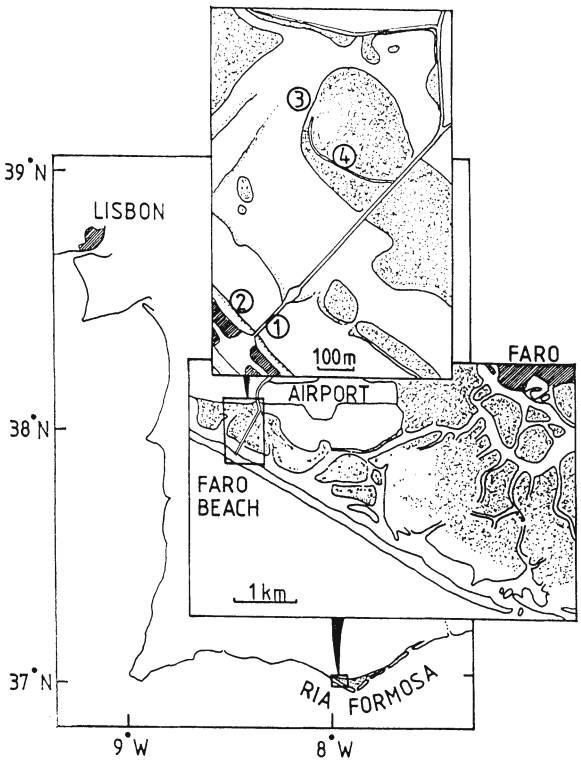

Figure 1. The Ria Formosa lagoon and the sample sites: 1: bridge leading to Faro Beach, 2: sand flat, 3: mud flat with *Zostera noltii* patches, 4: track crossing a salt marsh.

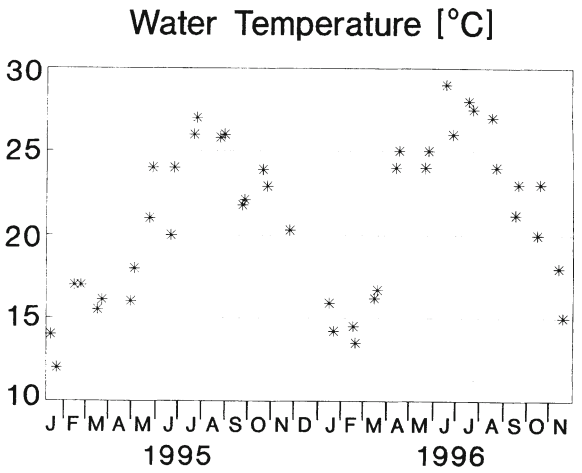

Figure 2. Records of the water temperature of the Ria Formosa at the sample sites.

the sample was taken in the morning at spring tide, the water was coming in, at the afternoon sampling the water was going out. At neap tide, the situation was reversed. In the morning, the tide was coming in, in the afternoon the tide was going out the lagoon. A conical plankton net of 200 μm mesh size was trawled from the bridge leading to Faro Beach (Praia de Faro) at walking pace for 5 or 10 min. The net had a cone mouth diameter of 9.3 cm and measured 40 cm in length. The water volume filtered during each trawl (1.2–2.3 m^3) was quantified by a current meter placed at the extremity of the net mouth. Samples were stored in a dilute formalin solution and sorted under a dissecting microscope typically already at the same sampling day. *C. maenas* larvae were identified according to the description by Rice & Ingle (1975).

Benthic stages have been sampled from spring 1995 to autumn 1996 monthly at shallow water level by delimiting an area of 0.1 m^2 by a cylindrical bottomless container. The surface of the sediment was screened by a 500 μm sieve equipped with a handle. It was originally constructed for aquarium use. According to empirical evidence, 3 screenings of the bottom were sufficient to catch all crabs. The content of the sieve was examined in a white plastic box on site. The crabs detected were preserved in formalin, examined and sized to $\pm$0.1 mm carapace width under a dissecting microscope in the laboratory. Three sites were evaluated, taking 25 replicates at each site: a sand flat in the immediate vicinity of the bridge to Faro Beach at neap low tide, a mud flat some 500 m away, 1–2 h before a moderate spring high tide at rising water and *Zostera noltii* patches on the mud flat. Due to the large amount of small crabs, the number of replicates had to be reduced on the mud flat and in the *Zostera* patches in spring 1996 to 10 each in April, 15 each in May and 20 each in June. Additionally, at high tide a track crossing a salt marsh was examined by sight, collecting all crabs from an area of 300–1280 m^2. Only crabs with a carapace width below 20 mm are contemplated in this analysis.

Results

The occurrence of *Carcinus maenas* larvae was limited to the period between October and May, i.e. when the water temperature stayed below 23 °C (Fig. 2). During autumn and winter months, *Carcinus* larvae formed the dominant fraction of the crab larvae in the plankton of the lagoon (Fig. 3). Exclusively zoea I stages were found in the plankton at the hours of sampling.

Compared to the afternoon sample, larval abundance was significantly higher in the morning with a maximum of up to 160 larvae m^{-3}, both at neap tide (13 from 15 pairs of observations; $p<0.001$)

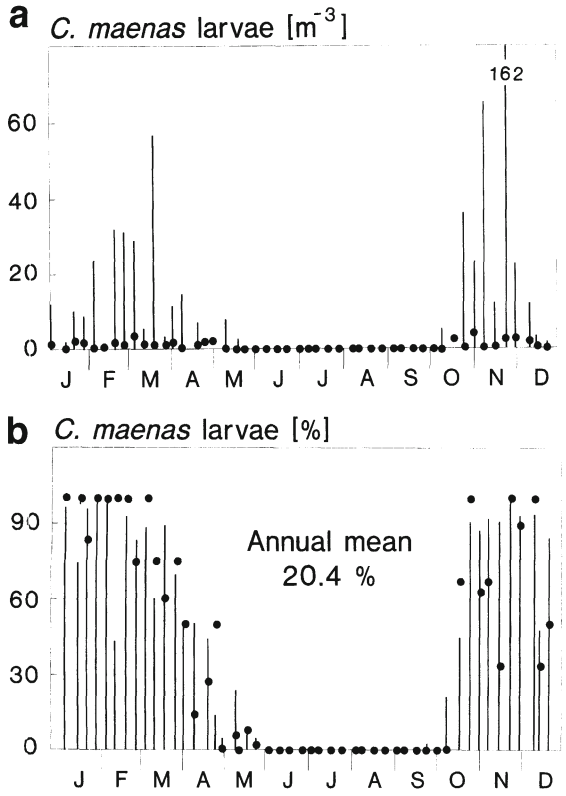

Figure 3. (a) *Carcinus maenas* larvae: abundances recorded in 1995 at the bridge leading to Faro beach; (b) contribution of *C. maenas* larvae to the decapod larvae present; bar: morning sample; dot: afternoon sample.

and at spring tide (16 from 16 pairs of observations; $p<0.001$; sign test of Dixon & Mood (1946)). At spring tide, the afternoon sample contained only a median of 3.8% of the *Carcinus* larvae of the morning sample (quartiles: 1.2 and 16.7%). In this case, the water mass moved to the inlet of the lagoon. At neap tide, there was a median of 20% left in the afternoon (quartiles: 9.3 and 36.3%). In this case, the water mass moved to the inner part of the lagoon.

A striking observation was that main recruitment started many months later than the appearance of the zoea larvae in the plankton (Fig. 4). Among the sites examined, *Zostera noltii* patches were preferred by young crabs. *Carcinus* abundance was in 19 attempts at various seasons always significantly higher when a *Zostera* patch was included in the screened area in comparison to an unvegetated area ($p<0.05$; U-test by Wilcoxin et al. (Mann & Whitney, 1947)). Although single observations of small crabs were already made during winter months (4.2 mm carapace width in January and 3–5 mm in February), main recruitment started in 1996 not before April. It was much more intense in 1996 than in 1995, where unfortunately screening was only initiated in April, when already a fairly high amount of small crabs was present.

Crabs of this new age class appeared at the other sites (track and sand flat) at carapace widths of 5–10 mm 1–2 months later. A considerable reserve of small crabs with a carapace width below 20 mm was present in *Zostera* patches nearly all year round.

Discussion

The expected seasonal shift from summer to winter months in more southern regions was confirmed for larval release. Observations of winter release of *C. maenas* larvae have already been reported by Paula (1993) in the nearby Mira estuary and by Drake et al. (1998) for the Bay of Cádiz. However, despite the geographic proximity, Paula (1993) classified *Carcinus* as a permanent irregular spawner, because some *Carcinus* larvae were present all year round in the Mira estuary.

Due to the geography of the Ria Formosa lagoon, peak larval abundances were high compared to the other studies for winter conditions (Mira estuary: peak at 43.9 larvae m^{-3}, means <12 m^{-3}; Bay of Cádiz: <20 m^{-3}). This justifies the comparatively small sample volume.

Queiroga (1996) documented for the north coast of Portugal that larval development takes place mainly in the ocean. There the larvae are subjected to conditions which are quite different from those in the lagoon in terms of predators, food and environmental conditions such as temperature, salinity and oxygen content. A return to the lagoon with potential currents along the shore may complicate completion of the life cycle to an unknown extent. It may even be the case that the recruits originate from other coastal environments nearby.

Losses under lagoon conditions are high, as evidenced by differences in abundance at the same sampling day. Losses between the 6 h of sampling reflect both mortality (probably by predation) and flushing (applies only to the observations at spring tide). Sampling has been limited to day time, where predators with visual orientation are most effective. Hence, similar calculations also including night samples may display different trends.

In estuaries, larvae of some decapod species perform directed vertical migrations which retain the lar-

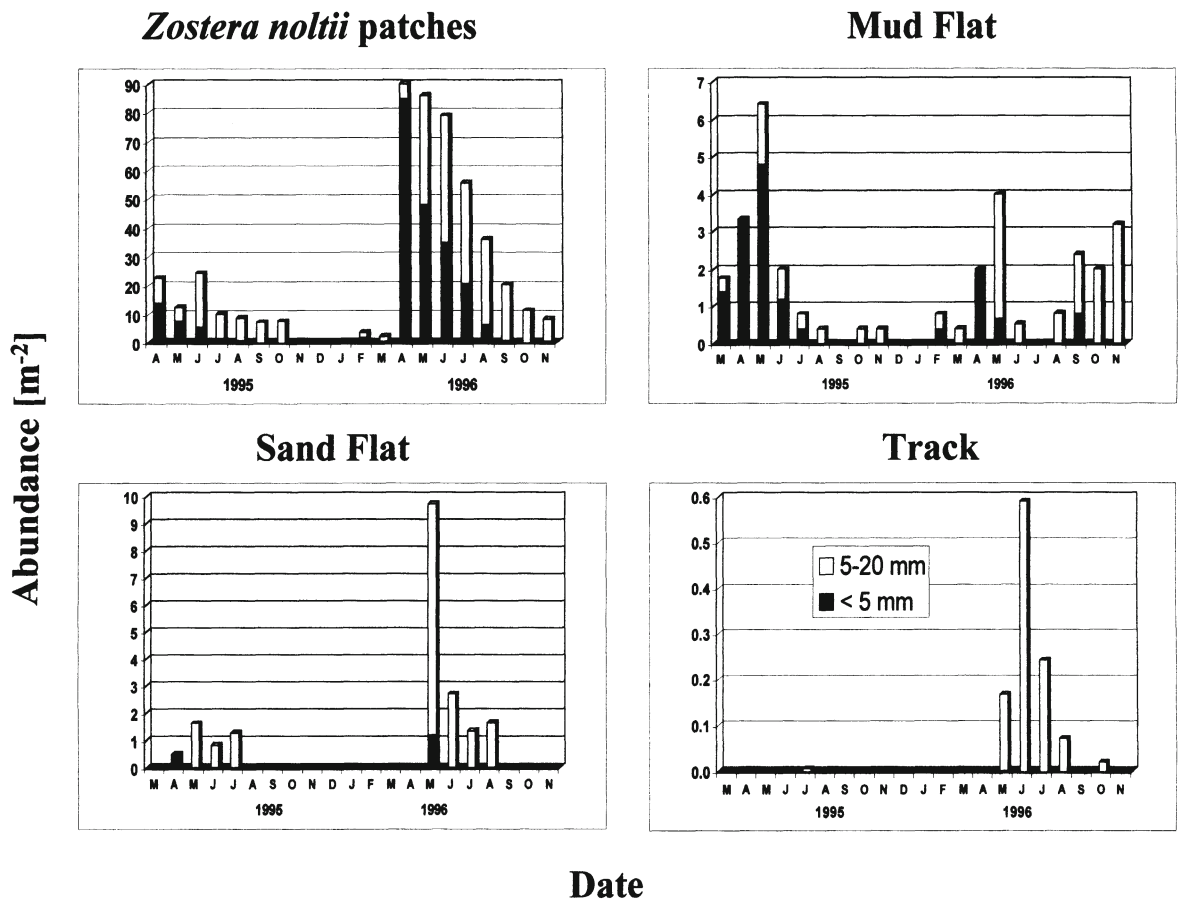

Figure 4. Carcinus maenas: abundance at 4 sites of observation in the Ria Formosa lagoon splitted into records of specimens below 5 mm carapace width and records of specimens between 5 and 20 mm carapace width.

vae in the system (e.g. described for *Rhithropanopeus harisii* by Cronin & Forward, 1986). Paula (1993) also attributed a certain potential of retention in the Mira estuary for *C. maenas*. This, however, would be a suicidal strategy for the Ria Formosa animals. In fact, vertical migration documented for the first instars of *C. maenas* larvae favour an export from coastal waters (Zeng & Naylor, 1996; Queiroga et al., 1997).

Although recruitment of *C. maenas* in the Ria Formosa is generally earlier than reported from most other European sites, the seasonal shift in a latitudinal comparison is not so drastic as that for larval release. On the Swedish west coast, for example, recruitment starts only between August and September (Eriksson & Edlund, 1977; Pihl & Rosenberg, 1982), in the Dutch Wadden Sea after mild winters in mid-June, after cold winters beginning of August (Beukema, 1991), in the estuary of the Gironde in June (Bachelet, 1987). Mostly, recruitment is in distinct brood waves (Klein Breteler, 1976; Scherer & Reise, 1981; Pihl & Rosenberg, 1982; Beukema, 1991). Brood waves have also been reported by Queiroga (1993) for the Ria de Aveiro in late April and late June, but were not evident in both years for the Ria Formosa lagoon.

Structured substrates of different kind are sites of reduced mortality particularly of small crabs (Wilson et al., 1990, Moksnes et al., 1998). They are, therefore, preferred by young recruits. This has also been documented for recruits of the blue crab *Callinectes sapidus* at coasts of the United States (Orth & v. Montfrans, 1990; Ryer et al., 1990). Structured substrates also serve as a reservoir of small forms. Thiel & Dernedde (1994) observed in analogy to the present data that the mean crab size caught in the tidal zone of the German Wadden Sea decreased in autumn. The authors attributed this phenomenon to a migration of larger crabs to the subtidal zone, while small forms still found a refuge in this case in *Mytilus* clumps. These small forms may represent late settling individuals which find shelter, and/or slow growing

specimens which can hardly compete with their larger congeners at unprotected sites.

Abundance of small crabs in these structured substrates can be extremely high. In a *Spartina anglica* salt marsh in east England, Jackson et al. (1985) reported up to 100 crabs m^{-2}, in *Mytilus edulis* clumps in the Wadden Sea, Thiel & Dernedde (1994) more than 200 m^{-2}, and Klein Breteler (1976) up to 600 m^{-2}. The highest documented abundance of recruits are from seagrass beds and mussel banks in the Wadden Sea near Sylt with about 500 crabs m^{-2}, with an extreme value of 2000 specimens m^{-2} (Scherer & Reise, 1981). Peak abundance will be most extreme when reproduction of the crabs is well co-ordinated and limited to a restricted period. This may be one reason why the highest crab densities observed for the Ria Formosa were modest in comparison to these observations.

The lag between first larval release and main recruitment may have contributed even more severely to the relatively poor recruitment, particularly during the first year of observation. Probably this is an analogous situation to that described by Cushing (1975) for the coincidence of fish egg release and the availability of adequate food at hatching. Factors which condition egg (or larval) release are primarily of physiological nature, amongst which temperature may play a prominent role. Factors which determine larval survival and success of recruitment are of an ecological nature and comprise the presence of predators, of adequate food in sufficient quantity, currents appropriate for completing the life cycle and probably many additional factors. Both physiological condition and ecological framework may match and lead to good recruitment or may not match and lead to poor or no recruitment. It may be that this match/mismatch is only obvious because *Carcinus* has been examined close to its limit of distribution. It can be speculated that the probability of a mismatch increases under these conditions, and leads to more variable recruitment and in the extreme case to the disappearance of the species.

Conclusions

Release of *Carcinus maenas* larvae is limited to winter months in the Ria Formosa lagoon. Larvae suffer high mortality in the lagoon and will only develop outside. Main recruitment starts at this site much later (April) than the onset larval release (October) – a restriction set probably by ecological conditions. *Zostera noltii* patches are colonized particularly by small crabs below 5 mm CW after recruitment, but also serve as a refuge for small crabs nearly all year round.

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Choice of prey size and species in *Carcinus maenas* (L.) feeding on four bivalves of contrasting shell morphology

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Key words: foraging behaviour, *Carcinus maenas*, selective predation, bivalve

Abstract

Experiments were designed to investigate selective predation by medium (40–55 mm carapace width: CW) and large (55–70 mm CW) *Carcinus maenas* when feeding on four bivalves of contrasting shell morphology. Size-selection was examined by presenting individual crabs with a wide size range of *Mytilus edulis*, *Ostrea edulis*, *Crassostrea gigas* and *Cerastoderma edule*. Medium-sized crabs preferred mussels 5–15 mm shell length (maximum shell dimension: SL) and cockles 5–10 mm SL, whereas large crabs preferred mussels 15–25 mm and cockles 10–20 mm SL. Crabs generally showed no preference for any particular size of either oyster species. Species-selection was examined by presenting individual crabs with paired combinations of the four bivalves in various proportions. When offered mussels and oysters simultaneously, both size categories of crabs consistently selected mussels, and food choice was independent of prey relative abundance. By contrast, *C. maenas* selected mussels and cockles as expected by the frequency in which each size category of crab encountered the preferred size ranges of prey. Crab preference clearly paralleled the rank order of prey profitability, which in turn was mainly determined by prey biomass, suggesting that active selection takes place at some point of the predation cycle. Experiments with epoxy resin models showed that initial reluctance of crabs to attack oysters was not associated with the ultimate energy reward. Moreover, they suggest that foraging decisions are partly based on evaluations of overall prey shape and volume, and that the minimum dimension of the shell constitutes an important feature which crabs recognise and associate with prey value.

Introduction

Crab predation is thought to be an important factor structuring marine benthic communities (Virnstein, 1977; Leber, 1985; Raffaelli et al., 1989). Prey size and species selection is a major aspect within this topic, given the direct influence that the removal of preferred prey types can have on the abundance and distribution of prey populations. Several studies have examined the behavioural and mechanical aspects of size-selective feeding by brachyuran crabs (e.g. Elner & Hughes, 1978; Jubb et al., 1983; Rheinallt, 1986; Hughes & Seed, 1995), and the results have often been considered within the context of Optimal Foraging Theory. According to this theory, a predator should choose its diet in order to maximise net energy intake per unit of handling time (for reviews of Optimal Foraging Theory see Hughes, 1980; Pyke, 1984). However, selection of small size ranges of hard-shelled molluscan prey reduces the risk of claw damage (Juanes, 1992) and minimises handling time (Hughes & Seed, 1981), thereby increasing the probable survival of foraging crabs which may themselves be vulnerable to predation.

Because handling times, and hence prey values or profitability, have a complex variation related to the morphological characteristics of both the crab chelae and the prey shell, foraging tactics can vary when crabs feed on different species of prey (Creswell & McLay, 1990; Seed & Hughes, 1997). Whilst size-selective predation has been extensively documented (e.g. Lawton & Hughes, 1985; Hughes & Elner, 1989;

Lin, 1990; Seed, 1990; Brown & Haight, 1992; Seed & Hughes, 1995), little information is available for selective predation amongst different species of hard-shelled prey.

The common shore crab, *Carcinus maenas* (L.), has been the subject of much research not only because of its abundance and widespread distribution in coastal and estuarine waters (Ingle 1980, Grosholz & Ruiz, 1996), but also because it is known to forage extensively on a variety of commercially exploited bivalve species (Ropes, 1968; Dare et al., 1983; Sanchez-Salazar et al., 1987). The present study compares size-related preferences of adult *C. maenas* when presented with each of four bivalves with contrasting shell morphologies: the mussel, *Mytilus edulis* L.; the flat oyster, *Ostrea edulis* L.; the Pacific oyster, *Crassostrea gigas* (Thunberg); and the cockle, *Cerastoderma edule* (L). Species-related preferences exhibited by adult crabs when feeding on various combinations and proportions of these bivalves are also examined. Experiments were designed in order to test the importance of prey shell shape in determining crab foraging decisions.

Materials and methods

Medium (40–55 mm carapace width: CW) and large (55–70 mm CW) *C. maenas* were collected by hand from the low shore in the Menai Strait, North Wales, and maintained individually in plastic aquaria (30×20 cm) filled to a depth of 10 cm with running sea water. Water temperature in the aquaria varied between 12 and 17°C. Only undamaged male crabs in the late inter-moult stage were used in the experiments in order to avoid any potential bias due to morphological and behavioural differences associated with sex and moult stage. Hunger levels were standardised by starving crabs for 48 h prior to experiments. Intertidal *M. edulis* and *C. edule* were collected from naturally occurring populations at various sites around Anglesey, North Wales, whilst *O. edulis* and *C. gigas* were obtained from CEFAS commercial oyster beds located in the Menai Strait. All prey were maintained in plastic trays with running seawater, and fed a mixture of microalgae once a day. Prior to trials, undamaged prey were chosen and any epizoic organisms removed from their shells.

Samples of all four prey species covering as wide a size range as possible were taken *(M. edulis, O. edulis, C. edule*: n = 35, *C. gigas*: n = 42), and shell length (SL: maximum linear dimension of the shell), shell height (SH: maximum linear dimension of the axis at right angles to SL), and shell width (SW: minimum linear dimension of the shell) of each individual were measured to the nearest 0.1 mm using vernier callipers. Soft tissues were removed following brief immersion in boiling water and dried to constant weight at 60 °C. Dry tissue weight (W) was then determined to the nearest 0.01 mg on a top loading balance. Relationships between SL (y) and SW SH, and W (x) were best described by the allometric equation

$$y = a \times x^b,$$

where a and b are constants. Linear relationships between these variables were obtained by least square regressions on logarithmically transformed data. In order to reveal any differences between the four prey species, regression lines were compared by analysis of variance using the General Linear Model with SL as the covariate. Pairwise comparisons between the regression slopes and intercepts were then performed using the Tukey's method.

Size-selection experiments were carried out by presenting individual crabs with *M. edulis*, *O. edulis*, *C. gigas* and *C. edule* ranging from 5 to 40 mm SL. Only one species of prey was offered to the crabs during any single feeding experiment. Each crab was simultaneously offered five prey items in each 5-mm size class. Prey items were scattered randomly over the floor of the aquaria and monitored twice daily. Any item consumed within each 12 h period was recorded and replaced by another of similar size in order to maintain constant prey availability. Experiments were run continuously until a consistent feeding pattern emerged (≈10 days). Results were analysed using a chi-square test to detect whether the distributions of eaten prey deviated from a random choice (Peterson & Renaud, 1989). Comparisons of the size ranges of prey preferred by both size categories of crabs were made on the basis of (i) shell length, (ii) shell width and (iii) relative prey size (RPS) which was obtained by dividing the median value of SW within each of the size classes of each prey species offered by the height (maximum cross-section) of the major chela. The height of the major chela in *C. maenas* was estimated for a range of crab sizes (n = 61) using the allometric equation

$$\mathrm{MH} = 0.13\mathrm{CW}^{1.21},$$

where MH (mm) is master chelal height and CW (mm) is carapace width (Mascaró, 1998).

Experiments to compare the breaking time and profitability of the preferred size ranges of each prey species were carried out by offering both medium and large crabs a prey item of known shell length, and recording (i) **breaking time** (**Tb**), the time from the first physical contact with the prey item, through the period of manipulation to the point where the shell was finally opened and the flesh exposed, and (ii) **handling time** (**Th**), the time from the first physical contact through the eating period to the point where the meal was completed and the empty shell abandoned. Handling time was then used to estimate prey profitability as dry flesh weight per unit of observed **Th** (mg s^{-1}). Analysis of variance and Scheffe's method for pairwise comparisons of breaking times and profitability values between prey species were performed on the basis of the size ranges of prey consumed in > 20% during single prey species experiments. In those cases where crabs exhibited no apparent size preference, the size range used was comparable to that for the preferred size range of mussels. Breaking times and profitability values were log-transformed before analysis of variance was applied to the data.

Once the preferred size ranges for each individual prey species had been established, paired combinations of a wide size range of prey species were offered to both medium and large *C. maenas*. The prey species combinations were *M. edulis-O. edulis*, *M. edulis-C. gigas*, *O. edulis-C. gigas* and *M. edulis-C. edule*, and each crab was offered 5 items in each size class of prey. The size classes of prey used for these experiments were based on those preferred by crabs in the experiments where prey species were offered individually. Prey consumption was monitored and prey items replaced as in single prey species experiments.

In order to establish whether crab preference for a certain prey species was determined by its relative abundance, each individual crab was offered equal and unequal numbers of the preferred size ranges of *M. edulis-O. edulis* and *M. edulis-C. edule*. The proportions of presented prey were altered so that the prey species that had been preferentially selected in the previous experiments was now at a lower relative abundance of 1:2 and 1:4 with respect to the less preferred species. Each time a prey item was consumed by a crab it was immediately replaced by another of similar size. Once a prey item was encountered and recognised as potential food, a crab could either reject (i.e. touch, manipulate and finally abandon) or accept (i.e. successfully open and consume) the prey. Each trial was run for 1 h and experiments were repeated on a daily basis until a consistent pattern emerged ($\approx$ 5 days). The total number of times that crabs encountered each prey species were then tested for goodness-of-fit to the expected values (assuming a probability of encounter: 1:1, 1:2 and 1:4). Differences in the total number of prey of each species that were successfully opened by each individual crab were tested using the same procedure.

The importance of shell shape in determining crab persistence was examined by presenting *C. maenas* of 50–60 mm CW with epoxy resin models of three contrasting geometric shapes: a 'cockle' (sphere: 904 mm^3), a 'mussel' (wedged rectangle: 420 mm^3) and an 'oyster' (flat disc: 530 mm^3; Fig. 1). The resin models were similar in length (all: 12–15 mm) but differed in their height (sphere: 12 mm; rectangle: 6 mm; disc: 15 mm) and width (sphere: 12 mm; rectangle: 6 mm; disc: 3 mm). On each day, 6 individually maintained crabs received a sequence of 5 models of one of the three model types and their **persistence time** (time elapsed from the moment a crab grasped a model, until it was finally abandoned) with these models were recorded. Over a three day period, each crab had experienced each of the different model types. After each trial, crabs were fed on mussel flesh for 1 h before being starved until the following day. The order in which each crab experienced the different model types was random. Data were logarithmically transformed before differences in persistence time were examined by analysis of variance using a balanced design with 'model in sequence' and 'model type' as fixed factors and 'crab' as a random factor.

Results

Table 1 shows the equation coefficients for the allometric relationships between shell length (SL) and shell width (SW), height (SH) and dry flesh weight (W) in *M. edulis*, *O. edulis*, *C. gigas* and *C. edule* together with pairwise comparisons between these relationships. Analysis of variance showed significant differences between the slopes ($F = 34.12$; $p < 0.001$) and intercepts ($F = 2.87$; $p < 0.05$) for the regressions between SW and SL of the four species examined. With increase in shell length, *C. edule* increases in shell width more rapidly than *M. edulis* and *C. gigas*, which in turn increase more rapidly than *O. edulis*. Analysis of variance on the regression lines of SH and SL showed significant differences between the slopes ($F = 11.5$; $p < 0.001$) and intercepts ($F = 6.88$; $p <$

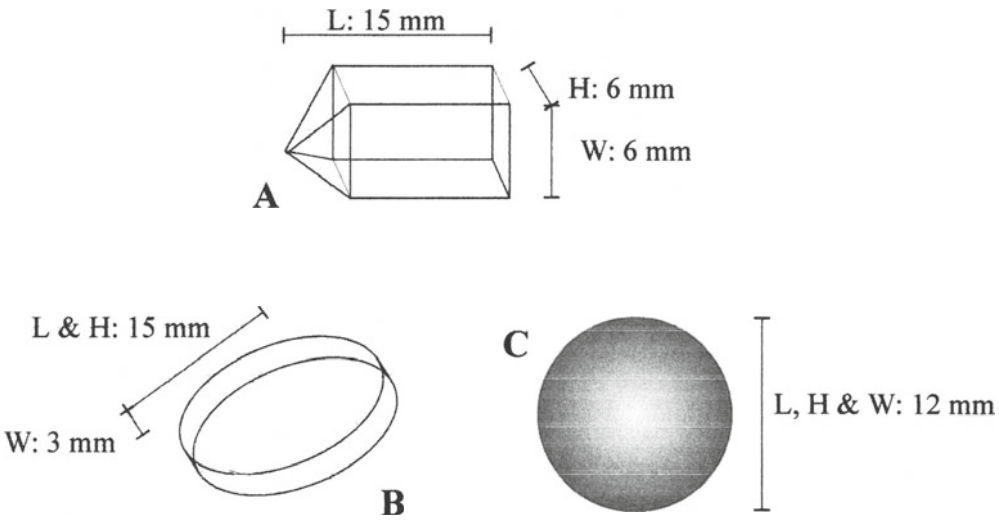

Figure 1. Epoxy resin models of contrasting geometric shapes that were offered to *Carcinus maenas* (50–60 mm CW) to examine the importance of shell shape in crab foraging behaviour: (A) wedged rectangle (420 mm ^{3}), (B) flat disc (530 mm ^{3}); (C) sphere (904 mm ^{3}); L: length, H: height, W: width.

0.001). With increase in shell length, *O. edulis* increases in shell height more rapidly than *C. edule*, which in turn increases more rapidly than both *M. edulis*, and *C. gigas*.

Results of the analysis of variance on the regression lines of weight (W) and shell length (SL) showed significant differences between the slopes ($F = 6.82$; $p < 0.001$) and intercepts ($F = 46.92$; $p < 0.001$). Pairwise comparisons between regression parameters revealed that the regression lines for *M. edulis* and *C. edule* are not significantly different from each other (Table 1), and that both have the same slope but a higher elevation than the regression line for *O. edulis*. These results suggest that both *M. edulis* and *C. edule* have significantly more biomass than *O. edulis* of comparable shell length. The regression line for *C. gigas* intersects that for *O. edulis*, indicating that amongst smaller prey Pacific oysters have less biomass than mussels, cockles and flat oysters. However, with increase in size the biomass of *C. gigas* increases more rapidly than in the other species (slope: 3.30±0.34) so that amongst prey > 20 mm SL, *C. gigas* has a greater biomass than *O. edulis* of comparable shell length.

C. maenas 40–55 mm CW preferred mussels 5–15 mm SL, and cockles 5–10 mm SL, whereas large crabs (55–70 mm CW) preferred slightly larger mussels (15–25 mm SL) and cockles (10–20 mm SL; Table 2). Neither medium nor large crabs included cockles > 20 mm, and > 25 mm SL, respectively, in their diets. When feeding on oysters, crabs generally showed no preference for any particular size class of prey. Only medium-sized *C. maenas* preferred *C. gigas* of 10–15 mm SL, but included Pacific oysters of all size classes offered. Differences between the preferred size ranges of prey were larger when expressed in terms of shell length than in terms of shell width, and crabs always preferred prey with a RPS < 1, suggesting that preferred prey was always smaller than the height of the largest chela. Mussels and cockles were consumed in higher numbers than either oyster species, which in turn were consumed in similar numbers by both size categories of crabs (Table 2).

Both the degree of crab selectivity (i.e. pattern of size selection) and the maximum length of prey varied from one species to another (Fig. 2). Whilst the percentage of mussels consumed by all crabs decreased slowly amongst mussels of increasing shell length, the percentage of cockles consumed declined steeply amongst the larger size classes of prey. The maximum shell length of *M. edulis* opened by medium and large crabs was 20–25 and 35–40 mm SL, respectively, whereas the maximum shell length of *C. edule* opened by both size categories of crabs was 20–25 mm SL. By contrast, crabs included *O. edulis* and *C. gigas* of up to 35–40 mm SL in their diet and were less size selective than when feeding on mussels and cockles, resulting in relatively more uniform distributions in both oyster species.

Table 3 shows the results of analysis of variance on the profitability and breaking time of the preferred size

Table 1. Equation coefficients of the allometric relationships between shell length (SL mm) and shell width (SW mm), shell height (SH mm), and dry flesh weight (*W* mg) in *Mytilus edulis (M)*, *Ostrea edulis (O)*, *Crassostrea gigas (C)* and *Cerastoderma edule (E)*. Coefficients *a* (intercept) and *b* (slope) in the linear model ($\log y = \log a + b \log x$) were obtained by least square regressions; r^2 is the coefficient of determination. Results of Tukey's pairwise comparison tests performed on the regression coefficients (slopes: normal type; intercepts: bold type) of each allometric relationship are also presented; * $p < 0.05$, ns = not significantly different

		Equation coefficients				Tukey's comparisons				
		a	*b*	r^2	*n*		*M*	*O*	*C*	*E*
Log SW - log SL	*M. edulis*	−0.42	1.03	0.99	35	*M*	– –	*	**ns**	**ns**
	O. edulis	−0.23	0.70	0.82	35	*O*	*	–	*	**ns**
	C. gigas	−0.39	0.96	0.97	42	*C*	ns	*	–	**ns**
	C. edule	−0.35	1.15	0.99	35	*E*	*	*	*	–
Log SH - log SL	*M. edulis*	−0.13	0.88	0.99	35	*M*	–	**ns**	*	**ns**
	O. edulis	−0.26	1.12	0.98	35	*O*	*	–	*	*
	C. gigas	−0.02	0.91	0.94	42	*C*	ns	*	–	**ns**
	C. edule	−0.04	1.00	0.99	35	*E*	*	*	*	–
Log W - log SL	*M. edulis*	−4.94	2.69	0.99	35	*M*	–	*	*	**ns**
	O. edulis	−5.99	2.89	0.96	35	*O*	ns	–	*	*
	C. gigas	−6.50	3.30	0.95	22	*C*	*	*	–	*
	C. edule	−4.86	2.82	0.99	25	*E*	ns	ns	*	–

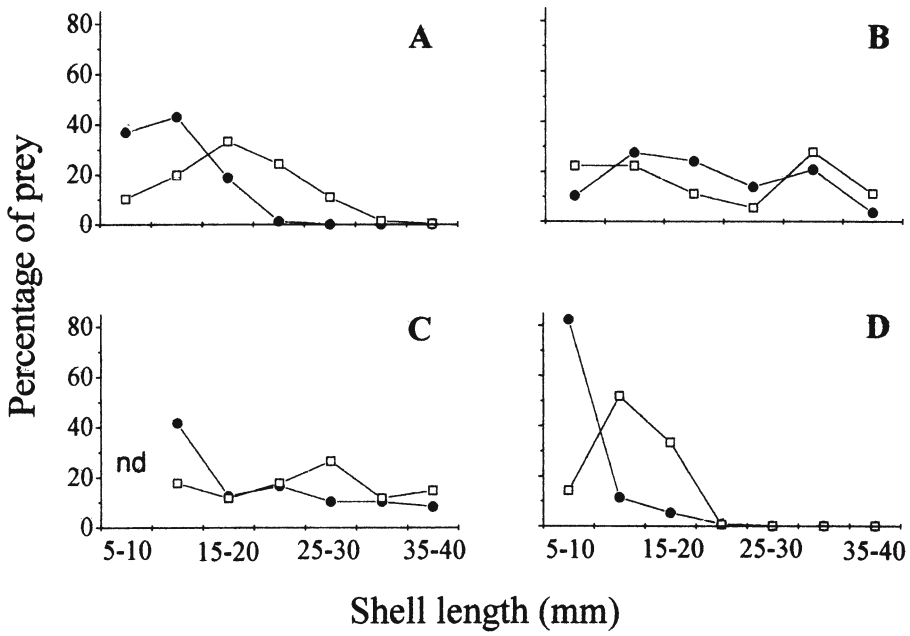

Figure 2. Percentage of (A) *Mytilus edulis*, (B) *Ostrea edulis*, (C) *Crassostrea gigas* and (D) *Cerastoderma edule* that were consumed daily by each *Carcinus maenas* 40–55 mm CW (solid circles) and 55–70 mm CW (open squares) during a period of 8–10 days. Note that *C. gigas* 5–10 mm in shell length were not available during these experiments (nd = no data).

ranges of prey. Results indicate that the ranking order of prey profitability paralleled the order in which prey were consumed by both size categories of crabs during feeding experiments. Analysis of variance showed that

Table 2. Results of chi-square tests on the mean number of prey consumed (No.) by medium (40–55 mm CW) and large (55–70 mm CW) *Carcinus maenas* during size-selection experiments. The preferred (i.e. consumed in > 20%) size ranges of each prey species are expressed in terms of shell length (SL mm) and relative prey size (RPS) when chi-square tests proved statistical significance; * significant at $p < 0.001$, ns = no significant departure from a random choice

	Medium					Large				
			Preferred size ranges					Preferred size ranges		
	χ^2	No.	SL	SW	RPS[1]	χ^2	No.	SL	SW	RPS[1]
M. edulis	699.5 *	19.4	5–15	2.0–6.3	0.15–0.46	235.9*	12.0	15–25	6.3–10.6	0.33–0.56
O. edulis	7.2 ns	1.8	–	–	–	4.0 ns	1.1	–	–	–
C. gigas	22.8 *	3.0	10–15	3.7–5.4	0.27–0.40	3.1 ns	2.1	–	–	–
C. edule	282.6 *	7.1	5–10	2.9–6.4	0.21–0.47	165.1 *	10.1	10–20	6.4–14.1	0.34–0.75

[1]RPS was calculated as the median value of shell width in each size class of prey divided by the height of the master chela in each size category of crab.

profitability of the preferred size range of *M. edulis* was significantly greater than that of both *O. edulis* and *C. gigas*. The preferred size ranges of mussels and cockles, however, provided similar profitability for crabs in both size categories. No significant differences were detected between profitability of the preferred size classes of *O. edulis* and *C. gigas* for either medium or large crabs. Analysis of variance of breaking time of the preferred size range of each prey species revealed that medium-sized crabs took significantly less time to break open cockles than mussels of the preferred size range (Table 3). Amongst large crabs, however, significant differences were only found between prey with extreme values (*C. gigas* > *C. edule*). No significant differences were detected between the breaking times of mussels and oysters or between the two oyster species. Because crabs generally took similar times to open all four bivalves, these results suggest that differences in profitability between the selected size ranges of prey were mainly due to differences in their biomass.

When crabs were offered a wide size range of *M. edulis* in combination with either *O. edulis* or *C. gigas*, both size categories of *C. maenas* showed a strong preference for mussels (Fig. 2). However, when crabs were offered a choice between *O. edulis* and *C. gigas*, neither size groups of crabs showed any preference for either oyster species. When given a choice between *M. edulis* and *C. edule*, medium-sized crabs clearly preferred mussels, whereas large crabs consumed similar numbers of both prey of 10–15 mm SL, but only consumed mussels from the larger size classes (Fig. 3).

When crabs were presented with both equal and unequal numbers of the preferred size ranges of *M. edulis*-*O. edulis*, the percentage of mussels opened by medium and large *C. maenas* (77–100%) was always significantly higher than that of oysters (Table 4). The proportion of *O. edulis* accepted by crabs on the other hand was never greater than 23%, even when the alternative species was scarce. Of all the mussels encountered very few were rejected, whilst any encountered *O. edulis* were only occasionally consumed; more frequently they were rejected before the crabs had attempted to open them. While the percentage of rejected oysters was always high, the already low percentage of rejected mussels in the 1:2 ratio experiments decreased even further in the 1:4 ratio experiments, where mussels were at their lowest relative abundance (Table 4). Results of goodness-of-fit tests showed that the number of observed encounters was not significantly different from those expected in all mussel-oyster combination trials (χ^2 from 0.22 to 2.84, all at $p > 0.05$), suggesting that consumption rates were not influenced by prey encounter rates.

In experiments with equal and unequal numbers of the preferred size ranges of *M. edulis*-*C. edule*, however, the percentage of accepted and rejected prey varied according to the rates in which prey species were encountered (Table 4). Of the total number of prey accepted by large crabs, the percentage of *M. edulis* decreased when mussels were less abundant. Similarly, the percentage of accepted *C. edule* increased as their relative abundance increased. Large crabs encountered both prey species as expected by the proportions in which they were presented (χ^2 from 0.03 to 1.63, all at $p > 0.05$). Although medium-sized crabs also accepted mussels and cockles in the same proportions as they encountered them, the encounter rates of mussels and cockles did not correspond to the relative abundance in which prey were offered (1:1 trial: $\chi^2 = 7.48, p < 0.05$; 1:2 trial: $\chi^2 = 9.6, p < 0.01$ and 1:4 trial: $\chi^2 = 25.77$, $p < 0.001$, respectively).

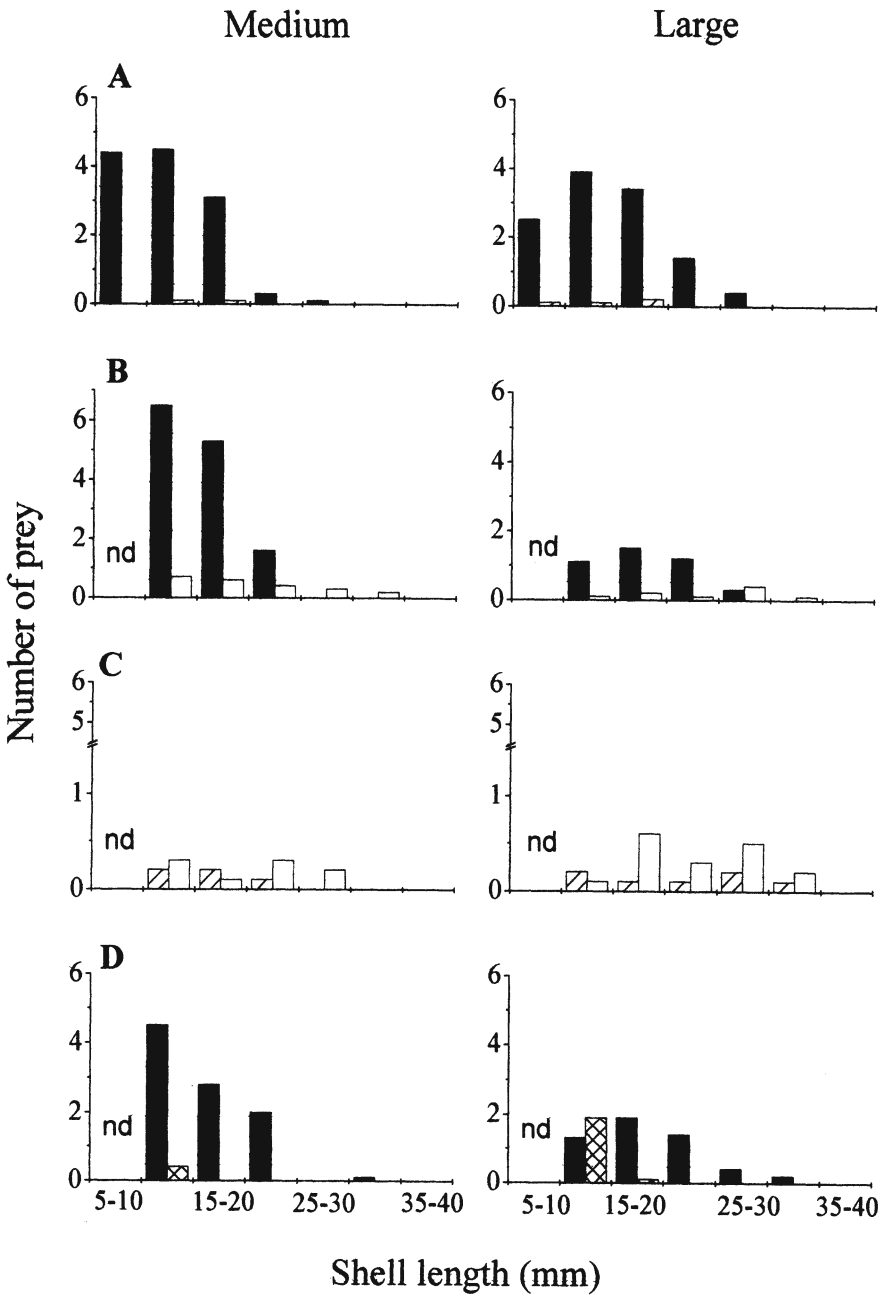

Figure 3. Number of *Mytilus edulis* (solid bars), *Ostrea edulis* (hatched bars), *Crassostrea gigas* (open bars) and *Cerastoderma edule* (crossed bars) of various size classes that were consumed by medium (40–55 mm CW) and large (55–70 mm CW) *Carcinus maenas* during experiments where crabs were offered prey species in paired combinations: (A) *M. edulis-O. edulis*, (B) *M. edulis-C. gigas*, (C) *O. edulis-C. gigas*, (D) *M. edulis-C. edule*. Values are mean consumption rates crab $^{-1}$ d^{-1}. Note that prey 5–10 mm in shell length were not available during some of these experiments (nd = no data).

Medium-sized crabs encountered mussels (49%) and cockles (51%) in statistically indistinguishable numbers in the 1:2 ratio trail ($\chi^2 = 0.05$, $p > 0.05$), and encountered mussels (39%) at approximately half the rate that they encountered cockles (61%) in the 1:4 ratio trial ($\chi^2 = 1.85, p > 0.05$).

When *C. maenas* (50–60 mm CW) were presented with epoxy resin models of three contrasting geometric shapes, persistence time decreased significantly

Table 3. Results of analyses of variance and selected pairwise comparisons using Scheffe's method on profitability and breaking time data of *Mytilus edulis* (*M*), *Ostrea edulis* (*O*), *Crassostrea gigas* (*C*), and *Cerastoderma edule* (*E*) consumed by medium (40–55 mm CW) and large (55–70 mm CW) *Carcinus maenas*; Diff. Mean=difference between means; SE=standard error of the mean; upper (UCI) and lower (LCI) limits of confidence interval; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = no significant difference between pairs of variables

	F	Pairwise Comp.	Diff. Mean	SE	LCI	UCI	*p*	Result
Profitability								
		M-O	0.31	0.089	0.05	0.56	*	*M* > *O*
40–55 mm	30.98 ***	*M-C*	0.58	0.070	0.37	0.78	*	*M* > *C*
		O-C	0.27	0.102	-0.03	0.56	ns	*O* = *C*
		M-E	-0.12	0.065	-0.31	0.07	ns	*M* = *E*
		M-O	0.60	0.072	0.39	0.80	*	*M* > *O*
55–70 mm	60.00 ***	*M-C*	0.66	0.078	0.44	0.89	*	*M* > *C*
		O-C	0.07	0.083	-0.17	0.31	ns	*O* = *C*
		M-E	-0.17	0.062	-0.35	0.01	ns	*M* = *E*
Breaking time								
		M-O	-0.17	0.138	-0.57	0.23	ns	*M* = *O*
40–55 mm	16.92 ***	*M-C*	-0.23	0.109	-0.54	0.09	ns	*M* = *C*
		O-C	-0.05	0.158	-0.51	0.41	ns	*O* = *C*
		M-E	0.58	0.101	0.29	0.87	*	*M* > *E*
		M–O	-0.19	0.136	-0.58	0.20	ns	*M* = *O*
55–70 mm	4.30 **	*M-C*	-0.24	0.148	-0.66	0.19	ns	*M* = *C*
		O-C	-0.05	0.157	-0.50	0.40	ns	*O* = *C*
		M-E	0.18	0.118	-0.16	0.52	ns	*M* = *E*

through the sequence of models regardless of their shape (Fig. 4; $F = 20.46$; $p < 0.001$). Although the decrease in persistence time was similar for all three geometric shapes ($F = 0.75$; $p > 0.05$), crabs persisted for a significantly longer period of time with the first sphere and wedged rectangle than with the first flat disc ($F = 7.31$; $p < 0.001$). No significant differences in persistence time between individual crabs were detected ($F = 2.00$; $p > 0.05$). These results indicate that crabs initially preferred those shapes with a larger minimum dimension. Persistence time, however, decreased with increasing number of models in the sequence in a similar rate regardless of model type, suggesting that shell shape does not influence the rate at which preference declines when crabs handle food items of zero profitability.

Discussion

Although crabs are known to be opportunistic predators, feeding on a wide variety of prey types (Ropes, 1968), prey size selection has been frequently reported in a diversity of brachyuran crabs feeding on various species of hard-shelled molluscan prey (e.g. Arnold, 1984; Hughes & Elner, 1989; Brown & Haight, 1992; Juanes, 1992). In the present study, *C. maenas* consumed a particular size range of *M. edulis* and *C. edule* in numbers higher than expected by chance (Table 2), but generally consumed all size classes of both oyster species in approximately equal numbers. The size ranges of selected prey and the patterns of size selection also varied from one species of prey to another (Fig. 2), suggesting that differences in size-selective predation amongst these bivalve prey are related to the contrasting morphological features of their shells and the way these features influence the vulnerability of such prey to predation by crabs. Since differences between the preferred size ranges of prey were larger

Table 4. Percentage and numbers (in parenthesis) of *Mytilus edulis*, *Ostrea edulis* and *Cerastoderma edule* that were accepted or rejected by *Carcinus maenas* 40–55 and 55–70 mm CW in experiments where crabs were presented with the preferred size classes of mussels and oysters, and mussels and cockles in proportions of 1:1, 1:2 and 1:4. Values are mean consumption rates per crab over 1 h periods during 4–5 consecutive days; * denotes prey species accepted in significantly higher numbers than expected ($p < 0.01$); NTA = cases in which results did not allow for chi-square tests to be applied; ns = no significant differences

		M. edulis			*O. edulis*		*M. edulis*			*C. edule*	
				1:1					**1:1**		
40–55 mm	acc	88	(7) *		13	(1)	70	(8) *		30	(3.4)
	rej	36	(3.6)		64	(6.4)	0	(0)		100	(0.4)
55–70 mm	acc	88	(7.4) *		12	(1)	50	(2.6) ns		50	(2.6)
	rej	6	(0.4)		94	(6)	44	(0.8)		56	(1)
				1:2					**1:2**		
40–55 mm	acc	91	(11.4) *		19	(2.6)	54	(8.4) *		46	(7.2)
	rej	4	(0.8)		96	(17.8)	10	(0.2)		90	(1.8)
55–70 mm	acc	100	(2) NTA		0	(0)	38	(4.2) ns		62	(6.8)
	rej	33	(3.8)		67	(7.8)	50	(0.8)		50	(0.8)
				1:4					**1:4**		
40–55 mm	acc	77	(4.6) *		23	(1.4)	49	(8.2) *		51	(8.6)
	rej	4	(0.6)		96	(15.4)	8	(0.4)		92	(4.6)
55–70 mm	acc	100	(1.4) NTA		0	(0)	9	(0.6) ns		91	(6.2)
	rej	11	(0.6)		89	(4.8)	56	(1)		44	(0.8)

when expressed in terms of shell length than in terms of shell width, it is suggested that prey size based solely on shell length is not an appropriate indicator of the morphological characteristics of the shell associated with crab preference, and the geometry and crushing resistance of prey shells should be taken into account when crab foraging behaviour is being examined.

Not only do these four bivalves have contrasting shell shapes, but, as they increase in size, their flesh content increases at different relative rates (Table 1). Sanchez-Salazar et al. (1987) attributed differences in the size selection of *M. edulis* and *C. edule* by *C. maenas* to variations in shell morphology and strength per unit length. These authors showed that the shell dimensions of cockles that could be opened by crabs of a given chelal strength were less, but the energy obtained was greater, than when feeding on mussels. Accordingly, they suggested that crabs could obtain better yields by consuming cockles than mussels of a similar linear size. In the present study, the size classes of *M. edulis* and *C. edule* that were selected by both size categories of crabs yielded similar biomass per unit time (Table 3). Crabs of both size categories preferred cockles of a shell length that was slightly smaller than the preferred mussels, but the preferred size ranges of both species were of similar shell width (Table 2). Furthermore, the ability of crabs to crush prey decreased more abruptly for cockles than for mussels as these increased in size (Fig. 2). As the more globular shaped cockles increase in length, shell width increases more rapidly than in the more elongate mussels (Table 1); consequently, cockles have a wider shell than mussels of similar shell length. Since dome-shaped shells are intrinsically stronger than flatter shells (Wainwright, 1969), the presence of a higher dome in the more convex cockle shell probably increased force applications required by crabs to open this infaunal bivalve. Shell features such as large size, increased thickness, greater inflation, and the absence of gape reduce the vulnerability of clams to predation by *Cancer productus* (Boulding, 1984), and have been shown to influence size-related preferences of *Callinectes sapidus* feeding on other infaunal prey (Blundon & Kennedy, 1982).

When crabs were offered a wide size range of oysters and mussels simultaneously, both medium (40–55 mm CW) and large (55–70 mm CW) *C. maenas* consistently selected mussels (Fig. 3). Furthermore, results of experiments where *M. edulis* and

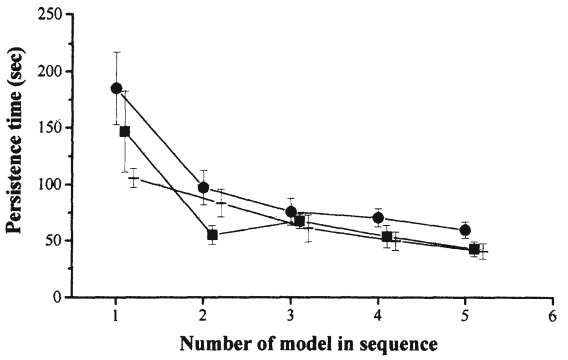

Figure 4. Mean persistence time (s ± se) for inedible models of three contrasting geometric shapes: a wedged rectangle (solid square), a flat disc (horizontal line) and a sphere (solid circle), that were presented sequentially to *Carcinus maenas* of 50–60 mm CW.

O. edulis were offered in equal and altered proportions suggest that preference for mussels, and the apparent lack of preference for oysters, are independent of the relative abundance in which either prey species are presented (Table 3). Previous authors have shown that *C. maenas* consumed *M. edulis* at more than twice the rate at which they consumed *C. gigas* (Dare et al., 1983), whilst *Cancer novaezelandiae* also preferred *M. edulis* when offered a choice of mussels and gastropods (Creswell & McLay, 1990). It can be argued that the lack of crab experience in handling a non-native prey species, such as the Pacific oyster, influenced the species-related preferences exhibited by *C. maenas* throughout these experiments. However, differences in prey species selection were mainly between mussels and both the European flat oyster and the Pacific oyster, whilst crabs consumed *O. edulis* and *C. gigas* in similar numbers.

Barbeau & Scheibling (1994) indicated that active selection can be considered to be an important component of predation when a predator selects a prey type more often than expected when given a choice of prey types than when not given a choice (see also Liszka & Underwood, 1990). In the present study, comparisons of prey consumption rates in single and multiple choice experiments could not be made, thus, active and passive components could not be analysed in this way. However, the preference for *M. edulis* exhibited by *C. maenas* was consistent throughout experiments where prey types were encountered in varying and contrasting proportions (Fig. 3; Table 4). In the single species experiments, the order in which prey species were ranked according to consumption rates clearly paralleled the rank order of species profitability, and differences in profitability between prey species were mainly due to differences in their biomass, rather than to differences in breaking time. These results suggest that prey value can influence prey species-selection, and that crab preference for mussels in the present study involves an active component of selection at some point of the predation cycle.

When *C. maenas* were offered *M. edulis* and *C. edule* in various proportions, species selection was strongly influenced by the frequency in which each size category of crabs encountered prey (Table 3). Although active selection could not be invalidated (*sensu* Barbeau & Scheibling, 1994), the close agreement in the proportions of accepted and encountered prey items suggests that the active component of selection in this particular prey combination is not important in determining crab preference. The observed differences in the foraging behaviour of *C. maenas* when feeding on a combination of mussels and oysters and a combination of mussels and cockles further supports the view that the relative importance of active and passive selection in explaining prey choice may differ with each predator-prey system (Abele et al., 1981).

Patterns of prey selection are the result of a sequence of specific behavioural components that a predator performs during a predation event, including the initial detection of prey (Hughes, 1990). Previous reports have shown that *C. maenas* is sensitive to different concentrations of mussel flesh filtrate (Kaiser et al., 1993), but decisions by crabs on whether to initially attack or reject prey are not influenced by differences between mussel and oyster flesh filtrates (Mascaró & Seed, 2000). In addition, Hughes and Seed (1995) showed that tonal contrast (colour) is important in prey selection by the blue crab, *Callinectes sapidus*. In the present study, epoxy dummies had all the same colour, but their contrasting geometric shapes could have influenced the visual target area detected by *C. maenas*.

Whilst extrinsic factors, such as hunger, experience and stimuli from alternative prey can modify predator selectivity, thus affecting predatory decisions (Hughes, 1980; Jubb et al., 1983), intrinsic factors such as the information gained by manipulating the prey in the chelae and mouthparts are crucial in deciding whether to continue or abort an attack (Akumfi & Hughes, 1987; Kaiser et al., 1993). In the present work, species-related preferences exhibited by crabs feeding on prey near the optimal size suggest that active selection might have taken place at some point in the predation cycle, and that this selection was related

to the amount of dry flesh weight gained per unit of handling time.

For crabs to be able to rank prey in the order of their profitability, mechanisms must exist by which they are able to recognise prey characteristics which are correlated with their potential value. In experiments where crabs were offered models of contrasting geometric shape, *C. maenas* persisted much longer with the first models that resembled both the shape of a cockle (sphere) and a mussel (wedged rectangle) than with the first model that resembled a flat oyster (disc), indicating that contrasting differences in shell shape can significantly influence persistence time on initial attack, and hence subsequent prey selection. Moreover, the resin models used had a similar maximum length, but differed in maximum height and width. If crabs evaluate prey value on the basis of shell width rather than length, they would be expected to persist longer on those models with the greatest shell width. A strong association between shell width and volume is indicated by the significantly greater increase in shell width as cockles and mussels rather than, as flat oysters, increase in shell length, and by flat oysters having significantly less biomass than both mussels and cockles of comparable shell length (Table 1). Furthermore, both *C. edule* and *M. edulis* were included in crab diets much more frequently than *O. edulis* throughout the experiments (Table 2), and *C. maenas* obtained the highest profitability when feeding on cockles and mussels (Table 3). These results are in accordance with those reported by Richardson et al. (1993), who suggested that the strong reluctance of *C. maenas* and *C. pagurus* to feed on another flat oyster, *Tiostrea* (= *Ostrea*) *lutaria*, could be related to characteristics in the shape of its shell.

It is interesting to note that shell shape was also related to dry flesh weight, since the relatively flatter oyster species has less biomass than the more voluminous shells of cockles and mussels (Table 1). Moreover, observations of crab handling techniques indicated that *C. maenas* had more difficulty grasping the relatively higher, flatter and more irregular shell of *O. edulis* than those of mussels and cockles, whose more globular shells fitted within the chelae in a position that allowed the predator to exert a greater compressive force (Mascaró, 1998). These results suggest that shell width constitutes an important morphological characteristic which crabs might be able to recognise and associate with potential prey value.

Previous works have reported that the minimum dimension of the shell is an important characteristic determining prey size and species selection in crabs (Boulding, 1984) and other decapods (Griffiths & Seiderer, 1980). In experiments where *C. maenas* was presented with Perspex models of different shape and size, Kaiser et al. (1993) found that changes in the length of models had little influence on handling time since mechanical efficiency of the chelae was determined by the cross-sectional profile of the prey. These authors further suggested that those models which more closely resembled the shape of a mussel (wedged rectangle) allowed the chelae to work with greatest mechanical advantage and improved handling efficiency. Furthermore, crabs consistently preferred prey with a RPS < 1, suggesting that the position in which the width of the shell fitted within the chelae is of considerable importance in determining attack success of the preferred size classes of prey. The flat disc model had a slightly smaller width, but had a much greater height than the wedged rectangle, suggesting that crabs did not persist with the flat disc as long as with the wedged rectangle probably because a large absolute height can reduce handling efficiency (see also Griffiths & Seiderer, 1980).

Foraging decisions by crabs based on shell characteristics associated with volume (i.e. shell width) might explain the patterns of species selection by *C. maenas* observed in the present study. The observed similarity in crab preference for *M. edulis* and *C. edule*, might be the result of the similarity in the volumes of the (different) size classes of both prey species that were selected. When crabs were offered similar size classes of *M. edulis* and *O. edulis*, they preferentially consumed mussels probably as a consequence of the differences in volume between the (similar) size classes of prey presented.

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Intertidal distribution and species composition of brachyuran crabs at two rocky shores in Central Portugal

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Key words: intertidal zonation, rocky shores, species composition, brachyuran crabs

Abstract

The objectives of the present study are to describe and compare the brachyuran community of rocky shores within the Central Portuguese coast and to examine the zonation patterns of the most representative species. For this, randomly placed transects were surveyed to obtain crab counts according to microhabitat and intertidal level. Repeated sampling in two different shores during two different seasons provided spatial and temporal replication for zonation analyses. Seven species were registered: *Pachygrapsus marmoratus, Eriphia verrucosa, Xantho incisus, Carcinus maenas, Necora puber, Pirimela denticulata* and *Pilumnus hirtellus*. Species density rankings are the same at both localities, but the less exposed shore presents higher diversity. While most species are mainly confined to specific microhabitats in the lower level, *P. marmoratus* and *E. verrucosa* can exploit the whole intertidal range. Regardless of shore and season, *E. verrucosa* is more abundant in the lower intertidal levels, while no such zonation patterns were recorded for *P. marmoratus*. Initial predictions concerning the effect of wave exposure and temperature on the zonation of those species are not validated after analysing the factorial model proposed. Between-shore contrasts were found instead, with higher densities recorded in the more exposed locality for both species. Possible causes of the observed patterns are discussed.

Introduction

In spite of several available studies on the distribution of intertidal brachyuran crabs (e.g. Bacon, 1971; Griffin, 1971; Hartnoll, 1975; Jones, 1976; Wada, 1983), there are no conclusive models explaining population density as a function of key environmental factors related to desiccation potential. Such factors are known to constrain the intertidal distribution of sessile invertebrates in which comprehensive descriptive models have been proposed and reviewed (see Lewis, 1964; Stephenson & Stephenson, 1970; Little & Smith, 1980; Rafaelli & Hawkins, 1996). Mobile fauna, however, may behave so as to minimise harsh environmental conditions. In addition to the remarkable osmoregulatory capacity of certain crab species (Gross, 1964; Barnes, 1967; Schubart & Diesel, 1998), locomotion may also allow the exploitation of different shelter resources (Cannicci et al., 1999), which may obscure eventual zonation patterns of these organisms.

As for sessile forms, most discussion has centered on the nature of limiting factors that set the upper and lower boundaries of a given species distribution. In this sense, it has been argued that tidal exposure time (McLay & McQueen, 1995) and substrate type (Menendez, 1987) may be the major factors causing zonation in brachyurans. However, there is evidence of active shelter exclusion (Navarrete & Castilla, 1990) and predator–prey interactions (Willason, 1980) acting as mechanisms of interspecific segregation.

In this study, we describe the composition of the brachyuran fauna at two rocky shores in the Central Portuguese coast and examine their distribution according to microhabitat and intertidal level. The vertical zonation of the most common species was studied in further detail by obtaining crab density estimates in replicate samples at different shores and seasons. By analysing the factorial model, results concerning the effect of temperature and wave exposure on zonation patterns are presented and discussed.

Materials and methods

The intertidal occupation patterns of brachyuran species were assessed by means of vertical transects over the entire tidal amplitude exposed at diurnal spring low tides. Within each transect, infra, meso and supralittoral areas were delimited according to prevailing sessile communities (Hawkins & Jones, 1992), and counts were recorded separately for each intertidal level. Nine replicate transects were randomly assigned at each of two rocky shores located within the Lisbon region, Cabo Raso (38° 42′ N; 09° 29′ W) and Avencas (38° 41′ N; 09° 21′ W), respectively, highly and moderately exposed to wave action, during two sampling seasons; winter (sampling dates centered on February, 1998) and summer (centered on September, 1998).

Transect demarcation and crab counts

Transects consisted of 15 m wide sections placed randomly on the shore, each providing three adjacent sampling units corresponding to the different intertidal levels. Due to particular characteristics of each sampling unit, namely the presence of sandy patches, topography constraints and slope, their actual sampling area varied from 40 to 450 m^2. The size of the smallest sampling unit was, however, large enough to minimize the effect of aggregation between conspecifics, thus allowing comparisons of density (calculated as individuals m^{-2}) between units of different size.

Seven distinct microhabitats within the rocky shores were distinguished: rocky areas (including crevices), tide pools, algal canopy, mussel beds, cobbles, *Sabellaria* reefs and barnacle beds. Transects were visually surveyed by a single observer. Time effort per sampling unit varied from 11 to 95 min according to their size. For each crab sight, the corresponding species and associated microhabitat were recorded. No methods other than visual inspection were used for crab recording.

Quantitative and statistical analyses

Shannon–Wiener Diversity indices were calculated for overall counts at both shores and a *t*-test was used to compare them as described in Poole (1974). Since natural logarithms were used in calculations, nits per individual (Krebs, 1989) is the diversity unit used hereafter.

The zonation patterns of the most representative species were analysed in a fully orthogonal model in which 'intertidal level', 'season' and 'shore' are all fixed factors. Significance of main factors and their interactions were evaluated in a three-way ANOVA, and SNK tests were run for *a posteriori* comparisons. Data were square-root transformed to achieve homoscedasticity, as usually applied in Poisson-like distributed variables (Underwood, 1997). Null hypotheses were rejected at probabilities of type I error lower than 0.05.

Results

Species composition and distribution

Seven species were recorded: *Pachygrapsus marmoratus* (Fabricius), *Eriphia verrucosa* (Forskål), *Xantho incisus* (Leach), *Carcinus maenas* (Linnaeus), *Necora puber* (Linnaeus), *Pirimela denticulata* (Montagu) and *Pilumnus hirtellus* (Linnaeus). A total of 2518 individuals were recorded, of which 78.5% were to the grapsid *P. marmoratus*. The xanthoids *E. verrucosa* and *X. incisus* made up 14.5 and 5.5% of all sampled crabs respectively, while each of the remaining species accounted for less than 1%. Despite its absence in the samples, the grapsid crab *Pachygrapsus transversus* (Gibbes) was observed on a few occasions at Avencas and once at Cabo Raso during nocturnal low tides.

Species density rankings are the same at both localities, but a higher diversity was found at Avencas Beach (H'_{av}=0.7479; H'_{cr}=0.5776; df=1462; t=4.24, $p \cong 0.000$). This difference is mainly due to the presence of *P. denticulata* and *P. hirtellus* and the higher relative frequency of *X. incisus* at that site (Fig. 1). While most species are predominantly confined to specific microhabitats in the lower level, namely tide pools and cobble areas (Table 1), *P. marmoratus* and *E. verrucosa* can exploit the whole intertidal range, preferably rocky crevices. A much reduced percentage of crab recordings was obtained in other potential shelter sources such as mussel and barnacle beds (Table 1) in spite of being these quite common at the study areas.

Zonation patterns of P. marmoratus *and* E. verrucosa

It was predicted that (1) zonation in these species would be less pronounced under conditions of higher wave exposure and (2) stratification would be more conspicuous during the warmer season due to physiologically stressful conditions in the upper levels. Nevertheless, the factorial model analysed in

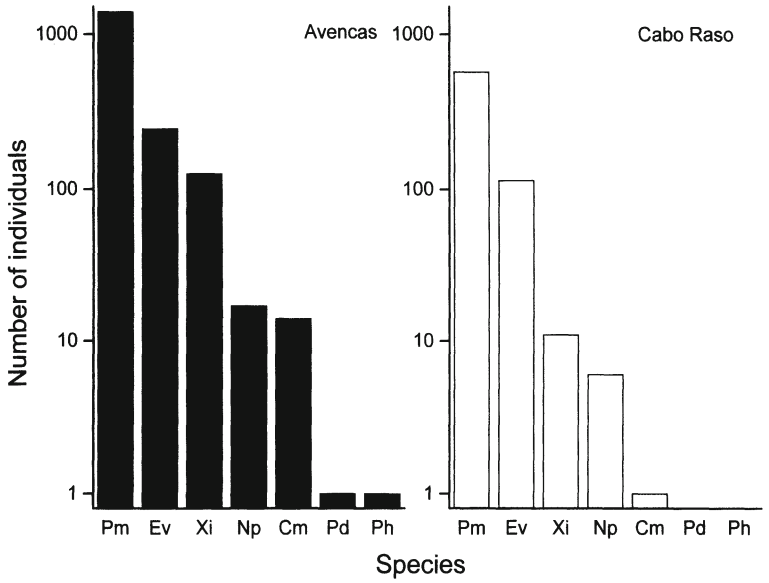

Figure 1. Number of individuals recorded for each species at the shores sampled. Pm: *Pachygrapsus marmoratus*; Ev: *Eriphia verrucosa*; cm: *Carcinus maenas*; Xi: *Xantho incisus*; Np: *Necora puber*; Pd: *Pirimela denticulata*; Ph: *Pilumnus hirtellus*.

Table 1. Percentage of crabs recorded in each microhabitat for each species. Species abbreviations as indicated in Figure 1

Species	Microhabitat						
	Rocky area	Tide pools	Algal canopy	Mussel beds	Cobble	*Sabellaria* reefs	Barnacle beds
Pm	69.0	13.5	1.7	3.3	12.2	0.1	0.2
Ev	90.1	8.8	0.3	0.8	0.0	0.0	0.0
Cm	6.7	93.3	0.0	0.0	0.0	0.0	0.0
Xi	10.1	36.2	0.0	0.0	53.6	0.0	0.0
Np	0.0	87.0	4.3	0.0	8.7	0.0	0.0
Pd	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Ph	0.0	100.0	0.0	0.0	0.0	0.0	0.0

Table 2. Three-way ANOVA describing intertidal density patterns of *Pachygrapsus marmoratus* (Fabricius) and *Eriphia verrucosa* (Forskål). SV: source of variation; MS: mean square; L: (intertidal) level; S: season; Sh: shore

SV	*Pachygrapsus marmoratus*			*Eriphia verrucosa*		
	MS	*F*-ratio	*p*	MS	*F*-ratio	*p*
Level	0.0039	0.39	0.6787	0.0029	4.45	0.0142
Season	0.0863	8.59	0.0042	0.0003	0.51	0.4761
Shore	0.0408	4.06	0.0466	0.0062	9.58	0.0026
L×S	0.0034	0.34	0.7156	0.0000	0.01	0.9909
L×Sh	0.0084	0.84	0.4361	0.0006	0.96	0.3879
S×Sh	0.081	8.06	0.0055	0.0004	0.61	0.4354
L×S×Sh	0.0036	0.36	0.6973	0.0002	0.26	0.7726
res.	0.01			0.0006		

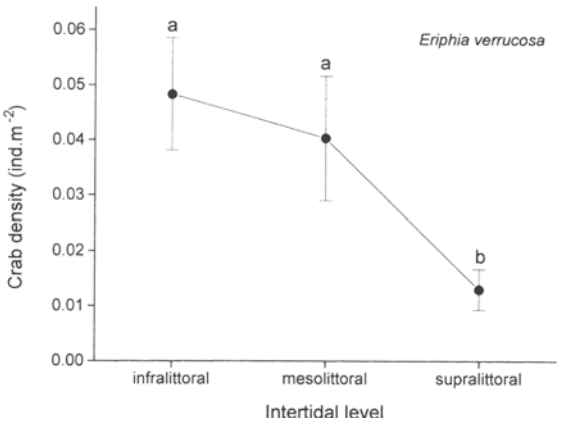

Figure 2. Zonation pattern of *Eriphia verrucosa* (Forskål). Bars indicate standard errors. Density between intertidal levels sharing a letter is not significantly different (SNK tests; $p>0.05$).

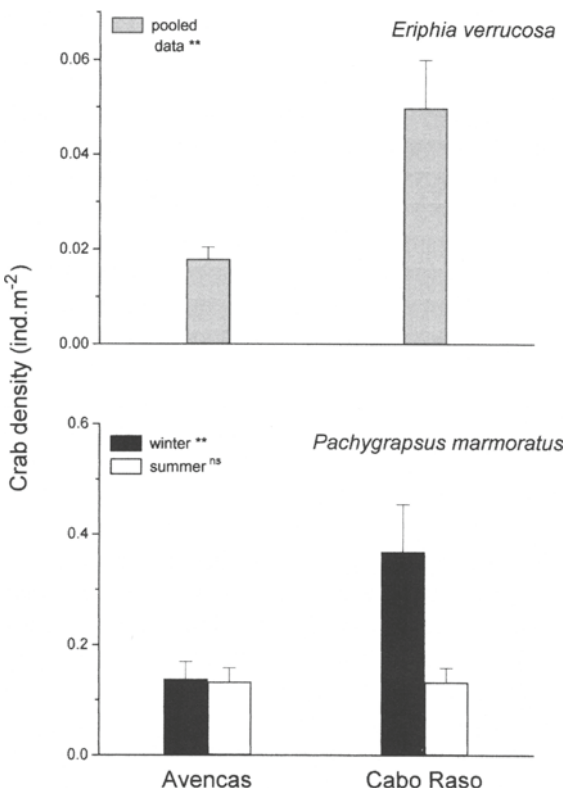

Figure 3. Density of *Eriphia verrucosa* (Forskål) and *Pachygrapsus marmoratus* (Fabricius) at the shores studied. Bars indicate standard errors. Results from SNK tests indicated as follows; ns: not significant; **: $p<0.01$.

this study does not support the above hypotheses since 'level' vs. 'shore' and 'level' vs. 'season' interactions are not significant (Table 2). Regardless of shore and season, the density of *E. verrucosa* is higher in the lower intertidal levels (Table 2, Fig. 2). This trend was not observed in *P. marmoratus*, in which density among levels did not show significant differences. Between-shore contrasts were also found in both species, which depend on the season sampled in the case of *P. marmoratus* as indicated by a significant 'season' vs. 'shore' interaction (Table 2). While *E. verrucosa* is clearly more abundant at Cabo Raso, this difference was only significant during winter for *P. marmoratus* (Fig. 3).

Discussion

The brachyuran taxocenoses found at the study sites are composed of two dominant warm temperate species, *P. marmoratus* and *E. verrucosa*, and five less common typical temperate species, using the biogeographical classification proposed by d'Udekem d'Acoz (1999). These less abundant species are restricted to specific microhabitats within the lower intertidal, as verified in this study, extending their bathymetric distribution to a few meters depth and occasionally to considerably deeper grounds (see Zariquiey Alvarez, 1968; Ingle, 1980; d'Udekem d'Acoz, 1999). The presence of the subtropical species *Pachygrapsus transversus* provides further indications of faunal affinity between the study area and other warm-temperate regions such as the Western Mediterranean. The significant difference found between diversity at both sites is probably related to shore profile. At Avencas Beach, the infralittoral area exposed at spring low tides may be notably larger because it extends over a gently sloped rocky platform. Therefore, the relative density of species restricted to that level is greater at Avencas than at Cabo Raso, which explains the higher diversity at the former shore due to higher species evenness. Abele (1974) found that number of substrates within a given habitat is the most important factor determining diversity of decapod crustaceans in marine habitats. Within a single marine habitat, however, the number of available substrates would be less variable and diversity differences would be thus largely dependent on the area each substrate actually covers. Using the present study as an example, tide pools is the only microhabitat where all recorded species were found (Table 1). If other conditions are

considered to be equivalent, which may seldom be the case, it could be expected that diversity of brachyuran crabs in rocky shores enclosing larger pool areas would be higher.

The values of species density presented here should only be considered valid for the specific purposes of this study. They are obviously underestimations since only visual sights were used for counts. Such bias would be more pronounced in cobble areas, where *Xantho incisus* is mainly found, and biogenic substrates where juvenile individuals of different species may remain concealed (Spivak et al., 1994; Flores & Negreiros-Fransozo, 1999). More accurate estimates would involve a combination of destructive methods for sorting individuals from mussel beds, *Sabellaria* reefs and algal associations, and marking-recapture techniques such as those detailed in Begon (1979) to quantify crab abundance in the bedrock populations. Although beyond the scope of the present study, gathering such information would be very valuable since such data are still lacking.

Truly intertidal species, *P. marmoratus* and *E. verrucosa* are both capable of exploiting the whole intertidal range at the shores studied. The present account does not demonstrate an effect of temperature or wave exposure on the zonation patterns of those species. Yet, it should be pointed out that not all the possible range of wave exposure conditions was covered by the study design. A more comprehensive model should add one level to the 'shore' factor, namely by taking samples at a typical sheltered site, and include replication at all that levels for proper comparisons. If this still not demonstrate a 'level' vs. 'shore' interaction it would more adequately indicate that exposure probably does not influence the zonation of shore crabs at the study region. Further research addressing the effect of biotic variables on the distribution patterns of these species would be equally useful at present, namely the availability of suitable foraging areas. Shelter displacing experiments would also help to verify whether competition for crevices between *P. marmoratus* and *E. verrucosa* exists under conditions of shelter shortage.

Unlike *P. marmoratus*, which does not show any zonation pattern at the study sites, *E. verrucosa* is significantly more abundant in the infra and mesolittoral zones than in the supralittoral. This contrast is hardly surprising since the means by which grapsid and xanthoid crabs had been able to exploit the rocky shore environment are quite distinct. Grapsids are known to be active, fast-moving animals exploiting a wide variety of food resources while intertidal xanthoids are less active, slow-moving crabs often specialized to prey on a very restricted number of food items. While *Pachygrapsus* may cope with high salinity variations (Alves, 1974) and osmoregulate under extreme conditions (Jones, 1941), low desiccation tolerance had been recorded in the xanthoid *Eurypanopeus depressus* (Smith), which survive in the intertidal zone by benefiting from the relatively moist shelter provided by oyster reefs (Grant & McDonald, 1979). *Eriphia* also shelters in very specific rock crevices (Vannini & Gherardi, 1979), usually only large enough to hold its occupant (AAVF, pers. obs.). Remaining in these specific refuges probably decreases the desiccation rates in those organisms, allowing them to reach the higher intertidal. However, as observed in this study, xanthoids are mainly found in the lower reaches of the intertidal zone (e.g. Snelling, 1959; Pellegrino, 1984), while grapsids are frequently dominant in the supralittoral (e.g. Hiatt, 1948; Little, 1990; Omori et al., 1997).

Between-shore density contrasts found in *P. marmoratus* and *E. verrucosa* are clear. In the case of *E. verrucosa*, this difference is not seasonal, but a consistent trend. This species would depend on a suitable supply of large rock crevices, since this may be a limiting resource in natural conditions (Beck, 1995), and relatively large areas per individual, as agressive interactions prevent conspecifics from sharing a common shelter (Warburg & Schwartz, 1989). Although far from its carrying capacity, it was apparent that the proportion of potential crevices actually occupied by crabs was higher at Cabo Raso than at Avencas, resulting in the differences found. Causes of this density contrast are still unclear, but anthropogenic impact may account for at least part of such difference, considering that Avencas Beach is closer to sites of pollutant discharge along the Tagus estuary and is more exposed to human collecting activities. Lack of between-shore differences in summer for *P. marmoratus* is very probably related to seasonal processes occurring in this species' populations. Recruitment of young crabs during late autumn – early winter (Flores & Paula, unpublished) seems to be the main factor contributing to the population increase in this season. Mortality in very young crabs would probably render its effect by summer, when abundance might be far below the species potential at both sites. This possibility would indicate that recruitment rates are higher at Cabo Raso, a question that needs to be addressed by specific research.

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Morphometric comparison between Mediterranean and Atlantic populations of *Pontophilus norvegicus* (Decapoda, Crangonidae)

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Abstract

A multivariate comparison of morphometric differences was undertaken on populations of *Pontophilus norvegicus* from four Atlantic and one Mediterranean locations. Multiple discriminant analysis revealed clear morphometric differences between Atlantic and Mediterranean populations, with the Atlantic populations exhibiting a low degree of separation. The underlying variables responsible for this discrimination are shown not to have any operational taxonomic utility and, hence, no sub-specific status is attached to the respective populations.

Introduction

Pontophilus norvegicus (Sars, 1861) is a widespread benthic crangonid shrimp in the eastern Atlantic Ocean, ranging from the Cantabrian Sea and the Gulf of Gascogne in the south (Lagardère, 1970; Rodríguez-Marín, 1993) to Iceland and Spitzbergen (Hansen, 1908) in the north. In the western Atlantic Ocean, it has been recorded from Greenland south to Maryland, U.S.A. (Williams, 1984; Squires, 1990). The species also occurs in the Mediterranean, where it is considered to be a glacial relict (Abelló & Vallarades, 1985); Mediterranean locations include Mallorca (Forest, 1965), Ligurian Sea (Relini-Orsi & Relini, 1972), Catalan Sea (Abelló & Vallarades, 1985; Cartes, 1993), the Sicilian Channel (Pipitone & Tumbiolo, 1993) and possibly the Adriatic Sea (Stevcic, 1990).

As regards depth, the species occurs from 50 to 1450 m in the Atlantic (Sivertsen & Holthuis, 1956), but is most common between 200 and 500 m (Smaldon, 1979). In the Mediterranean, it occurs in depths of 392–2261 m (Abelló & Vallarades, 1985; Cartes, 1993).

Following the original discovery of the species in the Mediterranean, Forest (1965) suggested that the specimens from Mallorca exhibited morphological differences in the relative lengths of the sub-orbital spines, rostral length and the antennal scale, compared with the Atlantic specimens figured by Kemp (1910). These differences were attributed to the smaller size of the specimens studied, compared to Kemp's material. Although Abelló & Vallarades (1985) concluded that no apparent morphological differences exist between Atlantic and Mediterranean specimens, they suggested that a morphometric and/or biochemical study may shed some light on possible regional differences. More recently, d'Udekem d'Acoz (1999) suggested that this matter deserved closer scrutiny and offered the suggestion of potential sub-specific differences between Atlantic and Mediterranean populations.

A review of Mediterranean/Atlantic vicariant forms in Decapoda is provided by d'Udekem d'Acoz (1999), who lists more than 40 taxa. However, the precise taxonomic status of many of the forms is poorly understood, with some forms allocated full specific or subspecific status (e.g. *Chaceon inglei* Manning & Holthuis vs. *C. mediterraneus* Manning & Holthuis), whilst others are simply recorded as vicariant forms of infraspecific rank (e.g. *longipes* form of *Macropodia tenuirostris* (Leach). In many of these taxa, intermediate populations in the Gibraltar area are know, raising doubt as to their separate taxonomic status. In contrast, several taxa have a discontinuous distribution, potentially limiting gene flow due to geographical isolation.

Table 1. Continuous variables and their abbreviations

Carapace length (CL)
Rostral length (RL)
Length of basal rostral spines (BASAL)
Distance between endpoint of rostrum and endpoint of sub-orbital spine (SUBORB)
Distance between orbit and insertion of first and last tooth on dorsal carina of carapace (CARINA1, CARINA2)
Distance between orbit and insertion of first tooth and second on first lateral carina (CARINA3, CARINA4)
Distance between orbit and insertion of tooth on second lateral carina (CARINA5)
Length, width of antennal scale (SCALEL, SCALEW)
Dorsal length of somites 1–6 (SOM1–6)
Maximum width and depth of pleuron 2 (PLEUR2W, PLEUR2D)
Length of telson (TELSON)
Insertion point of anterior and posterior dorsal spines (TELANT, TELPOST)
Length, width of chelae of first pereiopod (CHELAEL, CHELAEW)
Length of propodus of fourth and fifth pereiopod (PROP4, PROP5)
Length, width of uropodal endopod (ENDOL, ENDOW)
Length, width of uropodal exopod (EXOL, EXOW)

Table 2. Discrete variables and their abbreviations

Sex
Rostrum falling short, equal to or overreaching eye (ROSTRUM)
Rostrum falling short of, level with or overreaching proximal segment of antennular peduncle (ANTPED)
Number of teeth on dorsal carina (DORSAL)
Tubercle on dorsal carina present or absent (TUBERCLE)
Number of teeth on first lateral carina (LATFIRST)
Number of teeth on second lateral carina (LATSECOND)
Antennal scale tooth falling short, level with or overreaching antennal scale (SCALETOOTH)

The present study aims at elucidating potential sub-specific differences of Mediterranean from Atlantic populations of *P. norvegicus* by using morphometric measurements and the multivariate method of Multiple Discriminant Analysis (MDA).

Materials and methods

Shrimps (N = 189) were studied from one Mediterranean and four Atlantic locations: (1) Spain – NW Mediterranean (41° 04 N, 1° 45 E–41° 17 N 2° 50 E, 366–769 m, 16 males, 32 females), (2) Faroe Islands – Scotland (61° N, 7° E – 60° 42 N 4° 15 E, 300–500 m, 13 males, 20 females), (3) Lødingen & Ullsfjord, Norway (68° 20 N 15° 30 E, 200–270 m, 7 males, 20 females), (4) south of Ireland (51° 53 N 11° 59 W–56° 23 N 09° 18 W, 570–1000 m, 4 males, 33 females) and (5) off the western seaboard of U.S.A. and Canada (44° 49 N 61° 41 W–42° 16 N 69°56 W, 172–848 m, 7 males, 37 females). Individuals were grouped into these arbitrary regions, as some finer resolution regions did not harbour enough specimens to satisfy MDA assumptions.

For each individual, 30 different characters (continuous variables, Table 1) were measured, and categorical data recorded from a further eight characters (discrete variables, Table 2). Absent, regenerating or damaged body parts sometimes reduced the total number of variables for individual shrimps, these were treated statistically as missing data. Observations were made using a stereo microscope fitted with an ocular micrometer. Measurements were made with an accuracy of 0.05 mm, whilst discrete variables were recorded as counts or assigned dichotomous codes.

Within-regional analyses were carried out on the raw data to identify outliers using linear least-squares regression analyses with carapace length as the independent variable (Spotte, 1997). Individual measure-

Table 3. Position of group centroids in Discriminant Analysis

	Root 1	Root 2	Root 3	Root 4
Sexes combined				
NW Mediterranean	−4.215	−0.177	−0.177	0.006
Faroe-Scotland	2.287	0.103	−1.258	0.610
Norway	1.462	−0.173	−0.759	−0.948
Ireland	0.704	1.652	0.733	0.005
U.S.A.–Canada	1.549	−1.160	0.901	0.107
Females only				
NW Mediterranean	−5.857	0.528	0.116	0.006
Faroe-Scotland	2.488	−0.146	2.075	0.962
Norway	1.952	0.399	1.001	−1.295
Ireland	0.375	−2.055	−0.625	0.002
U.S.A.–Canada	2.533	1.228	−1.038	0.226

Table 4. Summary statistics for Discriminant Analysis

	Eigenvalue	% of variance	Cumulative %	Canonical correlation
Sexes combined				
Root 1	6.58	78.9	78.9	0.93
Root 2	0.90	10.8	89.7	0.69
Root 3	0.66	7.9	97.6	0.63
Root 4	0.20	2.4	100.0	0.41
Females only				
Root 1	11.35	79.0	79.0	0.96
Root 2	1.55	10.7	89.7	0.78
Root 3	1.09	7.6	97.3	0.72
Root 4	0.38	2.7	100.0	0.53

ments (outliers) were re-measured (if possible) and values corrected prior to subsequent analysis and only excluded from further analyses, if their values still exceeded 3 standard deviations (SD) in regression residual plots. All continuous variables were then divided by carapace length to minimise size effects. Carapace length was used as the variable indicating body size, as it is relatively unaffected by variation induced by growth and maturation (Lovett & Felder, 1989) and preservation fluid induced effects. Three sets of data in proportional form were retained as unstandardised values. These were rostral length (RL), already expressed as a ratio to carapace length and the insertion points of the anterior and posterior telson spines (TELANT, TELPOST), both distances measured from the proximal end of the telson and expressed as a percentage of telson length. Prior to further analysis, proportions were arcsin transformed to achieve univariate normality, with the exception of telson length (TELSON), which was arctan transformed, as several values exceeded unity.

Sexual variation was analysed first, using one-way ANOVA tests. Following this, the data set was subjected to Multiple Discriminant Analysis (MDA), a technique also known as canonical variate analysis. MDA finds linear combinations of variables (roots), that maximise differences among a priori defined groups (in this case regions), with the hit ratio (percentage correctly classified) providing a goodness of fit measure. In all MDA analyses, all variables were entered simultaneously, with the relative contributions of each variable assessed on the basis of the structure correlations (discriminant loadings), rather than the discriminant coefficients, as the former are considered more valid in interpreting the discriminating power of the independent varaibles. This technique has been successfully used in the discrimination of sibling species in *Alpheus* (Duffy, 1996), stock discrimination of lobsters (Cadrin, 1995) and to assess geographical variation in *Periclimenes* (Spotte, 1997).

Results

Several discrete variables were found not to exhibit any variation amongst regions, and hence they were excluded from further analysis. These were the number of teeth on the dorsal and both lateral carina, which in all individuals numbered four, two and one, respectively. On the dorsal carina, a small tooth was present close to the base of the rostrum followed by three large teeth. This small tooth has been overlooked in many earlier descriptions (e.g. Kemp, 1910), but its presence is identified in more recent descriptions (Williams, 1984; Squires, 1990).

Regarding carapace lengths, the across-region mean values for males was 8.60 mm (SD 1.82) and 10.21 (SD 2.87) for females, with this difference being statistically significant (one-way ANOVA, $F_{1,184}13.011$, $p<0.001$). A within-region analysis revealed that significant differences in carapace length of both sexes were only present in the Faroe-Scotland ($F_{1,28}14.251$, $p<0.005$) and U.S.A.-Canada ($F_{1,42}20.263$, $p<0.001$) populations, with in both instances males being on average smaller than females. An across-region analysis (sexes separate) revealed that significant differences existed in carapace

length of males ($F_{4,42}5.020$, $p<0.005$) and females ($F_{4,134}31.329$, $p<0.001$). Further post-hoc testing (Dunnett T3 test, $p<0.05$) demonstrated that males from the U.S.A. to Canada population are significantly larger than males from the Ireland population, whilst females from the U.S.A. to Canada population are significantly larger than from any of the other populations and females from the Ireland population are significantly smaller than females from either the Faroe-Scotland or the Norway populations.

Following standardisation with carapace length, all other variables were tested for sexual variation. Discrete variables were tested with Mann–Whitney tests, which revealed no sexual difference (all Z values <1.574). Continuous variables were assessed with one-way ANOVA tests, with the following variables, on average, being larger in males than in females: RL, BASAL, SUBORB and TELANT (all $F_{1,184}> 5.820$, $p< 0.05$), whilst the following variables, on average, were larger in females than in males: SOM1, SOM6 and PLEUR2W (all $F_{1,184}> 6.083, p<0.05$).

Given these sexual differences, two Multiple Discriminant Analyses were run; one on both sexes combined, but excluding any variables which exhibited significant sexual differences and one on females only, including all variables. As MDA requires a minimum within-region sample size of 20, a separate analysis could not be run on males only. MDA analysis on both sexes combined (Fig. 1) shows that Root 1 separates the NW Mediterranean population from the Atlantic populations as a whole, with along this root, overlap between the Atlantic populations being evident. Nevertheless, the position of all group centroids (Table 3) is significantly different from each other (Fig. 1), probably caused by the small separation of the Atlantic populations along the second root. Summary statistics demonstrated that the first root accounted for the majority of variance explained (Table 4), with the second root only accounting for an additional 10.8%. Examination of the structure correlation matrix (Table 5) reveals that four variables are highly loaded on the first root: TUBERCLE, TELSON, SOM and SCALEW, whilst nine variables exhibit their largest loading on Root 2. A classification matrix indicates that overall 88.2% of *a priori* grouped cases were correctly classified, with within-group classifications being: NW Mediterranean 100.0%, Faroes-Scotland 76.7%, Norway 70.4%, Ireland 89.2% and U.S.A.–Canada 93.2%. The MDA analysis on females only, essentially reveals the same structure in the data set, with the NW Mediterranean population separating from the Atlantic populations along Root 1 (Fig. 2), although a greater separation is evident between the Ireland and the U.S.A.–Canada populations on the Atlantic side (Fig. 2, Table 3). Similar eigenvalues and% variance explained are achieved (Table 4), whilst in the structure correlation matrix four variables are associated with the first root: TELSON, TUBERCLE, SCALEW and SOM1 (Table 6). A classification matrix indicates that overall 93.5% of a priori grouped cases were correctly classified, with within-group classifications being: NW Mediterranean 100.0%, Faroes-Scotland 88.2%, Norway 75.0%, Ireland 100.0% and U.S.A.–Canada 94.6%.

Table 5. Discriminant Analysis on sexes combined. Structure matrix of discriminant loadings. All variables entered simultaneously, largest absolute correlation between each variable and any discriminant function indicated by *

	Root 1	Root 2	Root 3	Root 4
TUBERCLE	0.380*	0.111	0.263	−0.107
TELSON	0.367*	0.054	−0.243	−0.154
SOM2	0.280*	−0.095	0.092	0.180
SCALEW	0.179*	−0.111	0.147	−0.146
ENDOL	0.171	0.488*	0.107	−0.242
PROP5	0.019	0.442*	−0.085	−0.139
EXOL	0.258	0.373*	0.024	−0.233
CARINA4	−0.092	0.319*	−0.053	0.286
SOM5	0.104	−0.299*	0.085	−0.161
PROP4	0.093	0.269*	−0.238	−0.118
SOM3	−0.079	−0.254*	−0.079	0.089
TELPOST	−0.070	−0.159*	−0.137	0.079
CARINA2	−0.063	0.143*	−0.027	0.042
CHELAW	0.335	0.197	−0.339*	−0.045
CARINA1	−0.194	0.118	0.254*	−0.156
EXOW	0.102	−0.129	0.221*	−0.027
PLEUR2D	−0.083	−0.170	0.210*	0.107
ROSTRUM	0.009	0.001	−0.112*	−0.101
CHELAEL	−0.119	0.418	−0.248	−0.541*
SCALEL	0.110	0.086	−0.059	−0.467*
CARINA3	−0.169	0.119	−0.010	0.420*
CARINA5	−0.036	−0.121	0.120	0.388*
SOM4	−0.070	−0.224	0.163	−0.350*
ENDOW	0.060	0.085	0.128	−0.255*
ANTPED	−0.048	−0.125	−0.160	0.196*

Discussion

When comparing geographically separated populations by means of a morphometric data set, factors

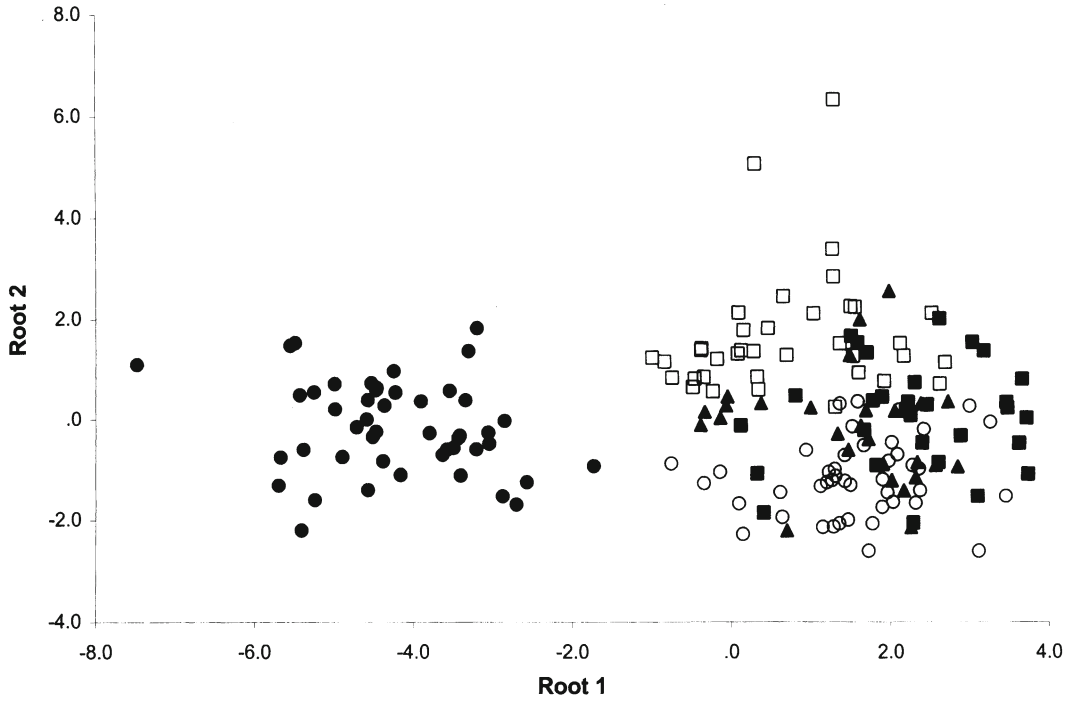

Figure 1. Scatterplot of MDA scores (first and second root only) for the five regions, both sexes combined. Symbols: NW Mediterranean (filled circle), Faroe-Scotland (filled square), Norway (filled triangle), Ireland (open square) and U.S.A.–Canada (open circle). Wilks's λ 0.035, χ^2 570.90, df 100, $p<0.001$

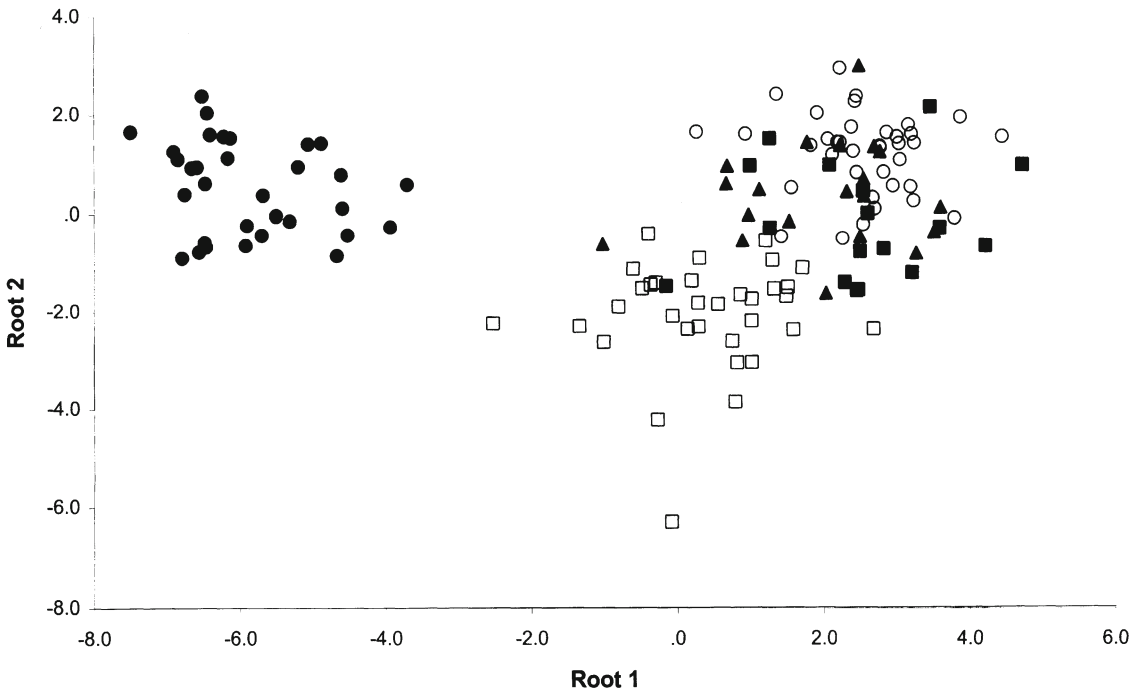

Figure 2. Scatterplot of MDA scores (first and second root only) for the five regions, females only. Symbols: NW Mediterranean (filled circle), Faroe-Scotland (filled square), Norway (filled triangle), Ireland (open square) and U.S.A.–Canada (open circle). Wilks's λ 0.011, χ^2 539.17, df 128, $p<0.001$.

Table 6. Discriminant Analysis on females only. Structure matrix of discriminant loadings. All variables entered simultaneously, largest absolute correlation between each variable and any discriminant function indicated by *

	Root 1	Root 2	Root 3	Root 4
TELSON	0.299*	−0.181	0.227	−0.213
TUBERCLE	0.237*	−0.160	−0.141	−0.062
SCALEW	0.139*	0.026	−0.123	−0.024
SOM1	0.096*	0.029	−0.076	−0.095
ENDOL	0.120	−0.411*	−0.046	−0.213
BASAL	−0.054	−0.384*	0.083	−0.122
EXOL	0.183	−0.373*	0.049	−0.232
PROP5	−0.033	−0.336*	0.036	−0.245
SOM6	0.127	−0.318*	−0.120	−0.175
SUBORB	−0.214	−0.275*	0.119	−0.086
CARINA4	−0.103	−0.264*	0.045	0.131
RL	−0.079	−0.248*	0.100	−0.034
TELPOST	−0.069	0.242*	0.215	0.082
PLEUR2W	−0.017	0.213*	−0.158	0.071
SOM3L	−0.045	0.176*	0.118	0.026
ANTPED	−0.012	0.167*	0.114	0.098
CARINA2	−0.044	−0.124*	−0.009	−0.001
CHELAEW	0.242	−0.219	0.327*	0.019
CARINA1	−0.117	−0.056	−0.205*	−0.174
PROP4	0.023	−0.143	0.167*	−0.126
EXOW	0.093	0.032	−0.157*	−0.092
CARINA3	−0.141	−0.086	0.004	0.453*
CHELAEL	−0.105	−0.301	0.138	−0.405*
CARINA5	−0.010	0.084	−0.127	0.339*
TELANT	0.054	0.235	0.233	0.290*
SCALEL	0.068	−0.046	0.057	−0.278*
SOM2	0.211	−0.057	0.002	0.248*
SOM4	−0.033	0.193	−0.079	−0.237*
ENDOW	0.047	−0.076	−0.088	−0.228*
SOM5	0.119	0.209	−0.072	−0.222*
PLEUR2D	−0.055	0.130	−0.171	0.199*
ROSTRUM	0.012	0.024	0.080	−0.164*

Table 7. Presence-absence of tubercle on dorsal carina of carapace per region, expressed as a percentage

	Presence	Absence
NW Mediterranean	83.3	16.7
Faroe-Scotland	13.3	86.7
Norway	14.8	85.2
Ireland	8.1	91.9
U.S.A.–Canada	6.8	93.2

such as sexual dimorphism, allometric growth and state of maturity can exert some influence on the observed differences (Mamuris et al., 1998). In addition, in the present data set, many of the collections contained few small and/or juvenile specimens, perhaps related to deployed mesh size and/or sampling gear selectivity in the different regions. The present study attempted to minimise additional variances through size standardisation, data transformation and by performing separate MDA analysis.

Extensive variation in morphometric variables existed between the studied Mediterranean population and the Atlantic populations as a whole. In addition, within the Atlantic populations studied the Faroe-Scotland and the Norwegian populations were most similar, whilst the south of Ireland and the U.S.A.–Canada populations were most dissimilar. This is supported by not only the MDA scores along the first two roots, but also the centroids of the regions and the percentage correctly re-classified individuals in the original groups.

The variables of primary importance in separating the Mediterranean and Atlantic populations as a whole, were related to telson length, the presence vs. absence of a tubercle on the dorsal carina of the carapace and the width of the antennal scale. However, the relatively low discriminant loadings on Root 1 of these variables (Tables 5 and 6), suggests that other variables may represent additional sources of variance. This is also the case for the separation of the Atlantic regions themselves, on which a strong influence of a score of variables associated with the second root is exerted. As the visibility of non-standardised, non-transformed variables is a necessary prelude to taxonomic, operational utility; the encountered differences in both the length of the telson and the width of the antennal scale (both transformed and standardised in the MDA analysis) should be interpreted as statistical constructs with no operational utility (Spotte, 1997). In contrast, the presence vs. absence of a tubercle has palpable reality and could be an operational variable. Although, it is more frequently present in the Mediterranean than in the Atlantic populations, 16.7% of Mediterranean individuals still lack a tubercle (Table 7) and hence this variable can also be discounted as being of operational utility. None of the other variables, with potential operational utility (e.g. carapace spines, rostral length) were identified by the MDA analyses as having any discriminatory power.

In general, morphological variability amongst different geographical populations is attributed to different genetic structure of populations and/or different environmental conditions prevailing in each geographic region. Certainly, the known Atlantic distribution of *P. norvegicus* only extends to the Bay of Biscay (Rodríguez-Marín, 1993), with the closest Mediterranean population being in the Catalan Sea (Abelló & Vallarades, 1985, 1988). At the present time, due to this geographical isolation and prevailing current patterns along the southern European coastline, gene flow and larval exchange between the Atlantic and Mediterranean population is, in all likelihood, severely restricted, if existent at all. Adaptations to environmental conditions may also play a role in this Atlantic-Mediterranean separation, as Lagardère (1970) formulated the hypothesis that along the eastern Atlantic seaboard, the amplitude of depth distribution becomes progressively lower with decreasing latitude, due to the upper limit of occurrence becoming progressively deeper; suggested to be in response to both temperature and substrate preferences (Lagardère, 1970). Although the depth range of the species extends from 50 to 1450 m in the Atlantic, it is most common between 200 and 500 m (Smaldon, 1979), whilst in the Mediterranean, Abelló & Vallarades (1985, 1988) consider the species to be a glacial relict species, typical of the bathyal zone (1020–1815 m) in the western Mediterranean, although it occurs here at higher temperatures than in the Atlantic. These differences in depth preferences and temperature regimes, and possibly substrate differences between the Atlantic and Mediterranean parts of the *P. norvegicus* range may thus play a role in the morphological variability between the regions.

Morphological variability between the Atlantic regions is much slighter, probably as a result of the absence of, present-day, geographical barriers and the resultant higher potential of larval exchange.

Given the present dataset, it can be concluded that the Mediterranean and Atlantic populations exhibit significant morphological differences as a result of geographical isolation with resultant limited gene flow and larval exchange. Although the populations are potentially approaching the subspecies stage in the evolutionary continuum of speciation, they at present do not appear to exhibit sufficient diagnostic morphological characters to be considered as subspecies, rather the results should be interpreted as geographical variation. As such, it is suggested that sub-specific status is presently not awarded to the populations.

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Notes on the distribution and biology of the deep-sea crab *Bathynectes maravigna* (Brachyura: Portunidae) in the Mediterranean Sea

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Key words: Bathynectes maravigna, distribution, biology, Mediterranean Sea

Abstract

Data on the distribution and biology of the deep-sea portunid crab *Bathynectes maravigna* are reported for the Mediterranean Sea, based on several fisheries research surveys. Densities are low and, therefore, biological data are scarce. In the western Mediterranean, the species is much commoner in Alborán Sea than in the Catalano-Balearic Sea. Occurrences are also scarce in the southern Adriatic and northwestern Thyrrenian Sea, as well as in the Ionian Sea. The Alborán Sea and the seas surrounding the southern Italian peninsula are the areas where densities are the highest. The occurrence depth range was found to be 245–786 m, but most of the occurrences took place deeper than 500 m. Sizes ranged between 9 and 51 mm carapace length in males and between 12 and 51 mm in females. Ovigerous females have been only reported in October–December and March–May. Eighty three percent of both males and females are right-handed. Sexual dimorphism was present in cheliped length with males having longer chelae than females. The species appears to be much commoner in those areas where Atlantic influence is the highest.

Introduction

The deep-sea portunid crab *Bathynectes maravigna* is distributed on both eastern and western parts of the North Atlantic Ocean from Norway, Iceland, Shetland, Hebrides and Faeroe islands to Morocco and the Canary islands on the East Atlantic and from Massachussetts to Florida on the West Atlantic. It is also found throughout the Mediterranean from Alborán Sea to Greece, including the Adriatic (Zariquiey-Álvarez, 1968; Lewis, 1977; Sardá et al., 1982; García-Raso, 1984, 1996; García-Socias & Gracia, 1988; Noël, 1992; Pipitone & Tumbiolo, 1993; Stevcic & Galil, 1994; González-Pérez, 1995; Pastorelli et al., 1996; Rinelli et al., 1998; Spanò, 1998). The species is usually recorded on the upper continental slope deeper than around 250 m, but there are records between 9 and 1455 m (Zariquiey-Álvarez, 1968; Noël, 1992). It has sometimes been recorded under the synonymy *Bathynectes superbus* (A. Costa, 1853), as in, for example, Zariquiey-Álvarez (1968) (see Manning & Holthuis, 1981). In the Mediterranean, the populations of *B. maravigna* are present in very low densities.

Another species of the genus, *Bathynectes longipes* (Risso, 1816), has also been recorded from the Mediterranean Sea, as well as from the northeastern Atlantic Ocean (Zariquiey Álvarez, 1968; Noël, 1992), but its populations seem to be much scarcer

than those of *B. maravigna*. Its habitat appears to be much shallower and on rocky bottoms. Most of the published data on the biology and ecology of any species of the genus *Bathynectes* concern *Bathynectes piperitus* Manning & Holthuis, 1981 from the southwestern coasts of Africa (Manning & Holthuis, 1981; Macpherson, 1983; Melville-Smith, 1985; Abelló & Macpherson, 1986, 1989; Abelló et al., 1990; Gili et al., 1993; Villanueva, 1993)

The present paper aims to provide some information on the distribution patterns of *Bathynectes maravigna* along the northern coasts of the whole Mediterranean Sea, as well as on some biological aspects of its populations.

Materials and methods

Occurrence and biological data on *Bathynectes maravigna* have been gathered from the fisheries research surveys performed within the European Union DGXIV MEDITS program (Bertrand et al., 1997). The MEDITS trawl surveys have been performed on a yearly basis in spring since 1994–1999. The study area encompassed the trawlable areas of the continental shelf and upper slope (depths between 30 and 800 m) along all the European Mediterranean coasts, from the Straits of Gibraltar to the Aegean Sea, including the Adriatic Sea. A total of 6336 trawls have been performed throughout the study area from 1994 to 1999. The same trawl net sampler and sampling strategy were used to uniformize data acquisition (Dremière et al., 1999; Fiorentini et al., 1999). Position (latitude and longitude) and depth of capture of *B. maravigna* were always recorded, as well as sex and size (carapace length, CL, in mm) in most study subareas. In the western Mediterranean, some additional morphometric data (handedness, crusher chela propodite length (CCPL, in mm)) were also recorded. Occurrence of ovigerous females was recorded in all areas. Additional information on the reproductive biology, especially on the occurrence of ovigerous females, has been gathered from other research surveys performed at other times of the year. A Kolmogorov-Smirnov test has been applied to test for significant differences in the size frequency distributions by sex.

Results

Distribution

Available data (Fig. 1) indicate that *Bathynectes maravigna* is commonest in Alborán Sea (the westernmost sector of the Mediterranean Sea), which is the area under the strongest Atlantic influence, and in the southern Adriatic. Other areas in which the species occurs regularly are the Eivissa Channel (between the island of Eivissa and the Iberian Peninsula), the seas surrounding the south of Italy and some areas of the Ionian Sea and Argosaronikos Gulf. The species is practically absent from the northwestern Mediterranean. Given the large amount of trawls performed, positions of negative occurrences are not presented in Figure 1 to clarify results.

Concerning depth distribution, *Bathynectes maravigna* has been found to occur at depths between 245 and 800 m (Table 1). The species occurs in shallower waters in Alborán Sea and in the southern Adriatic Sea, where it is sometimes found at depths of around 300 m. The median depth of occurrence is however found between 500 and 600 m in most areas.

Size structure

Size frequency distributions are presented for crabs collected from the Alborán Sea, North-West Ionian Sea and South Adriatic Sea (Fig. 2), the areas where the frequency of occurrence was the highest. Sizes ranged between 9 and 51 mm CL in males, and between 12 and 51 mm CL in females. Significant differences were only found between the size structure observed in males from the North-West Ionian Sea and those from Alborán Sea and South Adriatic Sea (d=0.4178, 16 df, p=0.043; d=0.524, 20 df, p=0.005; respectively). No significant differences were found between the females size structure. Large males were mainly present in Alborán Sea and in the southern Adriatic Sea, whereas small-sized crabs were found in a higher proportion in the NW Ionian Sea.

Reproductive biology

Very few data are available on the reproductive aspects of *Bathynectes maravigna*. These are limited so far to the occurrence of ovigerous females. Thus, ovigerous females have been reported in October, December and March in the northwestern Ionian Sea (n=3) with sizes ranging between 23 and 41 mm CL, in May in Alborán Sea (36 mm CL) and in November and April off

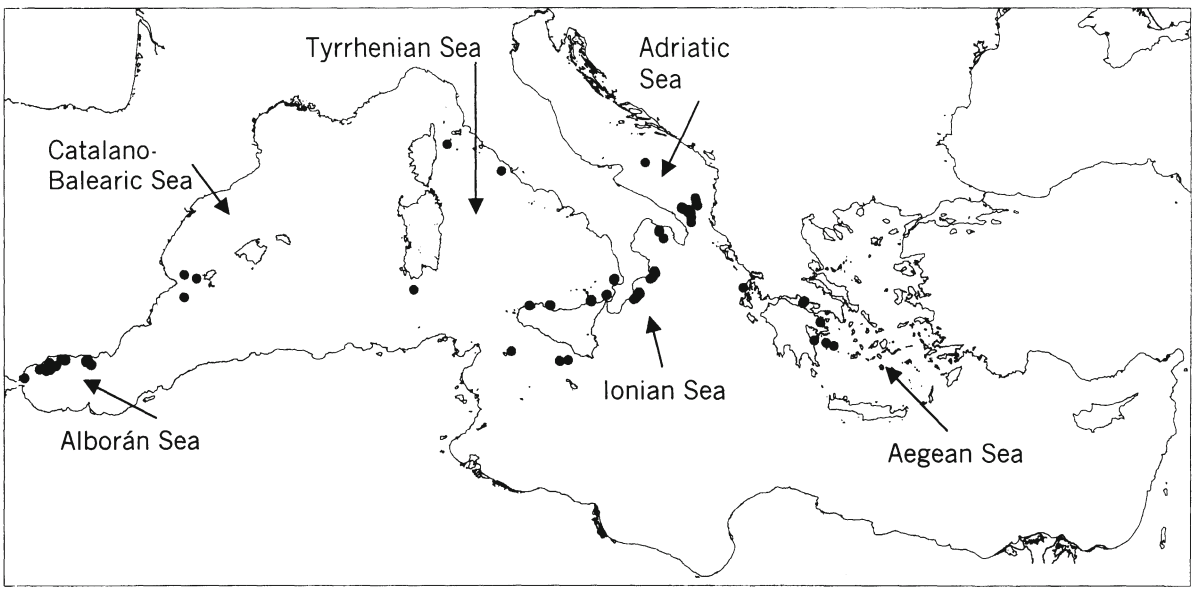

Figure 1. Occurrence of the crab *Bathynectes maravigna* along the northern coasts of the Mediterranean Sea, based in the data obtained during the MEDITS series trawl surveys. The study area encompassed the trawlable areas of the continental shelf and upper slope (depths between 30 and 800 m) along all the European Mediterranean coasts, from the Straits of Gibraltar to the Aegean Sea. A total of 6336 trawls have been performed throughout the study area from 1994 to 1999. Positions of negative occurrences are not presented to clarify results.

Table 2. Handedness (occurrence of a crusher cheliped on the right or left handside of the body) from crabs caught in the western Mediterranean

W-MED	Males		Females		total	
	%	*n*	%	*n*	%	*n*
Right-handed	84.6	33	80.0	40	82.0	73
Left-handed	15.4	6	12.0	6	13.5	12
Two cutters	0.0	0	8.0	4	4.5	4
Total		39		50		89

Table 3. Parameters of the linear regression equations fitted to the relationship between natural logarithm carapace length (in mm) and natural logarithm right crusher cheliped propodite length (in mm) for male and female *Bathynectes maravigna* from the Alborán Sea

	Constant	Slope	r^2	*n*	SE slope
Males	−0.7702	1.2227	0.99	22	0.0268
Females	0.3032	0.8927	0.97	23	0.0332

Mallorca (n=2) with sizes comprised between 40 and 41 mm CL.

Handedness

The occurrence of a crusher chela on the right or left handside of the body was determined in crabs collected in the western Mediterranean (Table 2). Right-handedness was predominant in the population, since 84.6% of males and 80.0% of females presented the crusher chela on the right handside of the body.

Morphometrics

Figure 3 shows the relationship between size of the crab and right crusher propodite length for both male and female *Bathynectes maravigna* from Alborán Sea. The data show the occurrence of sexual dimorphism in cheliped length, chelipeds being longer in males than in females at a given size, especially above the size at the puberty moult. The overall slopes of the potential regressions fitted to the data (Table 3) are significantly different for males and females ($p<0.001$). The regression lines start to diverge from sizes larger than 24–28 mm CL in crabs from the western Mediterranean population. A t-test for allometry indicates that males show a significant ($p<0.001$) positive allometry in chela length growth (the slope (b=1.2227) is significantly higher than the tested value b=1), whereas females show a significant negative allometry ($p<0.01$), with their slope (b=0.8927) being significantly smaller than the tested value b=1.

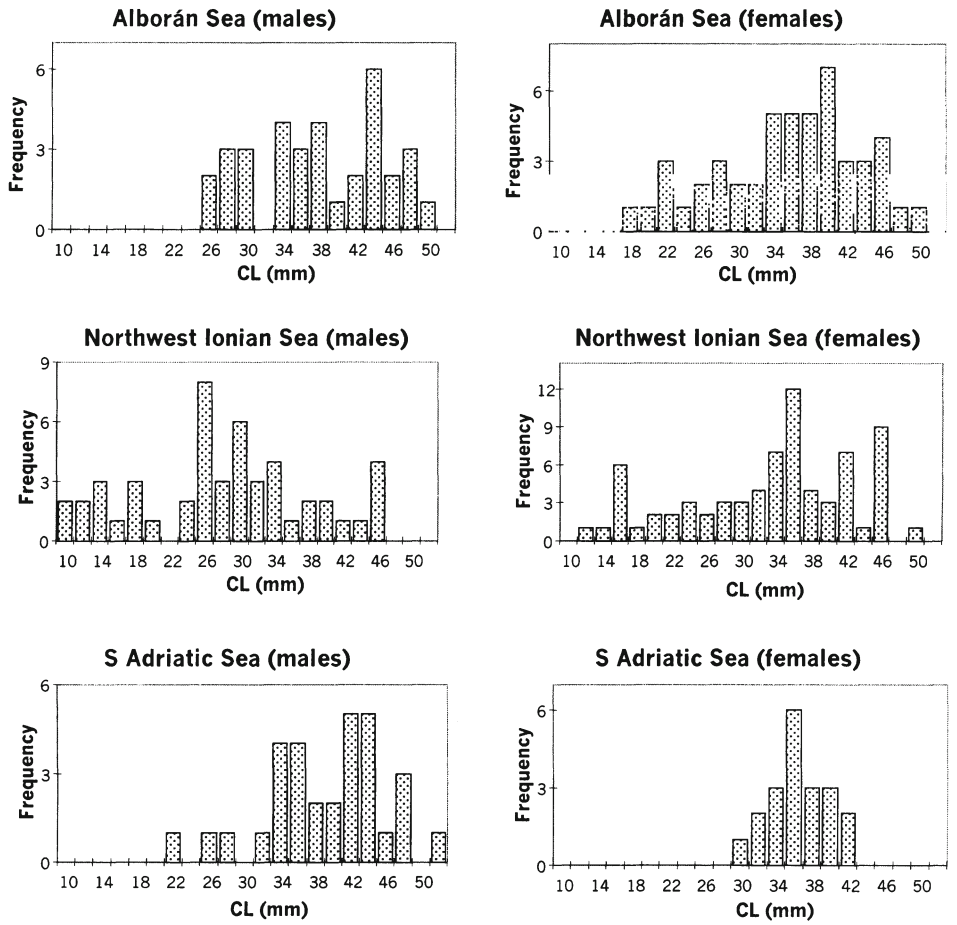

Figure 2. Size frequency distributions (carapace length, in mm) for male and female *Bathynectes maravigna* from the Alborán Sea, North-West Ionian Sea, and South Adriatic Sea.

Table 1. Distribution and size characteristics and occurrence of ovigerous females of *Bathynectes maravigna* in several subregions of the Mediterranean Sea (ns: not sampled)

	W-MED Alborán	W-MED Catalano-Balearic	C-MED N Thyrren	C-MED S Thyrren	C-MED NW Ionian	C-MED S Adriatic	E-MED Ionian-Arg-Sar
Number of occurrences	27	3	3	27	23	32	9
Min depth	245	577	570	500	330	304	384
Max depth	786	725	750	697	606	628	800
Median depth	558	590	601	556	535	513	539
Number examined	70	3	3	42	35	81	14
Proportion of males	0.40	0.67	ns	ns	ns	ns	0.64
Min CL males	25.8	34.2	ns	ns	9	22	16.4
Max CL males	49.2	41.3	ns	ns	46	51	43.5
Min CL females	19.1	39.4	ns	ns	12	30	18.7
Max CL females	49.0	39.4	ns	ns	51	42	34.3
Ovigerous females	May	Apr,Nov			Oct–Mar		

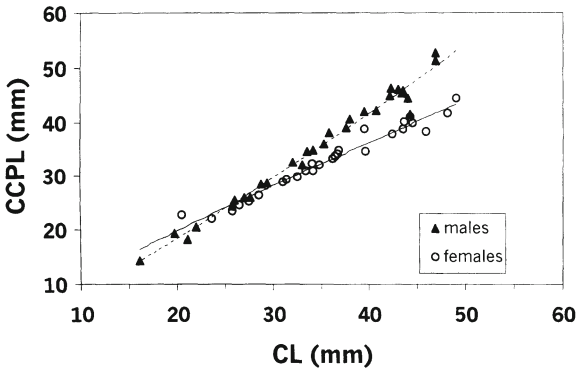

Figure 3. Relationship between carapace length (CL) and crusher cheliped propodite length (CCPL), in mm, for both male and female *Bathynectes maravigna* from the Alborán Sea.

Discussion

Present data, despite the relatively low number of specimens examined, have contributed to delimit the geographical and bathymetric distribution of *Bathynectes maravigna* throughout the northern coasts of the Mediterranean Sea and have also provided information on the size structure of its populations, handedness and morphometric characteristics as well as a preliminary approach to its size at sexual maturity.

Bathynectes maravigna is a very scarce species in the Mediterranean. Its populations appear to be more abundant in the westernmost part of the Mediterranean, in the Alborán Sea, as well as in the seas around the South of the Italian peninsula, which are the areas under the strongest Atlantic influence, besides the northern African coasts for which data are lacking. No data are available on the occurrence of the species on the North African coasts, but presumably it must also be present there. Present data suggest that the distribution of *B. maravigna* is linked to the areas which receive a strong influence of Atlantic water entering the Mediterranean, since the species is practically absent from the North-West Mediterranean, which is the area farthest away from the Atlantic influence. Atlantic water entering the Mediterranean flows along the North African coasts and reaches Sicily and the South of the Italian peninsula (Hopkins, 1985; Millot, 1987), which are the areas where occurrences of the species are the highest. Additionally, southern Adriatic waters have a lower salinity, which makes them show more similar characteristics to North Atlantic waters (Buljan & Zore-Armanda, 1979; Artegiani et al., 1981).

Ovigerous females of *Bathynectes maravigna* have been reported in February and March (Zariquiey-Álvarez, 1968) in the Iberian peninsula Mediterranean and from March to May along the western coasts of Africa (Capart, 1951). The diameter of the eggs ranges between 0.36 and 0.42 mm (Monod, 1956). In the present study, ovigerous females have been reported from Alborán Sea, Mallorca and southern Italy. This indicates that the species constitutes well established populations and that its occurrence in the Mediterranean is not only due to the influx of epipelagic larvae arising from Atlantic populations, as has been proposed for several invertebrates, including decapod crustaceans (see Bouchet & Taviani, 1992; Abelló & Torres, 1998; Cartes et al., 2000). *B. maravigna*, despite its strong affinity with Atlantic waters, does not constitute pseudopopulations in the Mediterranean Sea in the sense of Bouchet & Taviani (1992).

Morphometrics can provide useful information on sexual dimorphism as well as on the size at sexual maturity since, as usually found in crustaceans, the allometry level changes from the size at which puberty moult takes place. Present data indicate the occurrence of sexual dimorphism in right crusher chelar propodite length, chelipeds being longer in males than in females at a given size, as observed by Lewis (1977) in Atlantic specimens from off North-East America. Additionally, males present significant positive allometry in chela growth, whereas females show negative allometry. In Atlantic northamerican specimens, isometry was present in females (Lewis, 1977). The regression lines start to diverge from sizes larger than 24–28 mm CL in crabs from the western Mediterranean population. However, the low number of individuals examined, especially juveniles, has precluded further analyses of the data to better ascertain the precise size range at which puberty moult takes place in males and females.

Practically no data are available to date on the biology of this species, as is the case for many species whose occurrence is scarce and whose economical importance is low. Certainly more data are needed to provide more reliable estimations of all population characteristics, but we think that the collection of data on distribution and population characteristics of rare and little known species is necessary to understand the diversity and functioning of the Mediterranean ecosystems. The coordination of sampling efforts among different geographical areas may conduct to obtaining valuable information on very little known species, whose ecological importance may in some cases be great.

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Notes on the biology of *Cancer bellianus* (Brachyura, Cancridae) around the Canary Islands

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Abstract

Information on biometric and biological parameters of *Cancer bellianus* Johnson, 1861 (Crustacea, Decapoda, Brachyura, Cancridae) off the Canary Islands is given. Crabs examined were collected during experimental fishing surveys during 1974–1998. Carapace length, carapace width, total wet weight, sex and ovigerous condition were determined. This species was caught at depths from 153 to 750 m, the deepest ever recorded. Size frequency distributions were assembled and size-weight relationships were estimated by sex. Sex-ratio as a function of size and depth was determined. The size at first maturity was calculated by analysing the relative growth between the carapace length and the left chela width: 103.5 mm CL in males, 101.2 mm CL in females. Ovigerous females, egg size and fecundity estimates are reported apparently for the first time.

Introduction

The toothed rock crab *Cancer bellianus* Johnson, 1861 (Fig. 1) occurs in the North Eastern Atlantic. It ranges from the southern coast of Iceland to the Shetland Islands and Southwest Ireland and southwards to the Azores, Madeira, the Canary Islands, as well as and the West African coast off Morocco and the Sahara (El Aaiun, approx. 27° N). It is found at depths from 37 to 700 m on varied substrata (Holthuis, 1981; Manning & Holthuis, 1981; d'Udekem d'Acoz, 1999).

This common species has been included in the F.A.O. catalogue of species of interest to fisheries. Portuguese and Spanish bottom trawlers capture it off Portugal and NW Africa primarily as a by-catch; separate statistics are not reported for this species. It is marketed fresh mostly. The claws entire are removed, boxed on board and landed at markets in Portugal and Spain. Crabs are also occasionally landed (Holthuis, 1981). Despite its abundance, commercial significance and wide distribution, the biology of *C. bellianus* has not been studied in detail.

The results of many fishing surveys in deep waters around the Canary Islands have suggested that *C. bellianus* is occasionally abundant, which would possibly allow a restricted exploitation of this resource (e.g. González, 1989, 1995; Lozano et al., 1992). Around the Canary Islands, *C. bellianus* is obtained from bottom traps at 100–150 m depth, as a secondary item in an artisanal fishery to catch the narval shrimp *Plesionika narval* (J.C. Fabricius, 1787). This species is then usually sold as entire fresh crabs.

This study provides information on biometric and biological parameters of *C. bellianus* around the Canary Islands. The paper contributes to presently limited knowledge of the life history of this crab and provides information that may be of benefit in assessing and managing the stock.

Materials and methods

About 12 research surveys were conducted around in the Canary Islands of the Eastern Central Atlantic between 27° 40′–28° 32′ N and 14° 17′–17° 18′ W from 1974 to 1998 (Table 1). During these cruises, baited bottom traps and multiple shrimp traps were set on shelve and slope regions at depths ranging from 120 to 750 m (Santaella et al., 1975; González et al., 1988; González, 1989, 1995; Lozano et al., 1990, 1992; López-Abellán et al., 1994). A total sample of 428

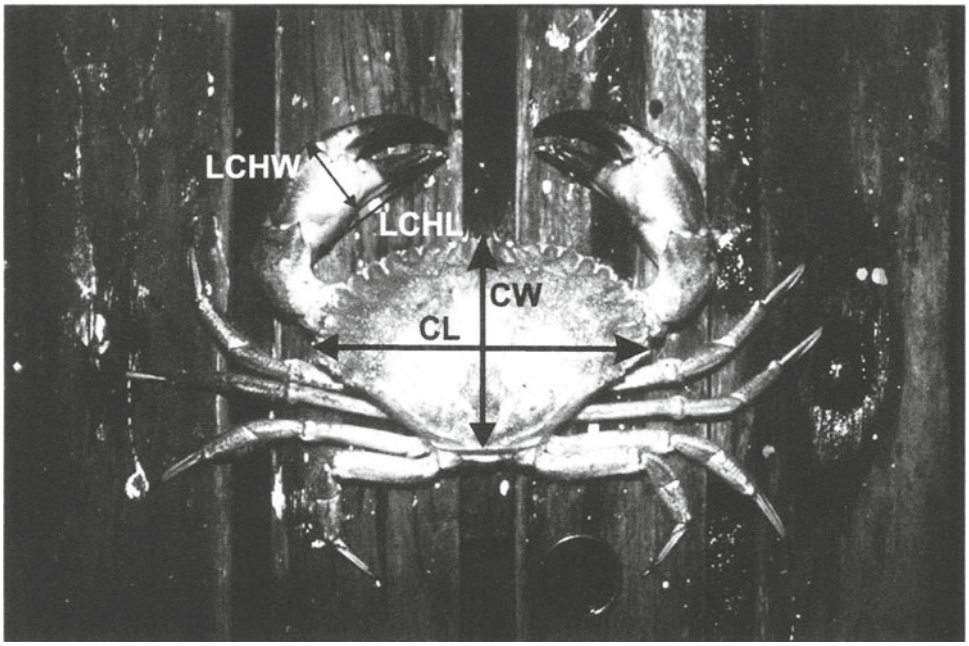

Figure 1. Cancer bellianus Johnson, 1861. Male with 135 mm CL, 190 mm CW and 1150 g WW collected off Tasarte (SW of Gran Canaria) at 277 m depth (cruise 'Camarón 9701'). (Photograph by second author).

individuals of *C. bellianus* were examined (size range 59–136 mm carapace length) (Table 1).

Fishing effort across the bathymetric range from about 150 to 700 m was more or less equally distributed at 100 m depth intervals. *C. bellianus* was not targeted during the research surveys, but was taken in the course of a larger study on the entire deep-sea pandalid shrimp and brachyuran crab assemblages.

Carapace length (CL), carapace width (CW), length of right chela (RCHL), length of left chela (LCHL), width of right chela (propodus) (RCHW), width of left chela (propodus) (LCHW), abdomen width (ABW), left pleopod length (LPL) and right pleopod length (RPL) in males were measured to the nearest mm (Fig. 1); wet weight (WW) was measured to the nearest 0.1 g. The ovigerous condition were noted (Fig. 2). To estimate fecundity, pleopods with attached eggs were removed from females and preserved in buffered 10% sea-water formalin. The presence or absence of dark embryonic eyes was noted for each egg mass. Eggs were dried to a constant weight at 60 °C, separated from the pleopods, rubbed gently to free them from connective tissue, and weighed to the nearest 0.001 g (Jewett et al., 1985).

All specimens collected were used for biometric and biological examination. Ranges for CL, CW, LCHW, LCHL, RCHW, RCHL, ABW, RPL and WW were determined for each sex and for all individuals. CL frequency histograms were obtained by 5-mm size classes. To investigate possible differences in size between left and right chelae by sex, as well as between left and right sexual pleopods in males, a *t*-test was applied. The allometric equation

$$Y = aX^b \quad \text{(Huxley, 1950)}$$

was used to estimate the allometric growth of all dimensions considered with respect to carapace length. Relationships obtained were linearized to the form $\log Y = \log a + b \log X$. Determination coefficients and *t*-tests for departures from isometry were calculated in all regresions obtained (Flores & Negreiros-Fransozo, 1999). Normality of the length data was determined using the Kolmogorov–Smirnov test prior to comparing the size between sexes. The same test was used to compare size frequency distributions (Sokal & Rohlf, 1981).

The overall ratios of males to females and the sex-ratios (males:females) by 10-mm size classes and 50-m depth intervals (from 100 to 750 m) were determined and tested by a chi-square analysis. Maturity of both sexes was determined by analysing the relative growth between CL and LCHW using the techniques of Somerton (1980) and Somerton & MacIntosh (1983). The computer program Mature I

Table 1. Origin and size range for the *Cancer bellianus* samples studied from the Canary Islands. Size: carapace length (CL) and carapace width (CW) in mm; depth in m. Is.: island; P: La Palma; C: Gran Canaria; F: Fuerteventura; L: Lanzarote; T: Tenerife; G: La Gomera; H: El Hierro; AI, all islands; *N*, sample size

Cruise	Date	Is.	Location (N–W)	*N* range	CL range	CW range	Depth
Agamenón 7406	06/74	AI	28° 36′–28° 37′				
			17° 57′–17° 45′	2	90–111	141–175	311–329
Canarias 85	01/85	C, T	27° 40′–29° 30′				
			13° 20′–18° 30′	86	70–136	–	153–476
Canarias 85	02/85	C, T	27° 40′–29° 30′				
			13° 20′–18° 30′	34	86–136	–	161–750
Mogán 8802	02/88	C	27° 43′–27° 45′				
			15° 47′–15° 49′	1	126	189	313
Mogán 8804	04/88	C	27° 43′–27° 45′				
			15° 47′–15° 49′	9	100–132	150–203	275–301
Canarias 9206	05/92	T	28° 01′–28° 19′				
			16° 43′–16° 57′	12	–	143–200	337
Canarias 9206	06/92	T	28° 01′–28° 19′				
			16° 43′–16° 57′	29	–	128–197	232–615
Taliarte 9301	02/93	C	27° 54′				
			15° 42′–16° 34′	2	98–99	163–168	620–677
Taliarte 9401	01/94	C	28° 01′–28° 52′				
			15° 51′–13° 13′	19	59–129	91–201	266–592
Taliarte 9402	02/94	C	27° 59′–27° 59′				
			15° 18′–15° 20′	45	68–129	103–186	231–465
Taliarte 9403	03/94	C	27° 59′–27° 59′				
			15° 18′–15° 20′	19	74–112	116–170	250–386
Camarón 9701	01/97	C	27° 40′–28° 11′				
			15° 18′–15° 51′	52	85–131	135–204	331–340
Camarón 9701	02/97	C	27° 40′–28° 11′				
			15° 18′–15° 51′	56	80–135	123–221	247–324
Taliarte 9709 (I)	09/97	C	27° 58′–28° 00′				
			15° 17′–15° 20′	22	75–110	114–174	300–601
Taliarte 9709 (II)	09/97	F	28° 10′–28° 16′				
			14° 17′–14° 23′	1	122–122	190–190	–
Camarón 9801	01/98	T	28° 00′–28° 32′				
			16° 06′–16° 54′	16	79–124	124–200	199–344
Camarón 9801	02/98	T	28° 00′–28° 32′				
			16° 06′–16° 54′	36	91–124	142–203	199–326
Camarón 9801	03/98	T	28° 00′–28° 32′				
			16° 06′–16° 54′	6	97–111	151–172	243–292
Totals	1974–98		27° 40′–29° 30′				
			13° 20′–18° 30′	428	59–136	91–221	153–750

(Somerton, 1980) was used to estimate the size at sexual maturity (which 50% of crabs are morphotype I or II).

Estimates of fecundity were obtained for each ovigerous female (only two specimens examined) by dividing the weight of the entire egg mass by the weight of a 200-egg subsample. Fecundity was then estimated as the mean of the two estimates (Jewett et al., 1985). The maximum length of 50 eggs obtained

Table 2. Cancer bellianus. Number of individuals and size range for males, females and all crabs

Variables	Males		Females		All individuals	
	N	range	*N*	range	*N*	range
Carapace length (mm)	251	59–136	156	68–129	407	59–136
Carapace width (mm)	233	91–221	95	110–189	328	91–221
Left chela width (mm)	138	22–68	46	20–38	184	20–68
Left chela length (mm)	136	42–126	46	38–120	182	38–126
Right chela width (mm)	138	3–73	46	20–37	184	3–73
Right chela length (mm)	136	5–131	46	37–73	182	5–131
Abdomen width (mm)	55	18–38	43	27–58	98	18–58
Right pleopod length (mm)	49	17–39	–	–	49	17–39
Wet weight (g)	266	90–1920	174	135–1200	440	90–1920

Table 3. Cancer bellianus. Results of Student's *t*-test applied to size of left and right chelae and to size of left and right males pleopods

Dimension	Males				Females			
	N	mean size (mm)	SD	*t*	*N*	mean size (mm)	SD	*t*
LCHW	138	43.41	9.84		46	30.11	3.72	
RCHW	138	42.13	12.40	0.95*	46	29.34	3.60	1.01*
LCHL	136	77.26	16.59		46	54.17	6.42	
RCHL	136	75.74	20.61	0.67*	46	54.02	6.75	0.12*
LPL	48	26.46	4.29					
RPL	49	26.38	4.34	0.09*				

*No significant differences ($P>0.05$).

Table 4. Cancer bellianus. Results of the allometric relationships examined. + = positive allometry; 0 = isometry; − = negative allometry; log indicates logarithms of base 10

Dimension	Sex	*N*	Linearized equation $\log Y = \log a + b \log X$	r^2	$t^{(1)}$ level	Allometric
WW	M	245	log WW =−4.337+3.544 log CL	0.849	5.63*	+
	F	154	log WW =−3.182+2.912 log CL	0.805	0.76	0
WW	M	227	log WW =−4.830+3.466 log CW	0.785	3.80*	+
	F	93	log WW =−4.123+3.105 log CW	0.802	0.64	0
CW	M	212	log CW =0.338+0.926 log CL	0.866	2.93*	−
	F	75	log CW =0.352+0.913 log CL	0.939	3.15*	−
LCHW	M	138	log CHW=−2.352+1.974 log CL	0.880	11.53*	+
	F	46	log CHW=−0.620+1.040 log CL	0.638	0.34	0
LCHL	M	136	log CHL =−1.690+1.770 log CL	0.795	10.05*	+
	F	46	log CHL =−0.595+1.159 log CL	0.773	1.63	0
AW	M	55	log AW =−0.632+1.046 log CL	0.896	0.95	0
	F	43	log AW =−1.172+1.396 log CL	0.913	5.96*	+
LPL	M	48	log PL =1.056+1.229 log CL	0.839	2.87*	+

(1) *t*-test for H_0: β =3 in WW/CL and WW/CW relationships; H_0: β =1 in the other relationships; * $P< 0.05$.

Figure 2. Cancer bellianus Johnson, 1861. Ovigerous female with 85 mm CL, 128 mm CW and 278 g WW collected off Gando Bay (E of Gran Canaria) at 210 m depth (cruise 'Taliarte 9709 (I)'). (Photograph by second author).

from different females were measured to 0.01 mm to calculate their mean-size.

Results and discussion

Cancer bellianus was caught at depths from 153 to 750 m (cruise 'Canarias 85', González et al., 1988) in waters around the Canary Islands, the deepest ever recorded for this species (see Table 1). *C. bellianus* appears to be common between 200 and 450 m depth. It was less common at 450–750 m depth, where it is gradually replaced by the deep-sea red crab *Chaceon affinis* (A. Milne-Edwards & Bouvier, 1894) (Geryonidae).

Maximum size recorded in this study (one male measuring 136 mm CL and 221 mm CW) (Table 2) overreaches the values referred by Holthuis (1981), who reported a maximum CL of 130 mm and CW of 200 mm. Females and males showed a unimodal distribution with peak at 100 and 100–110 mm CL, respectively (Fig. 3). Males (z=0.76) and females (z=0.60) exhibited a normal pattern and no significant differences in size were found between sexes (z=0.90). Crabs between 90 and 120 mm CL represented the 82.8% of the total sample (77.7 and 91.0% for males and females respectively).

Table 5. Cancer bellianus. Results of Somerton's F-test for the estimated size at 50% maturity

Males		Females	
	Cut point		Cut point
F value	(mm CL)	F value	(mm CL)
76.82*	104.9 [(1)]	22.053*	101.2 [(2)]

Parameters of the fitted logistic equation: [(1)] A = 1.01 × 10^5, B = −0.11; [(2)] A = 2.89 × 10^3, B = −0.08. * No significant differences (P> 0.05).

No significant differences were found between left and right size of chelae and between left and right size of pleopods in males (Table 3). Males showed positive allometry in weight (probably due to the typical growth pattern of the chelipeds), size of chelae and size of pleopod; isometry in reference at abdomen width; and negative allometry in carapace width (Table 4). Females showed positive allometry in abdomen width (as expected due to enlargement of females abdomen related to reproduction); isometry in weight and size of chelae; and negative allometry in carapace width (Table 4). At the same carapace length males have longer and wider chela than females. Significant differences (P<0.05) were found between sexes

Table 6. Means and ranges of body size and reproductive variables of *Cancer bellianus* (present work) and other *Cancer* species (from Hines 1991)

	Body size (CW, mm)	Egg size (μm)	Brood size (% body weight)	Fecundity (no. of eggs/brood)
	23	383		18 200
C. oregonensis	8–27	296–489	17.5	780–82 500
	68	329		453 700
C. gracilis	52–81	294–352	16.1	189 300–789 400
	78	406		205 200
C. irroratus	66–89	332–441	10.8	101 600–356 400
	100	333		1 156 000
C. antennarius	65–120	306–374	12.8	198 500–3 004 000
	116	367		877 300
C. productus	92–142	340–401	18.8	559 000–103 600
	120	428		652 300
C. borealis	105–135	353–470	11.1	311 400–1 045 000
	127	311		2 208 000
C. anthonyi	86–153	278–331	14.7	680 100–3 849 000
	155	442		938 300
C. magister	145–170	412–462	16.8	658 600–1 342 000
	160	438		484 800
C. bellianus	140–181	415–475	10	299 100–670 500
	163	396		1 408 000
C. pagurus	140–184	383–414	13.4	605 600–2 310 000

for all regressions with the exception of the CL-CW relationship (Table 4).

Of a total of 439 individuals, 267 (60.8%) were males and 172 (39.2%) females with a sex-ratio of 1:0.64. Males dominated all 10-mm size classes (55–135 mm CL). Significant differences were found between sexes by depth (P^2= 20.956); males were more abundant at 200–300 and 450–600 m depth intervals, whereas there were no significant differences from the expected 1:1 at 350–400 m.

The analysis of relative growth between CL and LCHW showed two regression lines. The lower line (morphotype I pattern) and the upper line (morphotype II pattern) represent the juvenile and the adult individuals respectively. The size at which 50% of the crabs are morphotype II specimens was 103.5 mm CL (in males) and 101.2 mm CL (in females) (Table 5). The smallest mature and largest mature crabs measured 85.4 and 120.0 mm CL (in males) and 89.8 and 114.5 mm CL (in females).

Two ovigerous females (84.5 and 104.5 mm CL) were caugth off the Bay of Gando (eastern coast of Gran Canaria) between 280 and 300 m depth in November and December of 1997. In the Madeiran archipelago, two ovigerous females and four post-ovigerous ones were found in November 1991 and March 1992, respectively (M.J. Biscoito, pers. comm.). Embryos in each egg mass attached to pleopods were early stages (eye pigmentation absent). Ovigerous females of this species have not been reported before in the Canary Islands waters or elsewhere. This apparent cryptic behaviour of ovigerous females could be partially explained by parasitic infestation of the oviducts (A. Rodríguez & J.I. González-Gordillo, pers. comm). The presence of yellowish protuding structures where gonopores should be located are been investigated in order to confirm the parasitic hypothesis.

The 84.5 mm CL female was bearing 670 000 eggs (mean diameter= 439 Fm ± 12.8), while the 104.5 mm CL female was carrying only 300 000 eggs (mean diameter= 437 Fm ± 15.4). Eggs from Madeiran ovigerous females measured 440 Fm in diameter (M.J. Biscoito, pers. comm.). Table 6 shows means and ranges of body size as well as reproductive variables for *C. bellianus* (present work) and other *Cancer* species (from Hines, 1991).

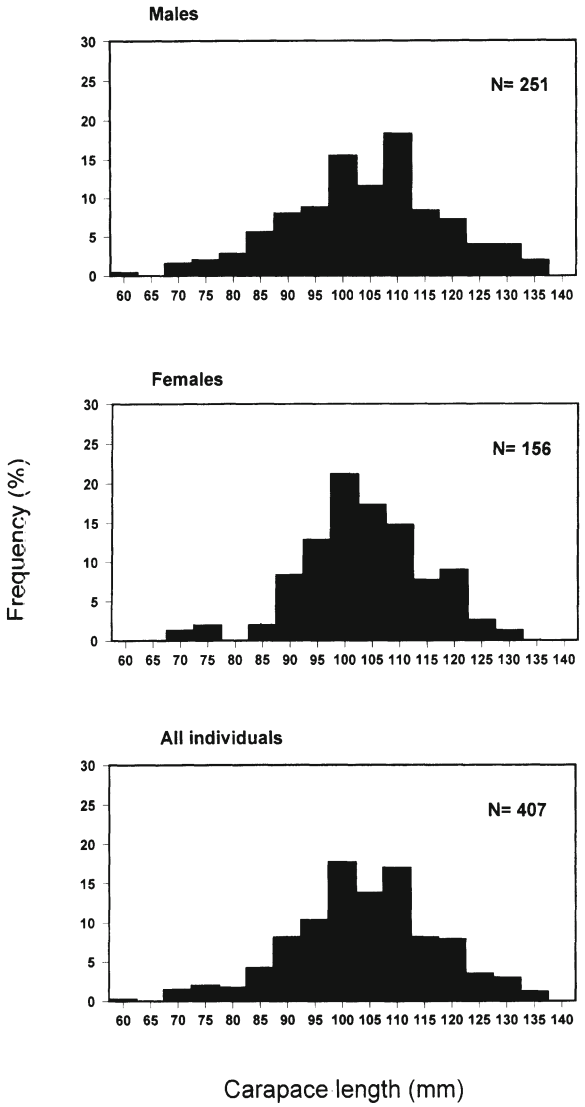

Figure 3. Cancer bellianus Johnson, 1861. Size frequency distributions for males, females and all individuals.

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Comparative suitability of binocular observation, burrow counting and excavation for the quantification of the mangrove fiddler crab *Uca annulipes* (H. Milne Edwards)

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Key words: Uca, census techniques, mangrove, East Africa

Abstract

Quantification of mangrove crabs is notoriously difficult. Several techniques have been applied in the past, but have rarely been tested. This paper looks at the use of burrow counts (BUR), binocular counts (BIN) and excavation (EX) for quantifying the fiddler crab *Uca annulipes* (H. Milne Edwards). Fieldwork took place at Maruhubi mangrove (Zanzibar, Tanzania). Twelve 1 m^2 quadrats were assessed on one spring cycle and one neap cycle. The results showed a strong correlation between carapace width (CW) and burrow diameter (BD), namely CW (mm) = 1.37BD (mm) −0.64 (r^2=0.98; p<0.001; DF=73). The mean spring tide densities were 77.8 m^{-2} (EX), 43.3 m^{-2} (BIN) and 104.8 m^{-2} (BUR). Of these, 37% (EX), 19% (BIN) and 42% (BUR) were juveniles (<7 mm CW). Census densities did not vary significantly between tides, although total BUR decreased from 1257 (spring) to 949 (neap). Whereas BIN significantly underestimated EX on both tides, BUR only significantly overestimated EX on the spring tide. Overall, burrow quantification matched EX better than binocular counts, suggesting the former is better for the quantification of *U. annulipes*. Whilst binocular census may be most appropriately carried out on spring tides, burrow quantification may be better on neap tides. A major reason for BIN underestimates and BUR overestimates were discrepancies in juvenile counts. Thus, 28% (spring) to 39% (neap) of adults, but 72% of excavated juveniles were unaccounted for by BIN. Spring tide BUR overestimated excavated adults by 25% but juveniles by 50%. Female BIN matched EX on the spring, but not the neap tide. Male binocular counts were significantly lower than EX on both tides. The potential influences on the results of crab size, sex, emigration and the method of sorting sediment during excavation are discussed.

Introduction

Fiddler crabs (Ocypodidae: genus *Uca*) constitute one of the most characteristic groups of animals associated with intertidal tropical shores, in particular with mangrove forests (Crane, 1975). Six species of *Uca* have been recorded in Tanzania: *U. annulipes* (H. Milne Edwards) *U. gaimardi* (=*U.chlorophthalmus*) (H. Milne Edwards), *U. inversa inversa* (Hoffmann), *U. vocans hesperiae* (Linnaeus), *U. tetragonon* (Herbst) and *U. urvillei* (H. Milne Edwards) (Hartnoll, 1975; Crane, 1975). Although *Uca annulipes* is the smallest of these species (adults from 7–17 mm carapace width), it is arguably the most numerous. It has an exceptionally large vertical shore range (Iceley & Jones, 1978) and is an omnipresent component of East African mangroves (McNae, 1968). Like other fiddlers, *U. annulipes* dwells in burrows, which it digs to a depth of up to 0.5 m depending on shore level. The species is diurnally active, emerging as the tide recedes. Surface activity terminates at the return of the tide, when burrows are re-entered and plugged. Burrow plugging also prevails during the night and on hot afternoons when the sediment surface is dry.

A persistent problem in estimating animal population size is the choice of sampling methods appropriate to the behaviour of the species under study (Hockett & Kritzler, 1972). Several techniques have been employed for burrowing mangrove crabs over the years, usually without prior testing or reference to application validity. The methods fall into five main categories: (1) Pitfall trapping has been frequently applied to the quantification of grapsids (e.g. Smith et al., 1991; McIvor & Smith, 1993; Frusher et al., 1994),

but less to ocypodid populations (Hockett & Kritzler, 1972; Salmon & Hyatt, 1983). The technique suffers from being entirely dependent on the degree of surface activity and catchability (Lee, 1998), which may vary greatly with time of day (Golley et al., 1962), lunar phase (Palmer, 1973; Zucker, 1978), reproductive phase, sex and size (Crane, 1975; Murai et al., 1982, 1983; Salmon, 1984, 1987). (2) Mark-release-recapture quantification was successfully applied by Hockett & Kritzler (1972) on Florida populations of *U. pugilator* (Bosc). Habitat disturbance was negligible because *U. pugilator* could be caught whilst feeding on down-shore sand flats (Hockett & Kritzler, 1972). Although *U. annulipes* at the present location frequently moves in such aggregations to forage in nearby areas, a behaviour known as droving (Crane, 1975), this activity is almost exclusively practiced by large males. Females feed in close proximity to the burrow and retreat instantly on disturbance. Mark-release-recapture census is, therefore, not applicable to *U. annulipes*, because sampling without bias towards size or sex would cause disturbance or destruction to burrows. (3) Excavation of crabs (e.g. Teal, 1958; Sasekumar, 1974; Frith & Brunenmeister, 1980; Macintosh, 1984) is not only very destructive to mangroves, but may in some habitats also be extremely difficult due to the abundance of underground roots. The method is labour intensive, time consuming and not applicable if repeated observations are required in the area. Excavation should nevertheless offer reliable density estimates for this species. (4) Burrow density, regardless of temporal variations in the ratio between crab numbers and burrow numbers, can within some error be correlated to crab densities (Mouton & Felder, 1996). Although burrow counting has been the predominant method for ocypodid quantification for some time (Krebs & Valiela, 1978; Icely & Jones, 1978; Snowdon & Ekwezor, 1990; Ens & Klaassen, 1993; Mouton & Felder, 1996; Dray & Paula, 1998), very few studies have examined its suitability (Ekwezor, 1985; Mouton & Felder, 1996). The method is relatively quick, non-invasive and may provide evidence of population size-structure through burrow width-distribution. (5) Visual counts of surface-active animals have been applied to the census of several populations of *Uca* (e.g. Golley et al., 1962; Zucker, 1978; Macintosh, 1984). This method depends entirely on the degree of surface activity, but it is quick, non-intrusive and descriptive. Nobbs & McGuinness (1999) recently tested the influence of various factors (e.g. quadrat size & observation duration) on visual counts of Australian fiddler crabs, but did not compare their results to absolute counts. Studies testing the accuracy of binocular census have, as far as we are aware, never been published.

Fiddler crabs are deposit feeders. Collected sediment passes to the mouthparts, which act as a filtering mechanism (Miller, 1961). This method demands a steady supply of water which, if the sediment is dry, originates from the burrow (MacNae, 1968). Water availability may be limited during neap tides and some species have been known to remain underground with their burrows plugged during dry periods of the semi-lunar cycle (MacNae, 1968; Zucker, 1978). Evidently, such activity could create differences between spring and neap tide surface and burrow counts.

Sexually determined differences in activity are well documented in fiddler crabs. Male *U. pugnax* feed slower than females (Weissburg, 1992) and spend more time on the surface to compensate for having only one functional chela with which to feed (Valiela et al., 1974). Droving is a predominantly male behaviour in *U. vocans* (Murai et al., 1983) and *U. tangeri* (Eydoux) (Crane, 1975). Ovigerous females of *U. annulipes* at the present location have never been caught on the surface, suggesting they hide underground when berried. It is possible that such differences in surface activity may lead to sexually biased counts in fiddler crabs.

This paper aims to test the suitability of binocular quantification and burrow counts for the census of *Uca annulipes*. Four questions are specifically addressed: (1) Which method matches the excavated density of *Uca annulipes* closest? (2) Does method suitability differ between spring and neap tides? (3) Do differences in surface activity between males and females mean that one sex is better accounted for by binocular census than the other? (4) Are all crab sizes estimated equally well by binocular and burrow counting?

Methods

Description of site

The Maruhubi mangrove forest is situated in close proximity to Zanzibar Town, Unguja, Tanzania (6° 09′ S, 39° 12′ E). Machiwa & Halberg (1995) and Machiwa (1998) give thorough descriptions of the physical and biological characteristics of the study area. Zanzibar has a mean annual spring tidal range of about 4 m. The survey was conducted within the

middle of the forest, 2.7–3.3 m above chart datum. This area is characterised by a stand of 3–5 m tall *Avicennia marina* (Forskål) trees and relatively sandy sediments with an organic content of 0.5–1.0%. The down-shore end is marked by a transition from *A. marina* to *Sonneratia alba* (J. Smith) domination. With the exception of 3–4 days during neap periods, the zone is flooded daily by high tides. The study area was limited to a 40 m (up/down shore) by 20 m (cross-shore) subsection of this area. The substrate of this subsection was relatively homogenous and *U. annulipes* was fairly evenly distributed (MEAM project observations). Stratification of the area prior to random allocation of quadrat plots was, therefore, not judged necessary.

The dominant brachyuran within the experimental site was *U. annulipes*. Other burrowing crabs included *Helice leachi* (Hess) and *Dotilla fenestrata* (Hilgendorf). The likelihood that the presence of these species may have had a significant influence on experimental conclusions was considered negligible. *H. leachi* and *D. fenestrata* together accounted for less than 2% of the excavated crabs. *Dotilla* burrows are easily distinguishable from *Uca* in being perfectly circular and vertical, whilst *H. leachi* is a bigger species than *U. annulipes* (adult CW 15–24 mm).

Correlation between burrow width and carapace width

Seventy-five *U. annulipes* burrows were randomly selected 1–2 h after habitat emersion by the spring tide. The diameter of each burrow entry was measured at its narrowest diameter by dial calipers (±0.1 mm), after which the burrow was excavated. The sex and the carapace width (±0.1 mm) of the resident crab were determined. The carapace widths of small juveniles (<3 mm) were measured in the laboratory using a stage micrometer. Although burrows as small as 1.7 mm diameter could be relatively easily recognised at Maruhubi, openings of less than 2 mm were not included in the analysis because of the difficulty in measuring such small diameters accurately in the field.

Binocular, burrow and excavation census

The study was carried out at Maruhubi during October 1998. The habitat was subdivided into a grid of 400 1 m (down shore) by 2 m (cross-shore) plots. Twelve spring and 12 neap plots were selected from this grid by computer randomisation. Within each plot a 1 m^2 quadrat was constructed from corner pegs connected by 5 mm coconut rope (as a rule the quadrat was placed in the left 1 m^2 of the 2 m^2 plot, unless this included a tree, in which case the quadrat was shifted to the right hand part of the plot).

Binocular observations took place between 0700 (0.5 h after tidal retreat during spring tides) and 0830. Twelve quadrats were observed on 2 successive spring tide days (6+6) and 12 on 2 days of the following neap tide. Crabs were counted from a distance of 3.5 m. The observer remained still and in position for 15 min before commencing observation. In order to eliminate crab migration prior to excavation, the quadrats were fenced by 15 cm tall wooden frames immediately after observation.

After completion of binocular observations, the diameters of all burrows inside the quadrats were measured by dial calipers (±0.1 mm). The quadrats were then excavated to a depth of 35 cm, or, if deeper, to the depth of the water table. The excavated soil was spread over a thick 4 m^2 polythene sheet and carefully hand-sifted. This technique was found very successful at extracting the maximum number of animals from the soil. Although wet sieving may seem an attractive alternative, it is often not practicable in the mangrove (Frith & Brunenmeister, 1980). Each quadrat, in being more than a third of a ton of excavated sand, represented an impossible quantity to wet sieve. Although smaller quadrats could have been chosen, this would have compromised crab numbers and raised variation of binocular counts beyond useful limits. Wet sieving also demands access to water, which is a requirement that cannot be fulfilled unless the study site borders a creek of considerable depth. The latter is not the case at Maruhubi.

The sex and carapace widths of excavated crabs were determined in the laboratory. Animals of less than 3 mm CW were measured using a stage micrometer.

Statistical analysis

All analyses were tested for significance at the two-tailed probability level of 0.05. Data sets were checked for homogeneity of variance (F-test) prior to t-test or analysis of variance. The degrees of freedom for analyses of covariance and t-tests will be noted as subscripts to F-values ($F_{(DF)}$) and t-values ($t_{(DF)}$), respectively.

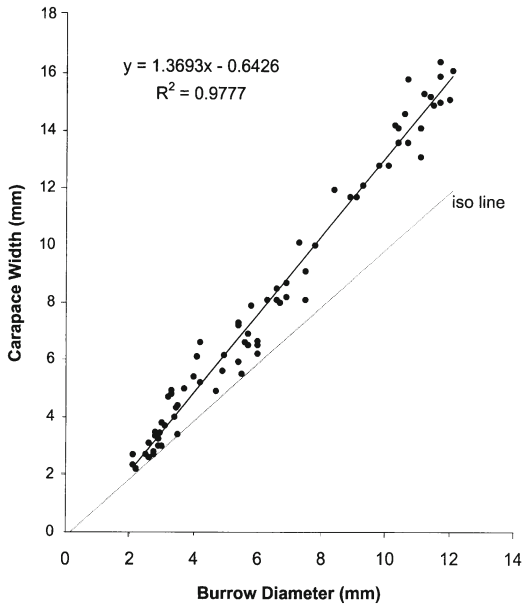

Figure 1. The relationship between carapace width (mm) and burrow diameter (mm) in *Uca annulipes*.

Results

Correlation between carapace width and burrow diameter

Figure 1 shows the regression between carapace width (CW) and burrow diameter (BD) in *U. annulipes*. The regression was based on the measurements of 75 burrows and tenant crabs and gained a highly significant coefficient of determination (r^2=0.98. p<0.001). Males and females were pooled in the regression because a prior analysis of covariance revealed no significant difference in CW:BD between the sexes ($F_{(73)}$=0.2. p>0.05).

The regression equation (see Fig. 1) was used to transform all burrow widths into crab carapace widths during the remaining part of the analysis. The result shows that the size of resident *U. annulipes* can be estimated very well from the diameter of burrow openings.

Comparison of spring and neap tide densities

Table 1 lists the results of estimating the density of *U. annulipes* by excavation (EX), binocular counts (BIN) and burrow counts (BUR) within 12 spring tide and 12 neap tide 1 m^2 quadrats. Density estimates of all crabs (ALL), adults (≥7 mm CW) and juveniles (<7 mm CW) were treated as separate groups. The mean excavated density of ALL *U. annulipes* (±95% confidence intervals) was 77.8±23.3 m^{-2} on the spring tide and 76.9±15.8 m^{-2} on the neap tide. This was compared to binocular counts of 43.3±8.8 m^{-2} (spring) and 38.0±11.7 m^{-2} (neap), and burrow counts of 104.8±30.6 m^{-2} (spring) and 79.1±24.1 m^{-2} (neap). Juveniles comprised 37.4% (spring) to 34.1% (neap) of the excavated animals and 42.0% (spring) to 40.9% (neap) of the burrows (Table 1: % of Total n). In comparison, binocular counts of juveniles were between 19.1% (spring) and 19.3% (neap), indicating a relative underestimation of juvenile numbers by the binocular census.

The three census methods (BIN, BUR & EX) were tested separately for significant differences between spring and neap tide densities (Table 1: comparison of tides). ALL animals and adults were tested by analysis of variance (ANOVA). The juvenile data sets remained heterogeneously varied despite logarithmic, square root or arcsin transformation ($F_{(23)}$-test: p<0.05), and were subsequently tried by two-sample t-test assuming unequal variances. The analyses did not reveal significant differences between spring and neap tide densities in any of the animal groups (p>0.05). Thus, although 32.5% more crabs unplugged burrows during the spring (n=1257) than the neap tide (n=949), there was no significant inter-tidal difference between burrow densities ($F_{(23)}$=1.67. p=0.21). The excavated density estimates showed minimal variation between the tides (e.g. ALL animals: $F_{(23)}$=0.0. p=0.95). This indicates that the density of the local population of *U. annulipes* was temporally stable and showed no signs of net immigration or emigration from the sample area over the semi-lunar cycle.

Comparison of Binocular and Burrow census

Table 2 lists the results of paired t-tests on the difference between BIN and EX and between BUR and EX. The subtractions (BIN-EX & BUR-EX) were tested for significant difference from a mean of zero. Table 2 also notes the mean BIN-EX or BUR-EX as a percentage of mean EX (% difference from EX). The latter was calculated as (e.g. BIN):

$$\%\ \text{difference from EX} = \text{mean BIN-EX/mean EX)} * 100\%$$

(The mean density of EX is listed in Table 1). Binocular counts were significantly lower than excavated counts on all occasions. The spring tide BIN was 44.5% lower than EX for ALL animals ($t_{(23)}$=−4.18. p=0.002), 28.3% lower for adults

Table 1. The results of statistical comparisons (Comparison of tides) between spring and neap tide density estimates of three groups of *U. annulipes*. Density was estimated by excavation (EX), binocular counting (BIN) and burrow counts (BUR). The counts per group are listed (n), as are the mean densities and 95% confidence intervals (95% CI) within twelve 1 m^2 quadrats per tide. Spring and neap tide densities were compared by Analyses of variance (ANOVA), except for juveniles where two-sample t-tests assuming unequal variances were used. Comparison of tides denote F-values and t-values. Superscripts refer to differences of significance between tides (probability)

Group	Spring tide			Neap tide		
	EX	BIN	BUR	EX	BIN	BUR
All animals						
Total n	934	519	1257	923	456	949
Mean density (m^{-2})	77.8	43.3	104.8	76.9	38.0	79.1
±95% CI	23.26	8.83	30.57	15.80	11.70	24.13
Comparison of tides	$0.0^{0.95}$	$0.49^{0.49}$	$1.67^{0.21}$	–	–	–
Adults						
n	585	420	729	608	368	561
% of Total n	62.6	80.9	58.0	65.9	80.7	59.1
Mean density (m^{-2})	48.8	35.0	60.7	50.7	30.7	46.8
±95% CI	8.63	8.62	10.52	8.32	9.82	11.53
Comparison of tides	$0.10^{0.76}$	$0.42^{0.52}$	$3.09^{0.09}$	–	–	–
Juveniles						
n	349	99	528	315	88	388
% of Total n	37.4	19.1	42.0	34.1	19.3	40.9
Mean density (m^{-2})	29.1	8.3	44.0	26.3	7.3	32.3
±95% CI	17.65	1.37	21.52	8.81	3.79	12.79
Comparison of tides	$0.28^{0.78}$	$0.45^{0.66}$	$0.91^{0.37}$	–	–	–

($t_{(23)}$=−3.65. p=0.004) and 71.5% lower for juveniles ($t_{(23)}$=−2.35. p=0.038). On the neap tide BIN was −50.6% lower than EX for ALL animals ($t_{(23)}$=−8.14. p<0.001), −39.4% for adults ($t_{(23)}$=−5.37. p<0.001) and 71.9% for juveniles ($t_{(23)}$=−4.71. p<0.001). Burrow counts were significantly higher than EX on the spring tide. BUR of ALL animals was 34.6% higher ($t_{(23)}$=3.39. p=0.006), adult BUR was 24.6% higher ($t_{(23)}$=3.78. p=0.003) and juvenile BUR was 51.2% higher than EX ($t_{(23)}$=2.51. p=0.029). BUR did not vary significantly from EX on the neap tide ($t_{(23)}$<1.5. p>0.2). During the spring tide where both BIN and BUR varied significantly from EX, the percentage difference from EX was always highest in binocular counts.

These results show that whereas binocular census always underestimated EX, burrow census tended to overestimate excavated densities. On the whole, BUR matched EX better than BIN. The fact that BUR was in agreement with EX during the neap tide suggests that burrow census of *U. annulipes* may be most suitable during this period.

The differences of BIN and BUR from EX were largest in juvenile counts (Table 2: % difference from EX). BIN of juveniles differed from EX by −72% (spring and neap), whereas BIN of adults differed by −28% (spring) to −39% (neap). This indicates that the juvenile percentage difference from EX was 2.5 (spring) to 1.8 (neap) times higher than the adult percentage difference in binocular counts. Similarly, burrow counts were from 51% (spring) to 23% (neap) higher than EX in juveniles, compared to 25% (spring) to 8% (neap) higher than EX in adults. The percentage differences of BUR from EX were thus between 2.1 (spring) and 3.0 (neap) times higher for juveniles than for adults. This summary suggests that a major reason for BIN underestimates and BUR overestimates of total densities (ALL) may have been due to large discrepancies in BIN and BUR counts of juveniles.

Table 2. The results of paired *t*-tests on whether binocular counts (BIN) or burrow counts (BUR) of *U. annulipes* differ significantly from excavated counts (EX). Null hypothesis: Mean BIN-EX or BUR-EX = zero. Counts represent twelve 1 m^2 quadrats per tide. 95% confidence intervals of the Mean Difference from EX are listed (±95% CI). Superscripts refer to differences of significance (ns=not significant. *=$p<0.05$. **=$p<0.01$. ***=$p<0.001$). % difference from EX = (Mean difference from EX/Mean EX)*100

Group	Spring tide		Neap tide	
	BIN-EX	BUR-EX	BIN-EX	BUR-EX
ALL animals				
Mean difference from EX	−34.6**	26.9 **	−38.90***	2.17^{ns}
±95% CI	16.22	15.56	9.37	16.83
% difference from EX	−44.5	34.6	−50.6	2.8
Adults				
Mean difference from EX	−13.8**	12.0**	−20.0***	-3.92^{ns}
±95% CI	7.37	6.22	7.31	10.64
% difference from EX	−28.3	24.6	−39.4	7.7
Juveniles				
Mean difference from EX	−20.8*	14.9*	−18.9***	6.1^{ns}
±95% CI	17.35	11.66	7.87	7.73
% difference from EX	−71.5	51.2	−71.9	23.2

Table 3. The results of paired *t*-tests on the difference between binocular counts (BIN) and excavated counts (EX) of male and female *U. annulipes* within twelve 1 m^2quadrats per tide. Null hypothesis: Mean BIN-EX = zero. The % Difference from EX = (Mean BIN-EX / Mean density of EX)*100. Superscripts refer to *t*-test differences of significance (ns=not significant. *=$p<0.05$. **=$p<0.01$. ***=$p<0.001$). Sex ratios were checked for Differences between tides and Differences between BIN & EX by *t*-tests assuming unequal variances: The t-values of these analyses are noted

Sex	Spring tide		Neap tide	
	Binoculars	Excavated	Binoculars	Excavated
Males				
Mean density	21.4*	28.3	21.2**	32.3
% Difference from EX	−24.1	–	−34.6	–
Females				
Mean density	14.4^{ns}	16.8	9.5***	14.7
% Difference from EX	−14.4	–	−35.2	–
Mean sex ratio	1.83	1.91	2.22	2.28
Difference between tides	0.95^{ns}	1.29^{ns}	–	–
Difference between BIN & EX	-0.19^{ns}	–	-0.28^{ns}	–

Comparisons of male and female counts

The binocular and excavated counts of male and female *U. annulipes* within spring and neap tide quadrats are listed in Table 3. Only adults (≥7 mm CW) were included in this analysis. The mean excavated densities were 28.3 males m^{-2} (spring) to 32.3 males m^{-2} (neap), and 16.8 females m^{-2} (spring) to 14.7 females m^{-2}(neap). Binocular counts were lower than excavated counts with mean densities of 21.4 males m^{-2} (spring) to 21.2 males m^{-2} (neap), and 14.4 females m^{-2} (spring) to 9.5 females m^{-2} (neap). BIN and EX mean sex ratios (males/females) were not significantly different from each other on either of the tides (*t*-tests: $p>0.5$) (Table 3), but rose from 1.83 (BIN) and 1.91 (EX) on the spring tide, to 2.22 (BIN) and 2.28 (EX) on the neap tide (Table 3: Mean sex ratio). Although the sex ratios from both census methods were highest on the neap tides, two *t*-tests assuming unequal variances indicated no significant differences between tides for either method ($p>0.2$) (Table 3).

The results of paired *t*-tests on the subtraction of excavated counts from binocular counts (BIN-EX) for male and female *U. annulipes* are shown in Table 3 (H_0: BIN-EX = zero). Male BIN were 24.1% significantly lower than EX on the spring tide ($t_{(23)}=-2.84$. $p=0.016$) and 34.6% significantly lower on the neap tide ($t_{(23)}=-3.89$. $p=0.003$) (Table 3: % difference from EX). Female BIN did not differ significantly from EX on the spring tide ($t_{(23)}=-1.32$. $p=0.21$), but were 35.2% significantly lower on the neap tide ($t_{(23)}=-4.45$. $p<0.001$).

These results suggest that the sex ratio of *U. annulipes* did not vary significantly between spring and neap. Equally, the surface sex ratios (BIN) never differed significantly from sub-surface ratios (EX). With the exception of spring tide females, significantly more males and females were underground than surface active. BIN of both sexes matched EX better on the spring than the neap tide, suggesting binocular census is most appropriately done during spring tides.

Discussion

The mean number of excavated *U. annulipes* (±95% confidence limits) ranged from 78±23.3 m^{-2} on the spring tide, to 77±15.8 m^{-2} on the neap tide. These quantities fall within the excavated density ranges reported for a west Thailand population of *U. annulipes* (58–335 m^{-2}) (Frith & Brunenmeister, 1980), as well as for other *Uca* species such as *U. rosea* (Tweedie) (80-203 m^{-2}) in a Malaysian mangrove (Macintosh, 1984). Burrow counts of a Kenyan mangrove population of *U. annulipes* by Icely & Jones (1978) gave density estimates of up to 100 individuals m^{-2}.

Ocypodids construct and occupy burrows as soon as the postlarvae settle on mangrove shores (Macintosh, 1988) and *U. annulipes* burrows as small as 1.7 mm diameter could be relatively easily recognised at Maruhubi. The diameters of *U. annulipes* burrows were highly correlated to the carapace widths (CW) of resident crabs (Fig. 1). Dray & Paula (1998) described similar results for *Dotilla fenestrata* (Hilgendorf) (Ocypodidae:Scopimerinae), as did Mouton & Felder (1996) working on *U. longisignalis* (Salmon & Atsaides) and *U. spinicarpa* (Rathbun) and Krebs & Valiela (1978) for *U. pugnax* (Smith). The strong relationship between burrow and animal size indicates that population structure may be estimated from the size distribution of burrow openings. This idea was put to use by Mouton & Felder (1996), who measured burrow size frequency distribution to investigate seasonal changes in the population size-composition and biomass of *U. longisignalis* and *U. spinicarpa*.

Comparison of binocular and burrow censuses

Binocular counts of all *U. annulipes* classes were significantly lower than excavated counts, on both the spring and the neap tides. Burrow counting, on the other hand, overestimated excavated densities during the spring tide. Binocular estimates of total densities were between 45% (spring) and 51% (neap) lower than excavated estimates, whereas burrow densities on the spring tide were 35% higher than excavated. These findings are in agreement with Macintosh (1988) who stated that counts of surface-active animals in Malaysia always underestimated densities, whereas burrow counts tended to overestimate population densities. During the spring tide, when both binocular and burrow counts differed significantly from excavated values, the percentage difference was always largest for the binocular census. Given also that burrow counts did not vary significantly from excavated ones during the neap tide, whereas binocular ones did, the overall results suggest that burrow counting is the most accurate of the two non-invasive techniques for the census of *U. annulipes*.

The fact that binocular census consistently underestimated excavated densities suggests that a significant part of the population (e.g. 28% of adults during

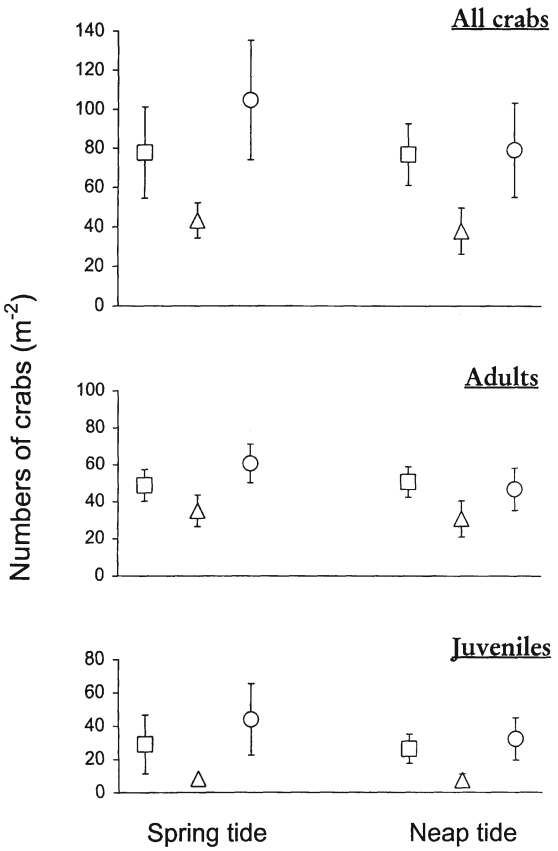

Figure 2. Mean densities of *Uca annulipes* within 12 spring and 12 neap tide 1 m^2 quadrats. Densities were assessed by excavation (squares), binocular quantification (triangles) and burrow counting (circles). Error bars depict 95% confidence limits.

spring) stayed underground. Visual counts are unlikely to capture the entire population on the surface at any one time. Zucker (1978) noted that the precise density of crabs could not be determined from binocular observations, since some individuals may have been temporarily within their burrows. At Maruhubi, disturbance, such as the intrusion of roaming predators like the ghost crab *Ocypode ceratophthalmus* (Pallas), may cause *U. annulipes* to retreat to burrows. Several authors have also noted how, during hot and/or dry days, *Uca* species frequently return to their burrows to replenish the water resources used in sorting sediments (Miller, 1961; MacNae, 1968; Macintosh, 1984). *U. annulipes* at the present site may frequently plug their burrows during hot periods of the day or at neap tides. This tendency has also been described in other *Uca* species, most notably during neap tides (MacNae, 1968; Zucker, 1978). Discrepancies between binocular and excavated counts may also have been due to emigration and the difficulty in spotting smaller animals (see below).

Burrow counting overestimated excavated densities during the spring tide by 35% (all animals), 25% (adults) and 51% (juveniles). The literature quotes very similar findings from quadrat excavations. Macintosh (1979) recorded about 15% more burrows than fiddler crabs in a Malaysian mangrove. Genoni (1991) reported *U. rapax* numbers as 74% of the burrow density. Mouton & Felder (1996) found the numbers of *U. longisignalis* and *U. spinicarpa* ranged from 75% to 100% of burrow numbers within estuarine marshes of Louisiana. Frith & Brunenmeister (1980) recorded very low burrow occupancy (11–51%) in a mixed-species population of fiddler crabs.

The excess of burrows in relation to animals may have three different origins, which will be dealt with in turn. None of these factors *alone* are likely to explain the majority of the discrepancy. Rather, differences between burrow and excavated counts are likely to have arisen from a combination of all three. (1) Some individuals emigrate from the observation area before excavation. Although, in this study, quadrats were fenced immediately after observation, emigration could have occurred previously during binocular counting (1–2.5 h). The upper levels of sandy habitats are generally poor in microorganisms, nitrogenous content and water supplies (Ono, 1965). Murai et al. (1983) suggested that in particular larger males of *U. vocans vocans* (Linnaeus) wander to low tide levels with high food productivity (*droving*) to compensate for lower feeding efficiency at their burrow sites. Miller (1961), Hocket & Kritzler (1972) and Crane (1975) state that *U. pugilator* may forage at a considerable distance from their burrows and that mature males higher up on the shore may leave their burrows to feed on lower grounds. In terms of estimating the mean population density, such droving behaviour is only important if crabs wander to areas outside the assessed habitat during census. Whereas droving to lower areas frequently occurs in *U. annulipes* at the present site, it is almost exclusively done by large males and particularly during spring tides. Thus, it is interesting that whereas burrow counts were significantly higher than excavated values on the spring tide, there was no significant difference on the neap tide; also that spring tide binocular counts of males were significantly different from excavated ones, whereas those for females were not (Table 3). However, the fact that male binocular counts were also significantly lower than excavated during neap, and that juvenile burrow

counts showed the biggest difference from excavated values (Table 2), suggest that emigration cannot explain all the differences. (2) Not all animals were extracted from the sediment during sorting. During the present study, sediments were hand-sorted *in situ* on a large tarpaulin. This technique was chosen because of the volume of sediment involved, and the lack of access to water made wet-sieving impossible (see 'Methods'). Krebs & Valiela (1978) found that without sieving, 20–50% of *U. pugnax* greater than 5 mm CW were missed by hand sorting, with the percent missed dependent on the stickiness of the mud. Powers (In Krebs & Valiela, 1978) in sandier sediments found that 20–25% of all *U. panacea* were missed without sieving. If the sorting technique used failed to extract all animals, it would have had greater bearing on the excavated counts of juveniles, because of the increased difficulty in seeing small animals. The fact that juvenile burrow and excavated counts displayed the largest difference suggests that this may have been so. Nevertheless, if poor sorting was the only reason for the underestimation of juveniles, we should expect the difference between burrow and excavated values to stay constant, irrespective of tidal phase. In fact, the percentage difference on the spring tide (51%) was more than double that of the neap tide (23%) (Table 2). Lack of complete extraction of animals is thus unlikely to be the only reason for discrepancies between burrow and excavated counts. (3) Some crabs dig more than one burrow. Crane (1975) observed that larger males sometimes wander low on the shore and take over burrows from smaller crabs, forcing some of the latter to dig new burrows. Olafsson & Ndaro (1997) found that individually kept laboratory specimens of *U. annulipes* over a period of 10 days made between 1 and 2 burrows per individual. *U. annulipes* plug burrows immediately before tidal immersion, and re-emerge only when the tide recedes. Burrow counts in the present case were made within 1.5–3.0 h after spring tide retreat, and gave an overall mean excess of 35% burrows (Table 2). If the excess of burrows was entirely due to crabs digging extra holes, 35% of all individuals (35 excess burrows per 100 excavated animals) would have dug an 'extra' burrow within this time. This seems unlikely, particularly since this part of the spring tide is typically characterised by intensive feeding (Macintosh, 1977; Zuker, 1978; Salmon & Hyatt, 1983).

Discrepancies between burrow and excavated counts are likely to result from a combination of all three factors. The first and second explanations could apply equally well to deficits in binocular counts, albeit their effect would be to decrease the difference from excavated values. There is no way of estimating whether, in the present study, this would have been the case. Studies usually assume that the number of crabs excavated equals the true density. This assumption is only valid if all crabs are extracted from the sediment and if emigration and immigration do not occur. Future studies could eliminate the chance of migration by fencing quadrats before tidal retreat. There would be little assurance, however, that such fencing might not influence crab behaviour.

Comparison of spring and neap tide results

The density estimates from binoculars, burrows and excavations did not show significant differences between the spring and the neap tide. Nevertheless, the differences between binocular and excavated counts in both adults and juveniles were largest and most significant on the neap tide compared to the spring tide. This, in addition to the fact that burrow exceeded excavated counts on the spring tide, but not on the neap tide, indicates overall lower activity levels during the neap tide. At neaps many animals may have stayed underground, perhaps not unplugging burrows. *Uca annulipes* may plug its burrow during hot and dry days. Zucker (1978) found that *U. latimanus* (Rathbun), *U. musica terpsichores* (Crane) and *U. beebei* (Crane) did not emerge from their burrows when the previous tide did not cover them. Macintosh (1977) collected fiddler crabs by excavating the burrows into which they retreated when disturbed and found that accurate population estimates were only obtained when sampling during spring tides. This observation may imply low surface activity outside spring tides. Naturally, such behaviour would cause a reduction in the burrow/excavated ratio during neap tides. The fact that burrow and excavated counts agreed during the neap tide, but not on the spring tide, suggests that burrow census is most appropriately done during neaps. Conversely, the fact that surface activity was higher during spring, that the discrepancy with excavated numbers was greatest during neap, and that there were no significant difference between female counts from binoculars and excavation during spring, suggest that binocular quantification is best done during springs. These conclusions are, however, based on the assumptions that migration was excluded and that all crabs were extracted from the sediment. This may not

have been the case (see 'Comparison of Binocular and Burrow censuses' section).

The influence of crab sex

The sex ratio (males/females) ranged between 1.8 (BIN, spring) and 2.3 (EX, neap). The ratio did not vary significantly between census techniques nor between the spring and the neap tide. Frith & Brunenmeister (1980) described a very similar sex ratio of 2.2 (males/females) in a Thailand mangrove population of *U. annulipes*. Male binocular counts were significantly lower than excavated ones on both the spring (24%) and the neap tide (35%), whereas females were lower on the neap tide only (35%). This may have been because females tend to stay covered during dry periods. Zucker (1978) in Panamanian populations of *U. latimanus*, *U. musica terpsichores* and *U. beebei* found that females were rarely observed from several days after the full moon to just before the new moon. Berried females may have a particularly strong tendency for this behaviour. Nakasone & Murai (1998) and Salmon & Hyatt (1983) describe how berried females of *U. lactea perplexa* (H. Milne Edwards) and *U. pugilator*, respectively, stay within plugged burrows for extensive periods. Females may resurface when the habitat becomes wet again and maximise surface time to compensate for not feeding during neap tide periods. Salmon (1984) noted how female *U. vocans* showed rapid and intensive feeding in close proximity to burrows as soon as the tide had receded, whereas male feeding was interrupted by wandering and sexual display. Large males of *U. annulipes* may wander to feed on lower areas that do get flooded during neap tides. This droving behaviour could in part explain why for males binocular counts were lower than excavated counts on both the spring and the neap tide.

The influence of Crab size

Juveniles (<7 mm CW) represented between 37% (spring) and 34% (neap) of the excavated *U. annulipes*. A main source of binocular underestimates and burrow overestimates was discrepancies in juvenile counts. Binocular estimates of juvenile densities were 72% lower than excavated (Table 2). This difference was 2.5 (spring) to 1.8 (neap) times higher for juveniles than for adults. The reason that binocular observations underestimated abundance is unlikely to be simply because many juveniles stayed underground during counting. The observation that burrow counts of juveniles were 51% higher than excavated ones during the spring tide certainly suggest high levels of activity in this size group. Macintosh (1988) states that counts of surface-active animals may underestimate densities because juveniles are easily overlooked. The difficulty in spotting these small animals is a more likely explanation for much of these very large discrepancies. Evidently, this problem does not exist for burrow counting, since juvenile burrow counts were 51% higher than excavated during the spring tide and, whilst not significantly so, 23% higher on the neap tide. The percentage difference between burrow and excavated counts was 2.1 (spring) to 3.0 (neap) times higher in juveniles than in adults. Droving by juveniles has, as far as we are aware, never been observed in *U. annulipes*, or in other species of *Uca*. Whilst it is possible that juveniles created proportionally more burrows than adults, it is probable that a major reason for the excess number of burrows on the spring tide was that not all juveniles were extracted from the sediment. Small animals certainly are the most likely to be missed during sorting.

Conclusions

Excavation of *Uca annulipes* burrows within Maruhubi mangrove showed that the carapace widths of tenant crabs could be well predicted from the width of burrow openings. Burrows may therefore be used in estimating population size-distribution and biomass.

The estimation of *U. annulipes* density from binocular and burrow census was compared to excavated densities. The overall results suggested that burrow counting was the more accurate of the two techniques. Binocular counts always showed greater deviation from excavated counts than burrow counts and consistently and significantly underestimated crab densities, in particularly juveniles. Burrow counts tended to overestimate excavated densities, but only significantly so on spring tides. Binocular counts are likely to have underestimated excavated densities because many crabs stayed underground during census. Different reasons for this behaviour are given. Burrow estimates may have overestimated excavated densities from a combination of three factors: (1) Some animals may have temporarily left the census area. (2) Not all animals were extracted from the sediment after excavation. (3) Some crabs make more than one burrow. None of these three are perceived as having had the most influence on burrow counts.

Binocular counts matched excavated densities best during the spring tide, whereas the burrow quantification was better during neap. These conclusions are based on the assumptions that migration was minimal and that all crabs were extracted during excavation. The validity of both assumptions is questioned.

The quality of the binocular census differed between males and females. Binocular counts of females did not differ significantly from excavated counts during the spring tide, but did during neap, whereas male counts were different on both tides. This observation, along with literature evidence on female behaviour, gives credence to the idea that binocular census is better on spring tides. Crab size also influenced the quality of density estimates. Density estimates of small animals showed the largest deviation from excavated counts in both the binocular and the burrow technique. Juvenile underestimates by binoculars may be because of the difficulty in seeing small animals; whereas missing small animals during sediment sorting following excavation may have led to apparent overestimates of juvenile numbers by the burrow census.

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A comparison of alternative methods for estimating population density of the fiddler crab *Uca annulipes* at Saco Mangrove, Inhaca Island (Mozambique)

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Key words: fiddler crabs, *Uca annulipes*, Density estimates, Mangroves, East Africa

Abstract

Visual counts of surface-active crabs both by binocular and burrow counting methods have been used in many studies to estimate population density. However, their reliability has not yet been assessed comparatively. Three methods for estimating the abundance of fiddler crabs *Uca annulipes* in a mangrove forest (Inhaca Island, Mozambique) were compared from three different sub-areas: two sub-areas inundated only during spring tides and one sub-area inundated in both spring and neap tides. Burrow, binocular and direct (excavation) counting methods were performed by plotting ten 0.25 m^2 quadrats in each sub-area over the four moon phases. Overall densities (per 0.25 m^2) differed according to method, sub-area and lunar phase. Burrow count overestimated crab density by up to 20%, while binocular count underestimated density by up to 41%. Correlation coefficient estimated for both counting methods showed that burrow count gives better density estimates than binocular count (0.91 and 0.56, respectively). Sex ratios were also investigated within the three sub-areas and at the moon phases. Males are dominant throughout the studied period except during new moon and first quarter, indicating that when the number of gravid females is low, sex ratio bias for binocular count is minimal.

Introduction

Distribution patterns and taxonomic status of fiddler crabs belonging to the genus *Uca* have been studied in great detail (Crane, 1975). The fiddler crab *Uca annulipes* has a widespread distribution in the southern hemisphere and typically is a dominant species in mangrove crab communities (MacIntosh, 1988). The activity patterns of *Uca* species are largely dependent on tidal periodicity, which is in turn moderated by moon phase.

Several methods have been used to estimate the population densities of crabs inhabiting mangroves (Cammen et al., 1984; Colby & Fonseca, 1984; Nobbs & Mcguinness, 1999). Due to their habit of emerging from burrows during ebb tide, population size can be estimated using a variety of methodologies. Absolute estimates can be produced through direct excavation of crabs from their burrows, while direct visual counts can be made of surface-active animals, or of their burrows.

Both visual counts of surface-active crabs and burrow counts have been used in many studies to estimate population density (e.g. Crane, 1975; Macintosh, 1988; Von Hagen, 1993; Nobbs & Mcguinness, 1999). The popularity of these methodologies is partly a consequence of the difficulties associated with excavating crab burrows in mangrove habitats. Furthermore, they are considered appropriate for generating accurate indices of relative abundance, whilst resulting in minimum impact on sensitive mangrove habitats.

A problem with visual counts of surface-active animals is that they tend to underestimate population size because some individuals (for example moulting crabs or ovigerous females) remain inside their burrows, and juveniles are easily overlooked (Crane, 1975; MacIntosh, 1988). Conversely, burrow counts tend to overestimate population density because a propor-

tion of burrows are unoccupied or have more than one opening (Macintosh, 1988). No previous studies have attempted to quantify the relationship between estimates generated by these different methods in relation to absolute counts.

In this study, we compare three methods of estimating the population density of the fiddler crab *Uca annulipes* through burrow, binocular and absolute counts at an East African subtropical mangrove, the Saco da Inhaca, Inhaca island, southern Mozambique. In addition, a comparison of the impact of tidal and lunar phases on the different counting methods and population sex ratios was made.

Materials and methods

Study area

The Saco da Inhaca (26° 07′ S, 32° 56′ E) is an enclosed bay fringed by extensive mangrove forest (Fig. 1) for which detailed descriptions of the general ecology, fauna and flora have been carried out (Kalk, 1995; Guerreiro et al., 1996; De Boer & Longamane, 1996; Abdurremane, 1998; Halare, 1999; Quincardete, 1999; De Boer, 2000).

The study was conducted in three different sub-areas characterised by differing tidal inundation periods. Two of the areas were in the mangrove forest, under the upper *Avicennia marina* (area A) and the mid *Avicennia marina* belts (area B). The third was a low sandbank in the inner part of the intertidal zone (area C). The two mangrove sub-areas were not inundated during neap tides, while the sandbank was inundated during all tides.

Sampling

Sampling was performed between January and March 1999 at the three sub-areas, and once at each of the four lunar phases (first quarter, full moon, last quarter and new moon). Each phase was sampled twice over a 2–3 day period, from the first day of each type of tide, starting 1–2 h after high tide. Twenty stations were allocated in each of the sub-areas, of which 10 were chosen randomly for sampling (quadrats of 0.25 m^2). Each randomised quadrat was observed for 5 min from a distance of 4 m, and the number of emerging crabs was counted using binoculars with 8×40 magnification. After each 5 min observation, the number of burrows within the plots was counted. Afterwards, the same plot was excavated with a corer to a depth of 25 cm. All crabs were collected, identified and sexed (females checked for eggs). Other species excavated while digging were recorded and discarded.

Burrow and binocular counts were converted to percentage of absolute count. Hourly sampling was also made in order to assess the variation of sex ratio, starting 1 h after high tide until low tide, during one spring tide at two sites, the upper *Avicennia marina* (A) and the mid *Avicennia marina* (B) areas. Regressions of absolute *versus* binocular counts and absolute *versus* burrow counts were computed for the three sub-areas. All quadrats without excavated crabs or burrows were excluded from this analysis. Pearson's correlation coefficients were calculated in order to assess the degree of relationship between the direct values (excavated) and the binocular and the burrow counts.

Sex ratio was calculated for male *versus* total crabs (M:T) for the three sub-areas and separately for each moon phase. One-way ANOVA was performed to test the hypothesis that there was no difference between the counting methods, the three areas and lunar phases. Post Hoc comparisons were made using the Tukey HSD test for unequal N.

Results

Average density estimates of crabs, based on binocular, burrow and absolute counts, varied between sub-areas (Fig. 2). The binocular method density estimates are lower than those based on direct counts, while burrow counts are higher than the direct counts.

It is clear that area C on the sand bank presented the lowest densities of crabs by all counting methods. Upper (A) and mid *Avicennia marina* (B) sub-areas contained the highest numbers of crabs, and presented no clear differences. Density estimates differed between sub-areas (Anova, $p<0.00001$, indicating significant differences between sub-areas A and B in relation to C), moon phases (Anova, $p<0.001$, indicating significant differences between full moon and last quarter in relation to new moon, and also between first quarter and full moon), and sampling method (Anova, $p<0.0001$, indicating significant differences between all 3 methods). Global results of the 3 performed Anovas are presented in Table 1.

On the whole, a total of 5952 individuals were counted by the direct excavation method. Binocular counts gave a total of 3533 individuals (59% of direct count), while burrow counts indicated 7487 individuals (126% of direct count). The results show

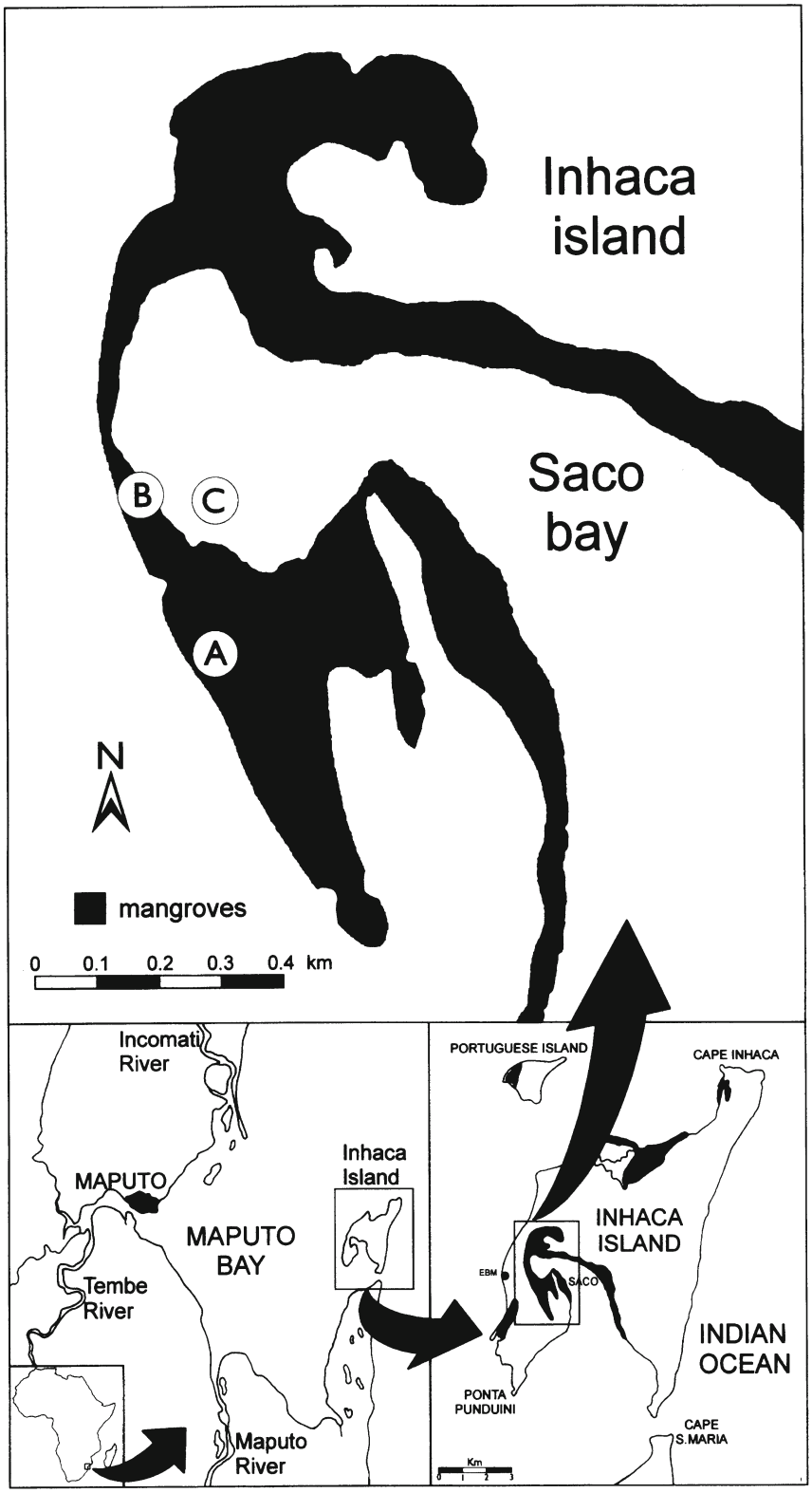

Figure 1. Map of Saco bay at Inhaca island, showing position of sampling areas.

Table 1. Results of 1-way ANOVA performed for the estimation method, area and moon phase

	df Effect	MS Effect	df Error	MS Error	*F*	*p*-level
Method	2	7736.061	2056	49.178	157.308	0.00001
Area	2	7661.165	2056	49.251	155.555	0.00001
Moon phase	3	376.215	2055	56.182	6.696	0.00017

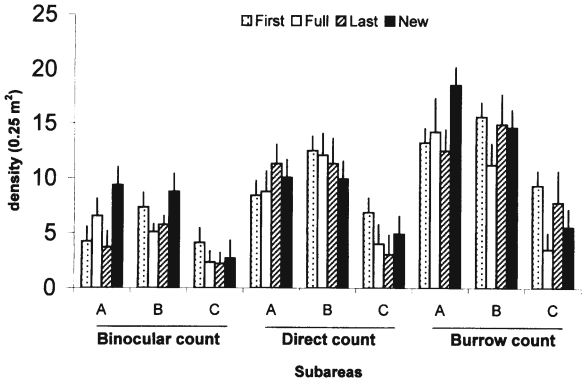

Figure 2. Density of *Uca annulipes* estimated by binocular, burrow and absolute counts, in the 3 sampling areas (Upper – A, Mid – B, Lower – C) during the 4 lunar phases. Vertical lines indicate +1 standard error.

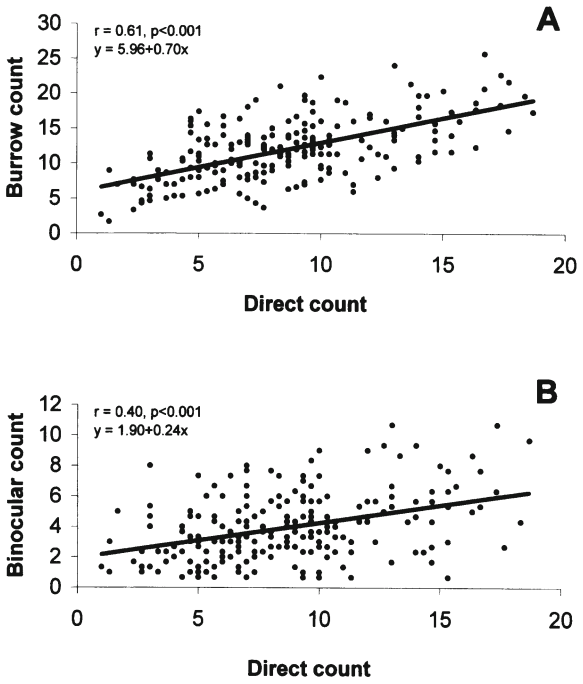

Figure 3. Regression in relation to direct excavation count for *Uca annulipes* of (A) burrow and (B) binocular counts.

that burrow counts overestimate density by around 25%, while binocular counts underestimate density by around 40%.

The relationships of binocular counts and burrow counts to excavated counts are shown in Figure 3. The correlation coefficients for these relationships were calculated. The correlation values are highly significant and strongly positive (for binocular *versus* direct counts r=0.40, p<0.001, and for burrow *versus* direct counts r=0.61, p<0.001).

Figure 4 presents the variations in sex ratio over the 4 lunar phases. At all areas sampled, there was a permanent male bias during the 4 lunar phases. Highest proportion of males is reached during full moon at areas B and C, and lowest was found during new moon at areas A and B.

Hourly variation in sex ratio during the ebb tide period is shown in Figure 5. Sex ratio is male-biased during all the ebbing tide period. This result is more pronounced at area B. In both areas, however, there is a slight increase of surface active male crabs throughout the ebb tide period.

The proportion of ovigerous females along the lunar phases is presented in Figure 6. The lowest val-

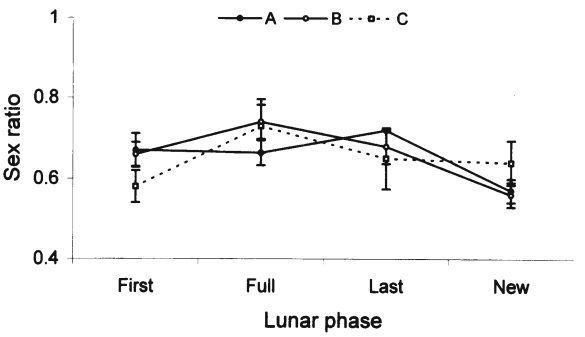

Figure 4. Fluctuation of sex ratio (males:total) for *Uca annulipes* along the lunar phases at the 3 sampling areas. Vertical lines indicate ±1 standard error.

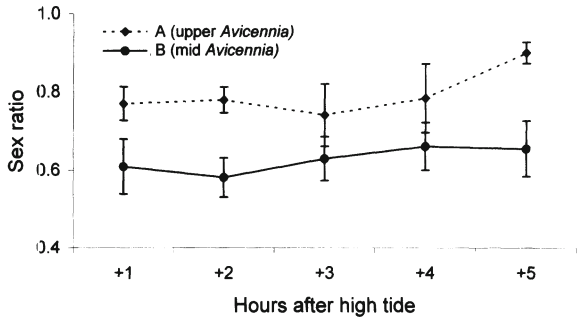

Figure 5. Fluctuation of sex ratio (males:total) for *Uca annulipes* during the ebb tide period for sampling areas A and B. Vertical lines indicate ±1 standard error.

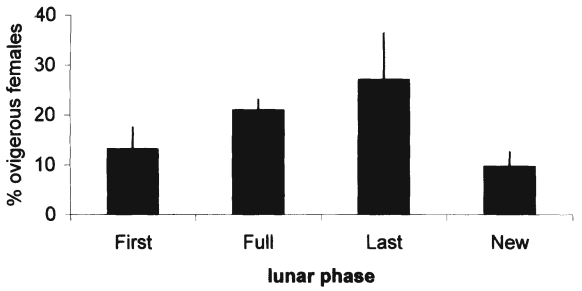

Figure 6. Fluctuation of proportion of ovigerous females of *Uca annulipes* along the lunar phases, the 3 sampling areas combined. Vertical lines indicate +1 standard error.

ues were obtained during new moon and first quarter counts, generally around 10%. Maximum values were observed during last moon quarter, reaching on average around 25%.

Discussion

The average densities differed greatly between the three sub-areas and between methods. The mid *Avicennia* zone seems to offer better suitability conditions compared to the other sub-areas, therefore the average number of crabs was higher compared to the other sub-areas. Some of the relevant conditions could be the amount of water and probably the organic content in the soil.

Density estimates obtained from the three different methodological approaches indicated that in relation to direct counts, burrow counts tend to overestimate crab population while binocular counts underestimate density of the crabs. MacIntosh (1988) and Firth & Firth (1979) reached a similar conclusion in their studies of fiddler crabs on mangrove shores in Malaysia and Thailand. These authors have suggested that the higher number of burrows in relation to the real number of the crabs can be attributed to the crabs' need to improve the opportunity for escape from predators and aggression from neighbouring conspecifics, and also because burrows are sometimes abandoned or lost.

Comparing binocular and burrow counting methods in relation to the direct count it is evident through the statistical tests and through the correlation coefficients obtained that the burrow count gives much better estimates of the real data than the binocular count. However, both methods have advantages and disadvantages that need to be discussed. Binocular count estimates fewer crabs per area, and the sexes can easily be confused especially for males lacking the larger chela and juveniles. The lunar phase, as well as the timing of the counting process in relation to ebbing tide, is also crucial. The incubation period for females, the distance from which the crabs are counted, as well as local disturbances can interfere seriously with crab behaviour, therefore resulting in a wrong interpretation of data. It is important to note that estimating population density using binocular counts should be confined to the non-breeding period in order to avoid missing gravid females. Females of *Uca annulipes* can be highly underestimated by the binocular method during incubation period. In this study, it was found that in periods with higher numbers of ovigerous females, fewer females were counted by the binocular method.

In spite of being a non-destructive method, the binocular method does not supply reliable results on the true density of crabs. Thus, it would not be recommended for rapid appraisal of crab density unless additional data (such as: emergence time, lunar phase, sex ratio variation in relation to tide, etc) on crabs in the area to be studied are carefully considered. Counting crabs after the ebbing tide shows variations in the sex ratios as the time increases. Lunar phase is also known to be a determinant for sex ratio variation in decapod crustaceans (see Crane, 1975; Macintosh, 1988; Emmerson, 1994).

Burrow count becomes an ambiguous method when the studied area is inhabited by several other burrowing crabs, even when burrow specific characteristics are well known. This is an important issue for crabs of the same genus. For example, in this study, *Uca inversa* coexists with *U. annulipes,* and it is quite difficult to determine which burrow belongs to which crab species before a survey is done, to check the dominant burrow sizes of both species. In this case, the exact percentage of occurrence of each species needs

to be known in advance. Another problem is that, usually, the number of crabs dwelling within the same burrow is not known and the sex ratio can never be assessed.

Salmon (1987) found in his behavioural study that males of *U.thayeri* could be found in relation to the number of burrows in the following proportions: 3/2, 1/1, 1/3, 1/4, 1/9 and 1/11 (number of crabs/number of burrows). This example illustrates that, in spite of the relatively good correlation between the direct and the burrow counts, there will remain an important source of error. Before trying to study population density for *Uca annulipes* a similar study would be required in order to assess the consistency of the relation between the number of crabs occupying a certain amount of burrows. This will always constitute a limitation when a rapid assessment of density is required. If the burrow characteristics are well distinguished, and the species inhabiting the studied area are known, then this method can easily be applied and give fair results on density estimate. However, it is still important to bear in mind that the values obtained will usually be higher than the real ones.

Another aspect that may interfere with the number of counted crabs, as well as in their relationship with the burrow number, is the droving behaviour of *Uca annulipes*. It is known that these crabs drove from their burrowing areas to feed in other areas, presumably with higher organic content (P.Blackwell, pers. com.). During this study, many crabs where seen in patches away from their burrows in areas without any burrows. On the other hand, for some quadrats no crabs were encountered inside the area as well as within the burrows during excavation. This suggests movement towards other surrounding areas for feeding.

Males dominated throughout the study period and in all sub-areas. Sex ratio variation is less pronounced during new moon and during the first quarter, indicating that when the number of gravid females is low, sex ratio bias is minimal. More females are active during the first and new moon, therefore sex ratios are closer to parity during these periods for the three sub-areas. Female *Uca annulipes* are known to change their pattern of emergence during the incubation period (P.Blackwell, pers. com.), therefore affecting sex ratio. During all lunar phases, except new moon, females are proportionally more abundant at the surface in the lower bank (sub-area C) than in the other areas studied. This fact may be related to the fact that sub-area C is inundated by all tides and thus ovigerous females do not suffer the usual desiccation problems encountered in higher areas.

These results show that both binocular and burrow counting methods can be used to estimate the apparent abundance of *Uca annulipes* in mangrove areas. Both methods are 'mangrove friendly' since they do not destroy the environment. However, for both cases, a number of factors have to be taken into account in order to get the best validated approach to population density.

Acknowledgements

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Aspects of the population dynamics of *Neosarmatium meinerti* at Mgazana, a warm temperate mangrove swamp in the East Cape, South Africa, investigated using an indirect method

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Key words: mangroves, crabs, growth, density, burrows, southern Africa

Abstract

Twelve 1 m^2 quadrats were randomly staked out in the *N. meinerti* zone throughout the mangrove swamp at Mgazana. Every month during 1995 and 1996, the numbers and positions of the holes falling within the quadrats were measured and recorded. A wide size range of *N. meinerti* was then caught within the mouths of their burrows and measured to yield a significant crab size–hole size relationship. Data was then analysed using cohort analysis and regression. Occupied burrow size ranged from 13 to 75 mm in diameter. Hole and hence crab frequency distribution was found to be polymodal throughout the year. *Neosarmatium meinerti* appears to live for about 5 years. There was a complete lack of holes or crabs in the 5–10 mm hole size class or Year 0 class. These 'missing' classes were found when resin casts of adult burrows revealed small burrows containing the missing size class looping off the main burrow shaft. Developing crabs can thus obtain both shelter and food here (*N. meinerti* is known to store leaves in burrows before consumption) before they are strong enough to break through to the surface and form their own fully independent burrows. These Year 1 cohorts emerge from April to August. The monthly position of holes did not vary considerably, but hoods produced over the holes did change position suggesting that burrows are relatively permanent. There was also evidence of re-occupation of existing holes by other crabs which represents a considerable saving of energy compared to *de novo* excavation. Density estimates ranged from 5.5 to 22 m^{-2}, with an overall mean of 11.4 m^{-2} and showed little seasonal trend. Crab holes were significantly denser directly under trees compared to open ground.

Introduction

In mangrove systems along the tropical East Coast of Africa and the Indo-West Pacific across to Australia, a major macrofaunal ecological dominant is the sesarmid crab *Neosarmatium meinerti* (De Man, 1887) (Jones, 1984; Lee, 1998). These large crabs generally construct deep burrows in the upper-intertidal to supratidal zones within mangrove areas in estuaries where they feed directly on abscised leaves which fall to the forest floor (Emmerson & McGwynne, 1992; Steinke et al., 1993).

At Mgazana on the east coast of southern Africa, *N. meinerti* is the most important crab species in terms of biomass, contributing up to 60 g dry mass per m^2 in the supra-tidal zone (Branch & Grindley, 1979). This averages out at around four *N. meinerti* .m $^{-2}$ (Emmerson & Mc Gwynne, 1992). However, the estimation of density has been difficult for this species as burrows are deep and usually constructed in hard, dry mud (Bright & Hogue, 1972) making excavation extremely difficult. As burrows have been used to identify (Zoutendyk & Bickerton, 1988) and estimate crab population densities in the past (Aspey, 1978; Krebs & Valiela, 1978; Montero et al., 1983; Warren, 1990; Steinke et al., 1993; Mouton & Felder, 1996), this approach was applied to estimate density, growth and recruitment of *N. meinerti* within the Mgazana system.

Methods

At the Mgazana Estuary (31° 42′ S; 29° 25′ E) on the Transkei coastline, East Cape, South Africa, sites were chosen within the *Avicennia marina* zone as this was

where *N. meinerti* were most common. This estuary was chosen as it has one of the most extensive mangrove systems in the subtropics south of Mozambique (Branch & Grindley, 1979) with an estimated 150 ha of forest (Ward & Steinke, 1982) which supports a large population of *N. meinerti* (estimated 6 million crabs, Emmerson & McGwynne, 1992). Localities for sites were chosen from a grid used in previous work which gave a spread along the estuary approximately every kilometer (Emmerson & McGwynne, 1992). As the estuary was approximately 6 km long, two sites were randomly chosen from a transect of 5 sites every kilometer yielding a total of 12 sites. At each site, a 1 m^2 quadrat was marked out at each corner using metal stakes driven into the substrate. Sites were visited every month for 2 years from January 1995 to December 1996 when all the holes in each quadrat were carefully drawn, measured and counted. Only open holes with evidence of occupation such as freshly cleared mud and dactyl imprints were used.

In order to establish the relationship between hole size and crab size, a wide size range of crabs was caught in the mouths of their burrows and the following variables recorded: sex, carapace width, carapace length and hole diameter using vernier calipers.

Resin casts were also randomly taken (one per site) to establish the morphology and depth of *N. meinerti* burrows using standard techniques applied for other decapod species (Dworschak, 1983; Montero et al., 1983; Stamhuis et al., 1997).

Monthly frequencies were analysed using cohort analysis according to the methods of Harding (1949) and Cassie (1954). Differences between male and female carapace width and hole diameter were tested by ANCOVA using BIOM after transformation to straighten the curve (Sokal & Rohlf, 1995). The regression between carapace width and burrow width was calculated using *Excel.* In comparing densities of quadrats from open sites and sites under mangrove canopies, a paired *t*-test was used.

Results

As no difference was found between male and female size and hole diameter (means F, 3.36; not significant; slope F, 3.6, not significant), data were pooled and a relationship established between hole diameter and crab size (Fig. 1). A significant correlation ($p<0.05$, $n = 133$) was found between hole width and *N. meinerti* carapace width ($R^2 = 0.9011$). This correlation was then used to convert hole sizes into crab sizes.

In order to obtain an overall index of the population dynamics of *N. meinerti* at Mgazana, monthly burrow size class frequencies were combined for each site. The histograms for each month during 1995 and 1996 (Fig. 2a–2d) were then analysed using cohort analysis. Modal means for each month were then converted to crab sizes using the burrow-crab relationship formula in Figure 1. Modal progressions showing cohort growth are summarised in Figure 3 and Table 1.

Crab densities were estimated from monthly burrow numbers per quadrat over the 2 years sampled. Quadrat means ranged from 5.5 m^{-2} to 22 m^{-2} with an overall mean of 11.4 m^{-2}. Crab holes tended to be much denser directly under mangrove trees, compared to open ground (mangrove cover mean, 14.56 m^{-2}, open mean, 6.32 m $^{--2}$, $t = 23.78$, $n = 312$, $p<0.01$) which may be related to the proximity to leaf-food supply. Numbers varied between quadrats, but means showed little seasonal trend (Fig. 4), although there were periodic 'recruitment' or emergence of small 10–15 mm CW size class crabs mainly from May to July (Fig. 3).

Hole and hence crab distribution was found to be polymodal with patterns changing throughout the year (Figs 2a–d). Cohort analysis (Fig. 3) showed the modal progression and growth for five distinct year classes (Table 1). *Neosarmatium meinerti* thus lives for around 5 years in Mgazana with growth fairly rapid during summer (November–April) and very slow during winter (May–August; Fig. 3). Additional research such as tagging is required to corroborate these indirect growth estimates with actual growth measurements. Conspicuous by their absence in Figures 2a–d and 3 are small holes in the 5–10 mm hole size class or Year 0 crab size class.

Discussion

Neosarmatium meinerti at Mgazana has a distinctive spawning peak every year during February (Emmerson, 1994), followed by offshore larval development from February to March (Pereyra-Lago, 1989), suggesting that the megalopae and first crab stages recruit back into the mangroves during March and April. These stages, however, were not apparent.

This result, together with a general observation of no small holes in the *N. meinerti* zone at Mgazana, led to a search for the 'missing' size classes. As many inter-tidal organisms have a differential zonal distribution during growth and development (Branch &

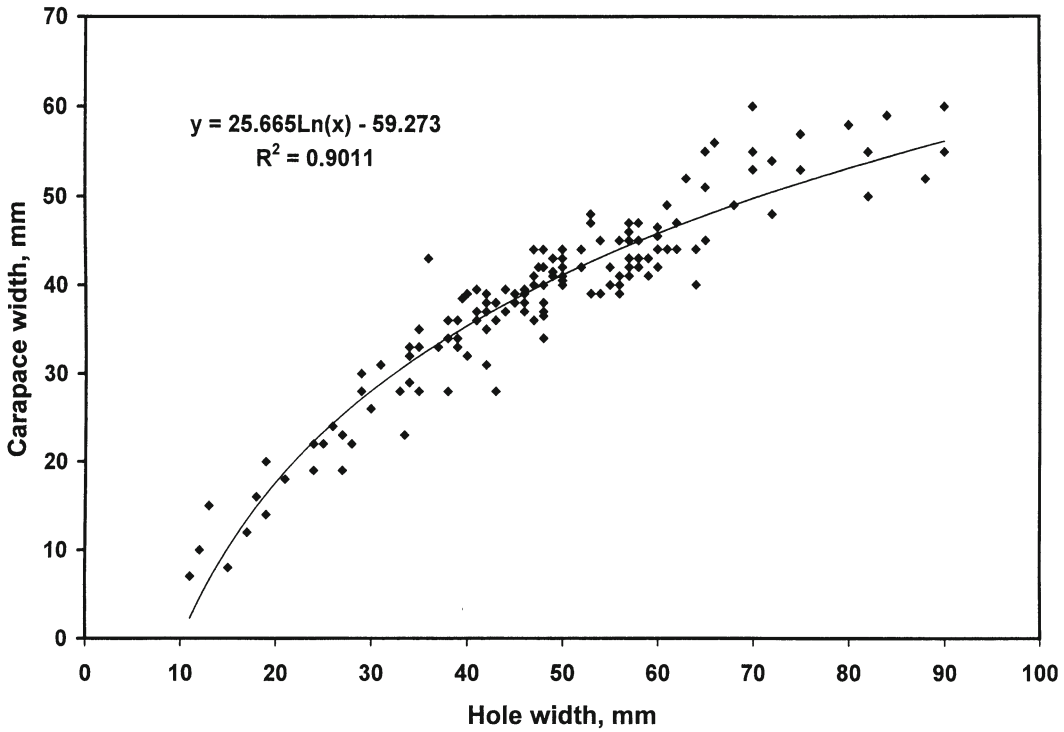

Figure 1. Relationship between burrow (hole) width and *N. meinerti* size (Carapace width).

Table 1. Growth and modal progression of for five year classses of *N. neinerti* from Mgazana

Class	January		December
Year 0	0	to	10–15 mm
Year 1	10–15	to	30 mm
Year 2	30	to	50 mm
Year 3	50	to	65 mm
Year 4	65	to	75 mm

Branch, 1983), it was thought that recruiting *N. meinerti,* from megalopa/first crab stage to 'emergence' in the 10–15 mm size class, may be distributed elsewhere within the mangrove zonation. Extensive sampling within the inter-tidal (Emmerson, 1994) yielded 9 associated species, but no small *N. meinerti*. However, when resin casts were taken of *N. meinerti* burrows, small side branches were found on many of them (Fig. 5). In one of them, a small crab was actually found embedded in the resin and positively identified as *N. meinerti.*

This pattern is different to those of other co-existing sesarmids within the Mgazana swamp system, such as *Sesarma catenata*, where thousands of tiny recruits invade the inter-tidal mudflats in late summer (Branch & Grindley, 1979). Because these inter-tidal mudflats are flooded every high tide, the sediments remain soft and it is relatively easy for crabs to excavate burrows. The *N. meinerti* zone, by contrast, lies within the upper inter-tidal and supra-tidal zone which, especially during the autumn and winter dries out forming a hard baked crust which would be difficult for a small crab recruit to breach. It would thus be a good strategy for vulnerable new recruits to enter adult burrows during spring flood tides and make small side burrows in the soft side wall. Not only would they obtain shelter by doing this, but also food as *N. meinerti*, like *N. smithi* (H. Milne Edwards, 1853), also stores collected mangrove leaves in their burrows (Giddins et al., 1986). This storage improves palatability prior to consumption by lowering tannins and increasing nitrogen/protein levels (Nielson et al., 1986; Emmerson & McGwynne, 1992). These burrows tend to be close to the surface and 'loop' back to the adult burrow (Fig. 4), but only break through directly to the surface once the crabs reach the 15–20 mm CW size class. This could be the stage when they are physically strong enough to breach the mud-crust and possibly

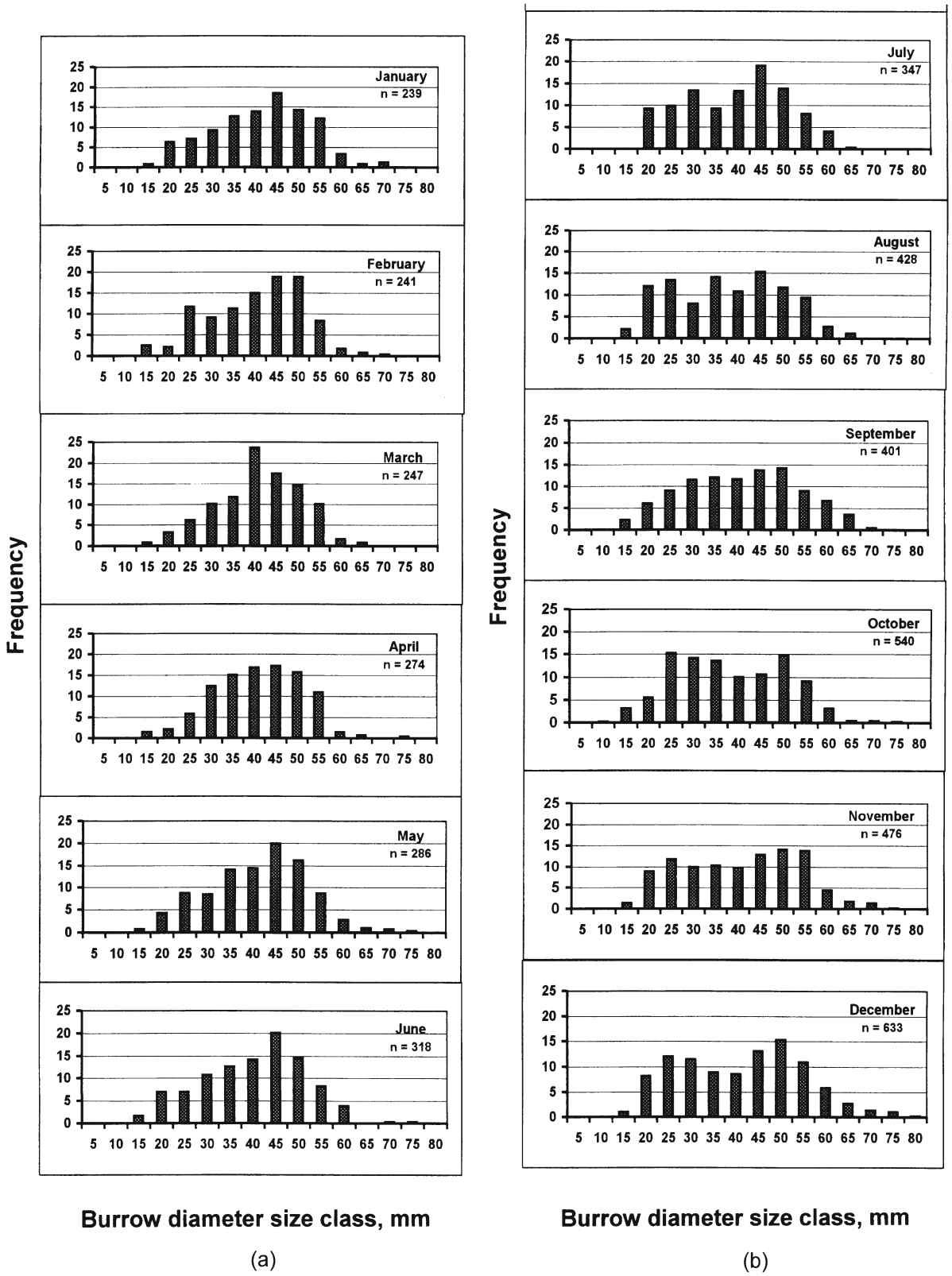

Figure 2a. Combined *N. meinerti* burrow size frequency distributions for Mgazana from January to June 1995. (b) Combined *N. meinerti* burrow size frequency distributions for Mgazana from July to December, 1995.

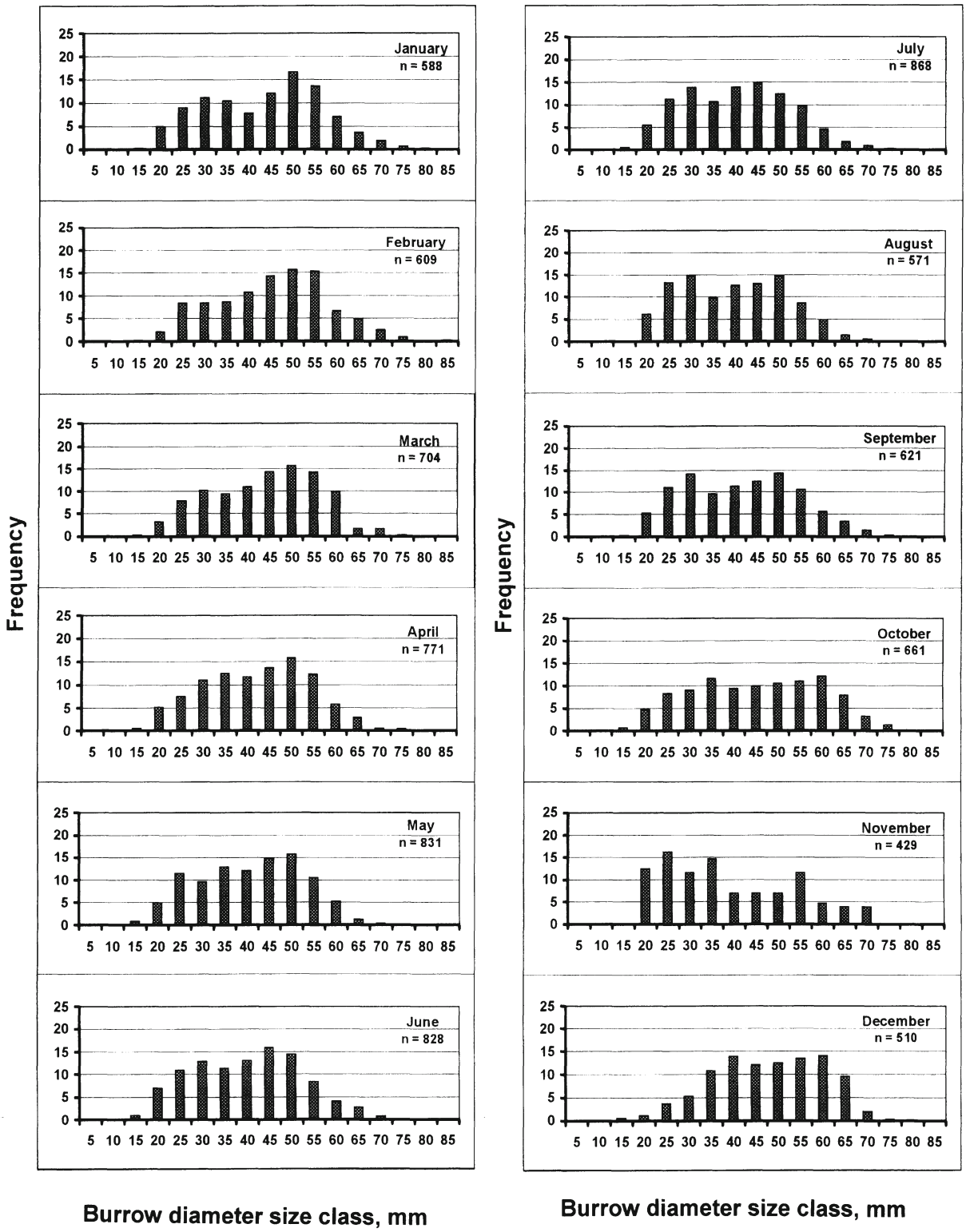

Figure 2c. Combined *N. meinerti* burrow size frequency distributions for Mgazana from January to June, 1996. (d) Combined *N. meinerti* burrow size frequency distributions for Mgazana from July, 1996 to December, 1996.

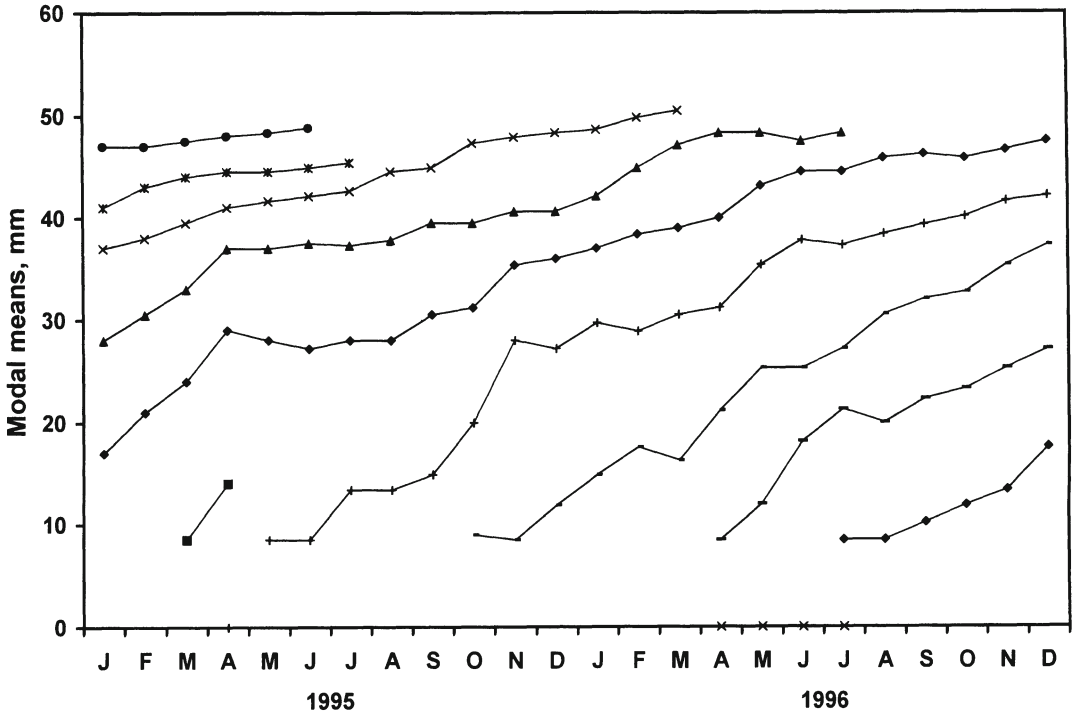

Figure 3. Monthly modal means of *N. meinerti* calculated from combined data for Mgazana. Different symbols represent separate cohorts.

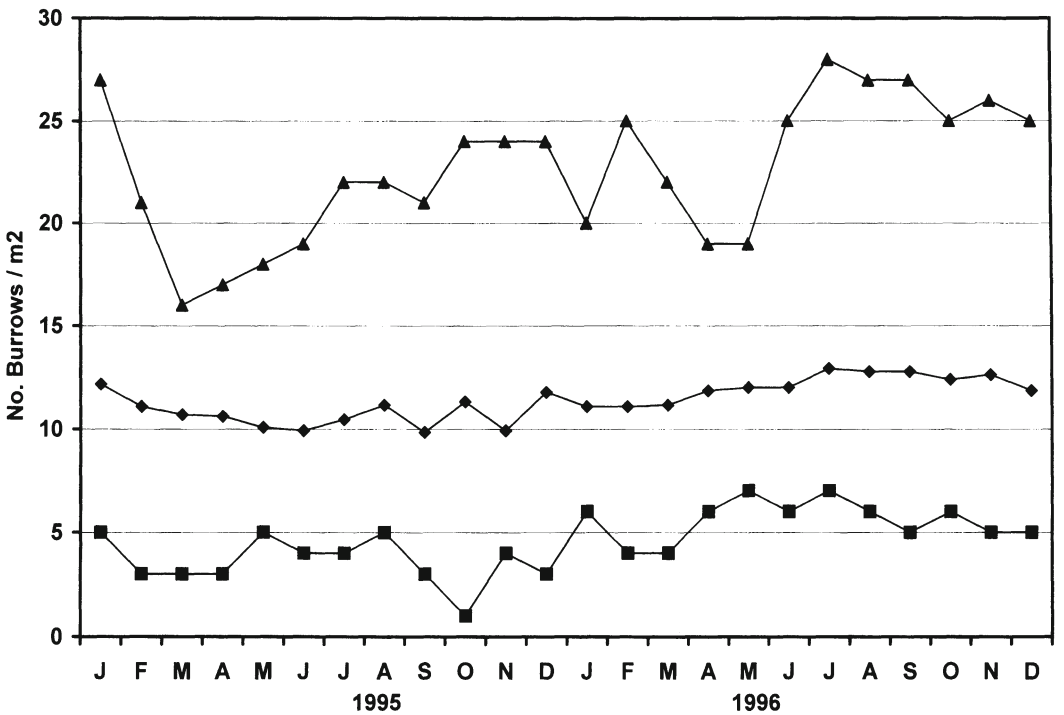

Figure 4. Mean monthly burrow densities (n .m^{-2}) of 12 sites at Mgazana for 1995 and 1996. Diamonds, mean; triangles, maximum; squares, minimum.

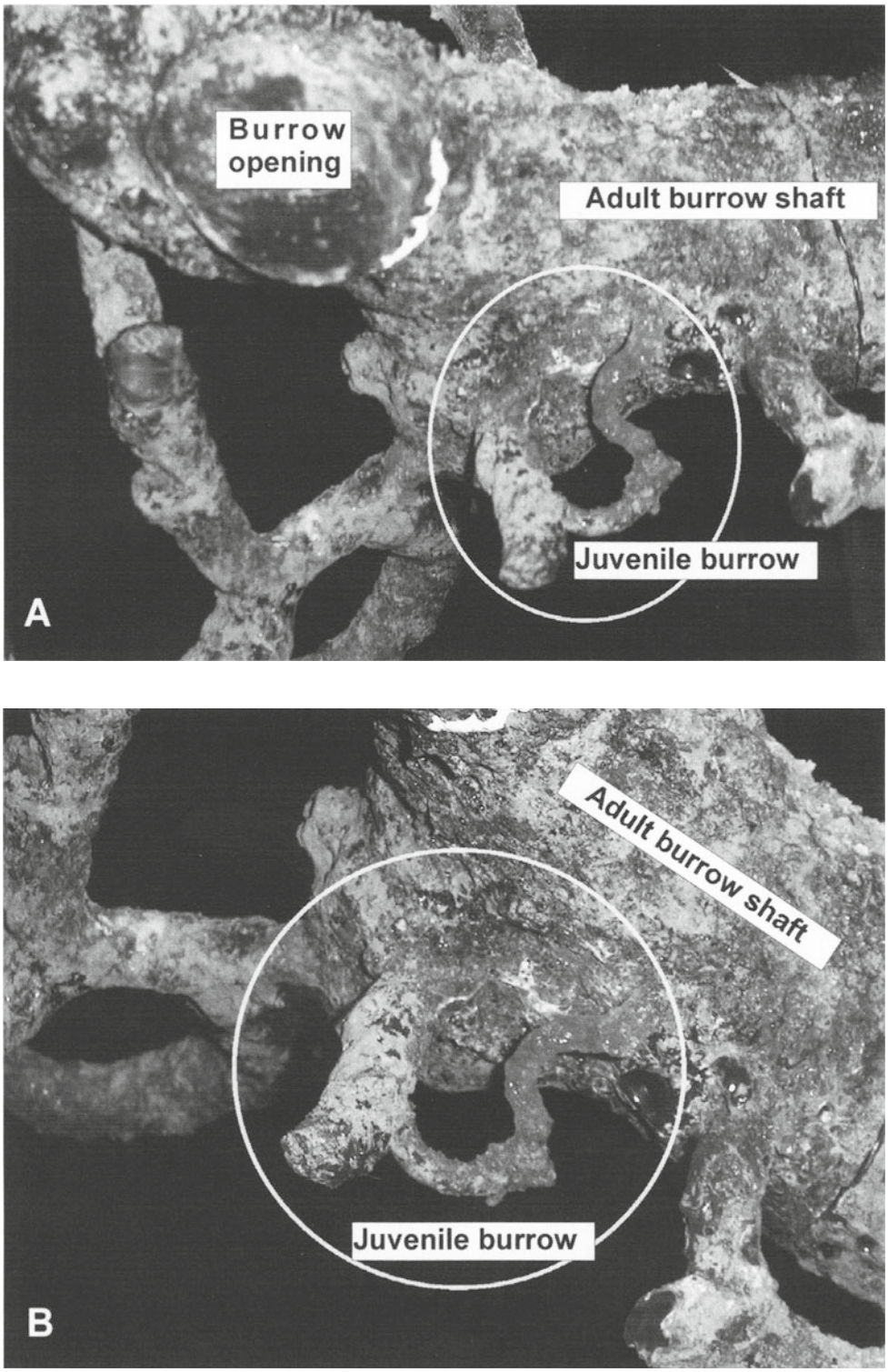

Figure 5. Photograph of a resin cast from a *N. meinerti* burrow showing the small 'nursery' burrow looping off the 'parent' burrow.

also explain why, after spending nearly a year underground, they suddenly emerge as young Year 1 cohorts (10–15 mm CW) from March to July (Fig. 3). The occurrence of 'nursery' burrows branching off adult burrows has been reported in other southern African estuarine decapods like the sand prawn *Callianassa kraussi* Stebbing, 1900 (Forbes, 1973), but not for crabs. Recent work on other mangrove-dwelling sesarmids by Knieb et al. (1999) has shown that there is a considerable amount of inter- and intra-specific cannibalism of adult crabs on juveniles and that juvenile survival may be regulated by differences in adult density and availability of refugia. If this applies to *N. meinerti* as well, then the presence of juveniles within 'nursery' burrows would protect against both inter- and intra-specific cannibalism.

The assumption of one crab per burrow for density estimates probably over-estimates the actual population density as it was observed that sometimes a hole had two entrances, usually of the same diameters. This has also been reported previously for *N. meinerti* (Steinke et al., 1993) as well as for other species (Warren, 1990). Hogue & Bright (1971) and Micheli et al. (1991) have described *N. meinerti* burrows as being straight, non-branching holes possibly more than a meter deep. This may be true in most cases, but branching burrows can occur in moister substrates as can be seen in Fig 5. Nonetheless, density estimates for *N. meinerti* at St. Lucia (5.1 m^{-2}) and Mgeni (0.3–5.3 m^{-2}) were similar to those found at the lower end of the scale for Mgazana, but it was not clear whether these sites were from open ground or under the mangrove canopy. The maximum estimates for Mgazana were high, especially when compared with the conservative overall estimate of 4 m^{-2} used previously for this system (Emmerson & McGwynne, 1992). However, in Kenya Micheli et al. (1991) estimated the highest densities of *N. meinerti* burrows to be 11.64 m^{-2} which is similar to the overall mean for Mgazana. Mouton & Felder (1996) have similarly used burrows for crab population estimates and they found that burrow numbers exhibited seasonal variations for two species of *Uca,* namely *U. spinicarpa* Rathbun, 1900 and *U. longisignalis* Salmon & Atsaides, 1968.

Small holes were often found to disappear after 1 or 2 months perhaps indicating that the occupant died or had moved. This was noticeable after a large recruitment of small Year 1 crabs, particularly around May and June. Many holes were found to be plugged in winter. Winter temperatures at Mgazana drop to 16 °C (Branch & Grindley, 1979), while at 15 °C aerial respiration of *N. meinerti* is extremely low (Emmerson, in prep.), so this could explain the nil growth and plugged holes. Mgazana is a warm temperate estuary close to the southern limit of distribution for *N. meinerti,* which is essentially a tropical species, suggesting that this may have an effect on growth during this time (Fig. 3).

The monthly positions of holes generally did not vary very much, but when they did, it was noted that the hood had changed position, but that the main shaft remained where it had previously been suggesting stability. This differs with the findings of Micheli et al. (1991) for *N. meinerti* burrows in Kenya where they estimated the average burrow life to average 25 days. It could be that this southern population at Mgazana is less active than their tropical counterparts, but it may also be due to the hoods changing position or differences in sediment type. According to Micheli et al. (1991), *N. meinerti* holes are never hooded in Kenya, whereas this is common at Mgazana. Hoods have the effect of increasing the distance between the occupants of adjacent burrows and are associated with high crab population densities (Zucker, 1981; Clayton, 1988). This may be a factor at Mgazana where densities are relatively high and there is strong intra-specific competition for space, compared with other mangrove areas along the south-east African coastline (Steinke et al., 1993). The sediment from Mgazana has a high clay content which would also make the burrows relatively more permanent. Micheli et al. (1991) also noted that most activity was probably confined to the top 20 cm of substrate and that the lower shaft remained intact, as was observed here. The entrances at Mgazana, however, did not collapse as they did in Kenya when dry, indicating that soil type was different which would affect burrow turnover rates.

It was also noted that holes positioned in the drier upper supra-tidal were more stable than ones in the wetter inter-tidal, presumably as the moister mud was easier to dig and shape as well as also being subject to more damage by tidal movement. Holes of older, larger crabs around the 50 mm CW size (L-infinity; Fig. 3) were often found to be subsequently 'resized' and occupied by smaller crabs, presumable following the death of the previous occupant. Burrow usurpation by conspecifics, without the previous occupant dying, is another possibility as this is common in other species such as *Scopimera globosa* (Wada, 1986). This re-occupation of existing holes by other crabs would

represent a saving of energy compared to *de novo* excavation of the holes.

Acknowledgements

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Seasonal abundance and recruitment in an estuarine population of mud crabs, *Scylla paramamosain,* in the Mekong Delta, Vietnam

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Key words: Scylla mangrove fishery

Abstract

Artisanal fisheries for mud crabs are an important source of income in coastal communities of the Mekong Delta. However, populations are subject to pressure from increasing fishing effort to provide seed crabs for pond culture, as well as diminishing mangrove habitats. In the present study, *Scylla paramamosain* was found to be the dominant mud crab species within an estuarine mangal system, representing over 96.8% of mud crab fishery landings between February 1998 and March 1999. *S. olivacea* was also present in relatively low abundance (3.2% of landings). Analysis of catch-per-unit-effort (CPUE) data collected throughout 1997 and 1998, for an intertidal hand-fishery, shows that mud crabs were present in a fairly narrow range of mean abundance throughout the year, despite protracted periods of freshwater conditions during the rainy season. Recruitment of juvenile *S. paramamosain* (3–4 cm carapace width) was continuous through 1998, with a significant peak at the beginning of the dry season. No juvenile *S. olivacea* (< 5 cm carapace width) were found during the study. The mangal appears to provide a nursery habitat for *S. paramamosain,* with predominantly juveniles and sub-adults present, while a higher proportion of adults were recorded in the sub-tidal component of the population. Mature females were present throughout the year, with a peak in September–October 1997. The CPUE analysis provides a baseline of seasonal variation in crab abundance that can be used to monitor the effectiveness of resource management and potential impacts of future changes in habitat.

Introduction

Over the last two decades, exploitation of mud crab populations has increased in many countries in South East Asia (Angell, 1992; Keenan, 1999). In the absence of regulation, fishing activities may target all size-classes, including seed crabs for pond culture, adult and sub-adult crabs for fattening, as well as mature females. Although it is not yet clear to what extent fishing might affect crab abundance, in heavily fished areas, declining crab landings and reductions in maximum sizes have been reported (Angell, 1992) and, in some regions, pond culture is now limited by seed supply, implying intense fishing for juveniles (Keenan, 1999; Fortes, 1999). Furthermore, as the most abundant *Scylla* populations are usually associated with mangroves, especially in estuaries, loss of habitat may also contribute to a decline in crab abundance.

The recent revision of the genus *Scylla* by Keenan et al. (1998), describes four species of mud crab. Previously, the taxonomy of the genus has been confused and the identity of species reported in prior studies is often uncertain. Consequently, very little is known about differences between the species in any aspect of their biology and ecology, despite their widespread distribution across the Indo-Pacific and their importance both in mangal faunal communities and as a fisheries resource. Only where the range of *S. serrata* extends beyond those of the other *Scylla* species, on the east coast of Queensland, Australia and South Africa, can previously studied species be identified from the literature with confidence (Keenan et al., 1998).

The aim of the present study was to use data collected from an artisanal fishery to investigate seasonal variation in abundance and recruitment of mud crab species in an estuarine mangal habitat in the Mekong

Delta. Estimation of abundance of mud crabs can be problematic, as traps have been reported to give underestimates due to bias against juveniles and moulting crabs, as well as trap-saturation and temperature-dependent effects (Williams & Hill, 1982; Robertson, 1989). Instead of direct sampling, we have used catch per unit effort (CPUE) as an indirect measure of abundance. This addresses the need for time-series CPUE data, to develop a baseline for monitoring the effectiveness of resource management and potential impacts of further habitat change on crab populations.

Table 1. Commercial size classes used in recording landings of mud crabs from intertidal and subtidal fisheries

Sex	Class
Female	Mature ovary
Female	>400 g
Male	>400 g
Female	200–400 g
Male	200–400 g
Male/female	<200 g

Study area

In the Mekong Delta, mud crabs are important species in small-scale artisanal fisheries that supply an increasing demand for seed crabs for pond culture. In addition, the majority of mangrove forests were cleared from the 1950s to 1991 (Hong & Hoang, 1993), with continuing rapid losses in some areas. The study area is situated on an island that divides the mouth of the Hau River, a branch of the Mekong River, to form the Tran De and Dinh An estuaries (Fig. 1). A belt of about 1000 ha of managed mangal, predominantly comprising *Sonneratia* and *Avicennia* spp., covers the seaward-facing SE shore of the island, with a tidal mud flat of 7000–10 000 ha. The mangal habitat is subject to strong seasonal variation in salinity, due to high freshwater flow during the rainy season. Several methods are used to fish for mud crabs on the island, including intertidal collection by hand and use of gill nets or small seine nets over the mud flats. From 1995 to 1999, one licence-holder controlled all fishing activities within the mangal and subcontracted rights to a group of 20–30 intertidal hand collectors, keeping detailed daily records of crabs collected by each individual.

Materials and methods

Salinity

Samples were taken from both surface and bottom water, at a station 100 m from the mouth of a creek opening on the Tran De estuary (see Fig. 1). Samples were collected at both high and low water on all new-moon spring tides from December 1997 to December 1998. Salinity was measured with a hand-held refractometer. During the monsoon season, samples were also taken from crab burrows at low tide, on the fringe of the mangal on the SE shore of the study area.

Species present

Crabs collected within the study area by hand-fishers were sampled over a period of 13 months from February 1998 to March 1999. Between 100 and 400 crabs were sampled each month. Species were identified following Keenan et al. (1998) and sex and carapace width (CW), including lateral spines, recorded in 1 cm classes.

Historical data

Historical records of crab landings from 1995 to 1998 were provided by the fishery licence-holder. These comprised data for daily catches of crabs collected by individual fishers in the intertidal mangrove, using metal hooks to collect crabs from burrows. This form of fishing is non-selective in terms of crab size, as all crabs encountered are collected. The collectors travel as a group to the island on one boat, so that they all spend the same amount of time fishing on a given day. As the fishing methods were standardised, data for hand-collected crabs was expressed as catch-per-unit effort (CPUE, kg person^{-1} d^{-1}). Only the most complete annual data sets (1997–1998) were selected for analysis. In addition, a separate analysis was carried out for juvenile crabs of 3–4 cm carapace width, which are collected by hand on the fringe of the mangrove forest at night. In this case, data were only recorded during 1998 and CPUE was calculated as numbers of juveniles collected person^{-1} d^{-1}. To examine seasonal trends, for each data set CPUE values were pooled by month and median CPUE values ranked by a Kruskall-Wallis test.

Figure 1. Location of study area in the Mekong Delta, Vietnam. Inset map shows the location on the study site between the Tran De and Dinh An estuaries (■ = mangrove study area).

Outside the intertidal study area, a variety of fishing gear is employed over the mud flats at high tide, including gill nets and hand-pulled seine nets. For both intertidal (mangrove) and subtidal (mudflat) fishing activities, total monthly landings are recorded in commercial size-classes, as shown in Table 1. Mature females, as determined by lifting the posterior edge of the carapace to examine the fullness and colour of the ovary, are recorded separately.

Results

Mean salinity for surface and bottom samples, at high and low tides, ranged from 21±1.9 ppt in April 1998 down to 0 ppt during the months of peak freshwater flow (Fig. 2). During this period, freshwater conditions exist at all water depths within the forest at high tide and within crab burrows at low tide.

Two species of mud crab were identified in the samples collected in the intertidal mangrove area. A total of 2696 *S. paramamosain* and 89 *S. olivacea* were examined, representing 96.8% and 3.2% of the total sample, respectively. Size-frequency histograms for male and female *S. paramamosain* are shown in Figure 3. *S. paramamosain* ranged in size from 3 to 14 cm (CW), though few crabs larger than 10 cm CW were recorded. *S. olivacea* sampled had a similar upper limit in size distribution, but no juveniles

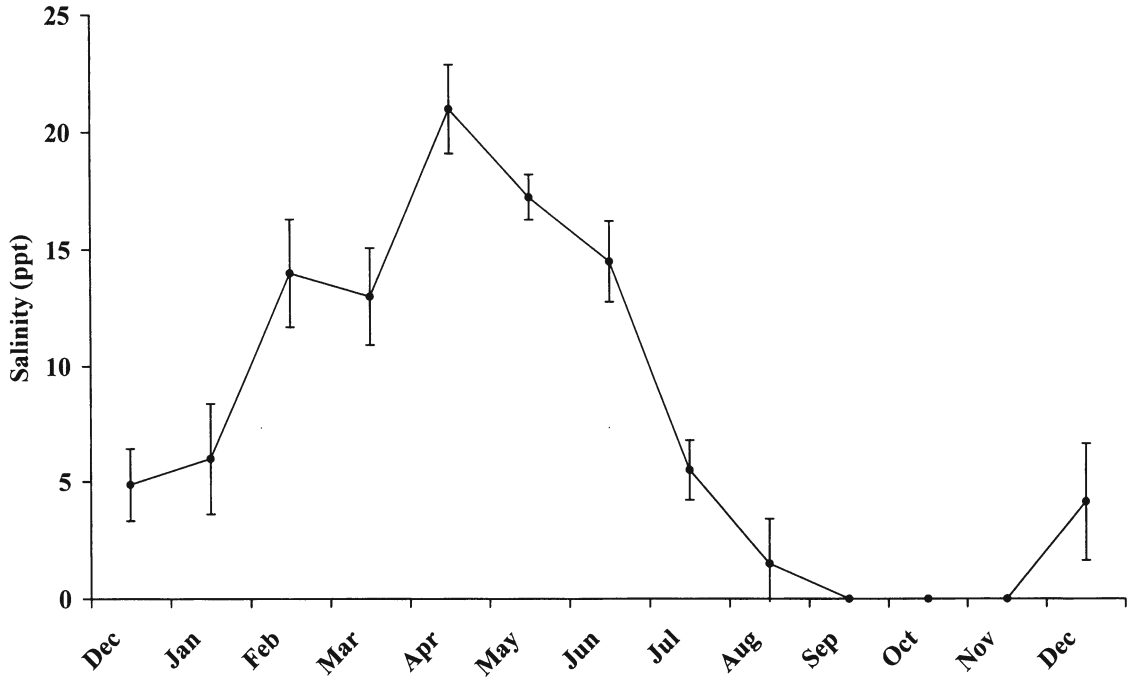

Figure 2. Seasonal variation in salinity (ppt) measured on the fringe at the western edge of the mangrove study site during 1997–1998. Each value is mean (± sd) of measurements taken at high and low tide, from the surface and bottom (n = 4-8 for each value).

of less than 5 cm CW were observed for this species. In both species, females were found to be in the puberty moult from 9 to 10 cm CW. This indicates that mud crabs collected intertidally are predominantly *S. paramamosain* sub-adults or juveniles. There was no clear modal progression in the size-frequency data for this species, with the modes for both sexes remaining between 4 and 7 cm CW throughout the sampling period (Fig. 3). This indicates year-round recruitment of *S. paramamosain* of 3–4 cm CW and continuous mortality or emigration.

Combined landings of both species of mud crab from the mangrove hand-fishery during 1997 were highest during the dry season, reaching a maximum of 900 kg month^{-1} in May (Fig. 4). However, this trend partly reflected increased fishing effort, as the range of mean monthly CPUE was fairly narrow between 1.6 and 3.1 kg person^{-1} d^{-1} (Table 2). However, there was significant variation in CPUE through the year (H=264.0, p<0.001), characterised by high median values for the dry season (February–May) and low values in the early part of the rainy season (June–August). However, median CPUE values returned to close to the overall median in September–December, despite this being a period of very low salinity.

Table 2. Catch per unit effort analysis for mud crabs (predominantly *S. paramamosain*) in the intertidal hand-fishery during 1997. Mean and median values are expressed as CPUE (kg person^{-1} d^{-1}), N = number of unit fishing days each month. Average rankings and z-scores show output from Kruskall–Wallis test (H = 264.00, DF = 11, P < 0.001 adjusted for ties)

	N	Mean ± se	Median	Average ranking	Z
January	77	2.08±0.14	1.95	1333.3	0.02
February	186	3.13±0.10	2.80	1901.6	10.48
March	310	2.53±0.11	2.10	1468.7	3.33
April	349	2.36±0.07	2.30	1490.6	4.13
May	418	2.24±0.06	2.20	1416.9	2.46
June	170	1.56±0.06	1.40	1018.5	−5.49
July	247	1.57±0.10	1.20	927.2	−8.69
August	249	1.65±0.07	1.40	1053.5	−6.00
September	128	1.92±0.09	1.92	1270.2	−0.93
October	138	2.07±0.09	1.90	1358.4	0.41
November	157	2.09±0.10	1.90	1349.1	0.29
December	234	2.02±0.09	1.80	1267.7	−1.34
Overall	2663	2.13±0.03	1.90	1332.0	

Comparison of landings from the mangrove hand-fishery and from the estuary outside the study area, mostly caught subtidally using nets over the mud flat,

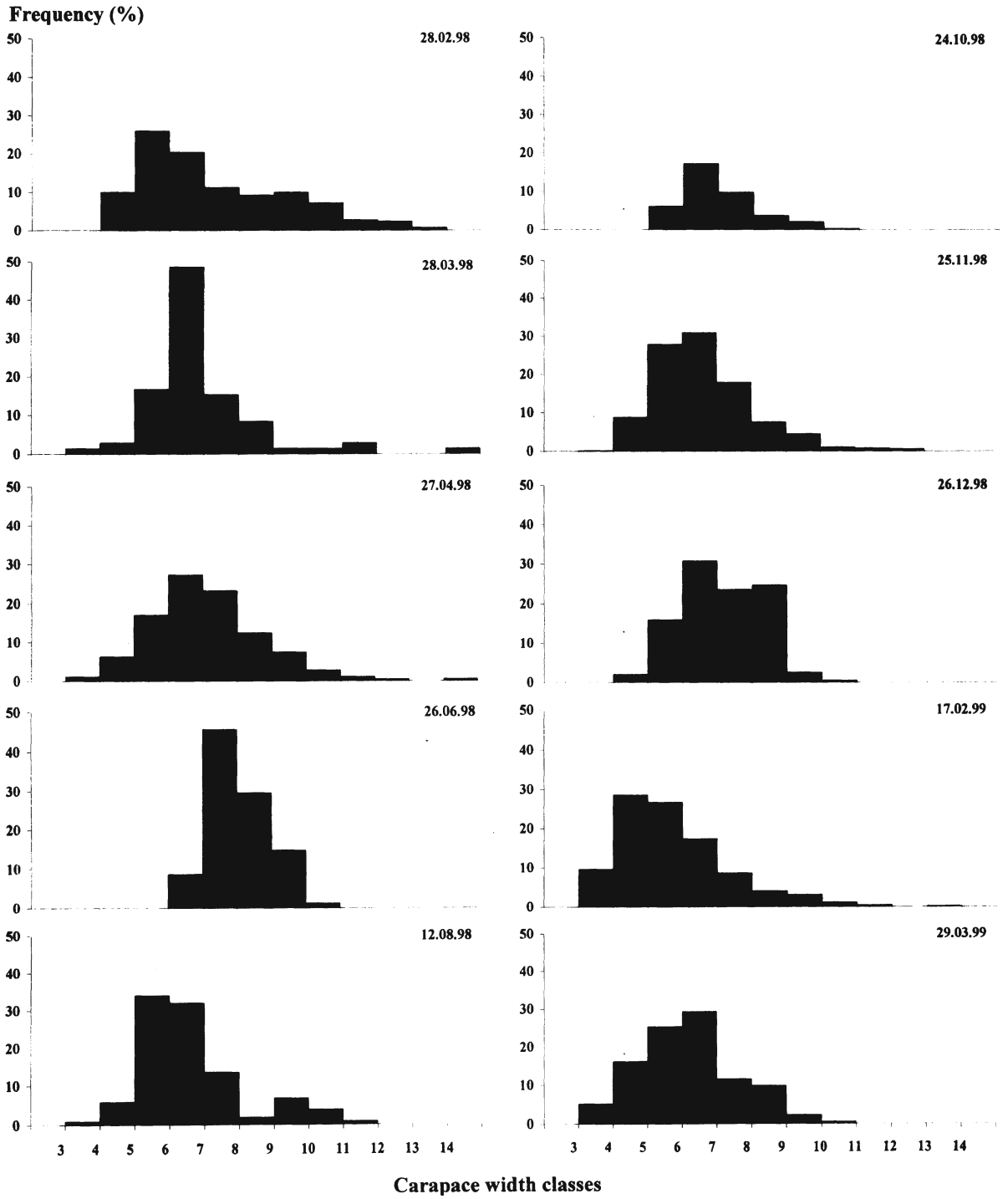

Figure 3. Size-frequency distributions for samples of *S. paramamosain* examined from intertidal hand-fishery landings during 1997–1999. Pooled males and females. N = 100–400 for each date.

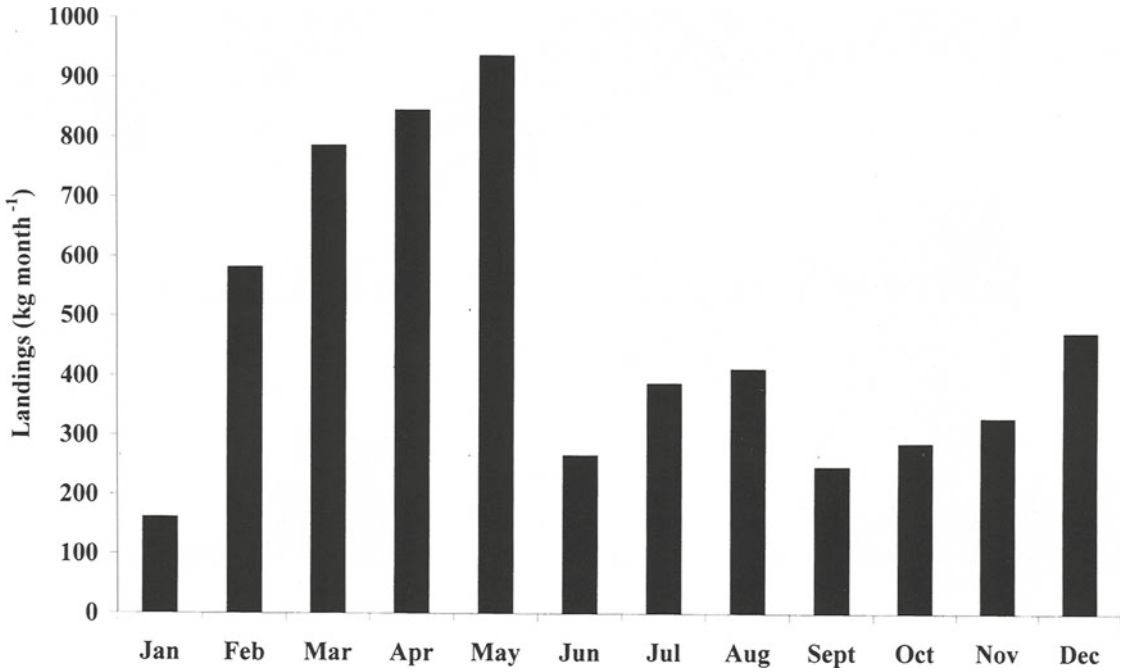

Figure 4. Total landings of mud crabs, predominantly S. *paramamosain,* in the intertidal hand-fishery during 1997.

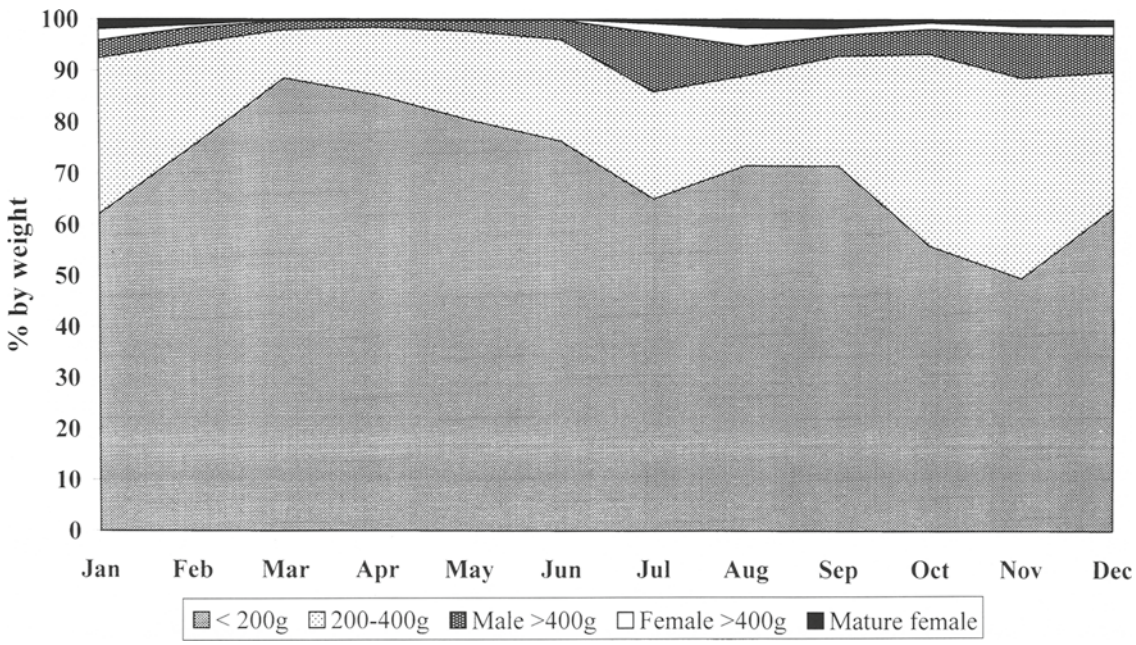

Figure 5. Commercial size-classes (% of landings) for mud crabs, predominantly *S. paramamosain,* collected at low tide in the intertidal mangrove study area during 1997.

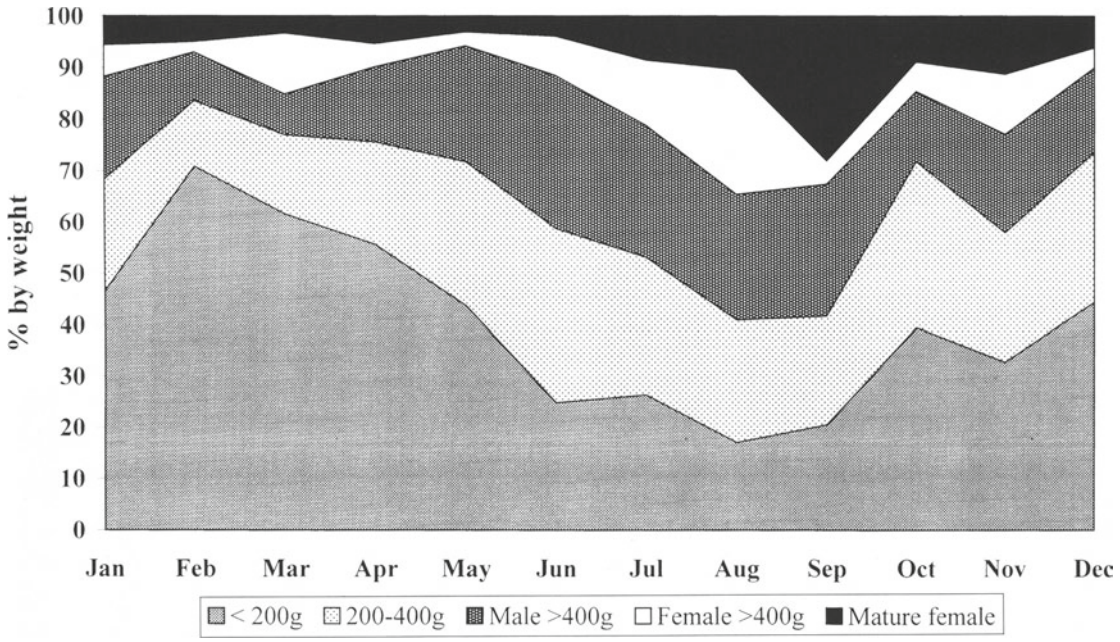

Figure 6. Commercial size classes (% of landings by weight) for mud crabs, predominantly *S. paramamosain,* fished at high tide over the mud flats adjacent to the mangrove study area during 1997.

indicates differences in crab size. As seen in the size-frequency data, juvenile crabs (<200 g) predominate in the intertidal catches, representing 50–89% of recorded landings (Fig. 5), while relatively few large adult crabs (>400 g) were collected (1.6–11.3%). In contrast, Figure 6 shows the composition of catches outside the study area. Adult crabs (>200 g) represented between 29.2 and 82.9% of landings by weight through the year, including a greater proportion of large crabs (>400 g), ranging between 16.4 and 58.3% of total landings. However, there is an indication of greater seasonality than in the intertidal area, with the greatest proportion of adult crabs recorded during the monsoon season (June–September), while juvenile and sub-adult crabs (<200 g) were predominant in January–March. Some seasonality in reproduction is indicated by an increased proportion of mature females recorded in the landing data, with a strong peak in September, when they represented 28% of landings by weight.

During March–December 1998 (no data was available for January–February), mean monthly CPUE for night collection of small juvenile crabs ranged between 12.5 and 41.4 crabs person^{-1} d^{-1} (Table 3). As indicated by the results of size-frequency sampling during 1998, crabs of less than 5 cm CW were all *S. paramamosain*. There was significant variability in monthly median CPUE values (H=707.3, $p<0.001$), reflecting a significant peak in abundance in August–September followed by a decline towards the end of the rainy season in November–December.

Table 3. Catch-per-unit effort analysis for juvenile *S. paramamosain* (3–4 cm CW) collected on the fringe of the mangal study area during 1998. Mean and median values are CPUE (crabs person^{-1} night^{-1}), N = number of unit fishing nights each month. Average rankings and z-scores show output from Kruskall–Wallis test (H = 707.3, DF = 9, $P < 0.001$ adjusted for ties)

	N	Mean ± se	Median	Average ranking	Z
March	59	26.9±2.18	25.0	2465.5	3.71
April	152	12.6±0.96	10.0	1256.1	−7.62
May	334	19.5±0.64	17.0	1991.8	1.04
June	323	15.8±0.61	14.0	1636.6	−4.96
July	138	12.7±0.74	12.0	1353.1	−6.20
August	326	41.4±1.49	36.0	2902.4	16.44
September	462	32.2±1.01	28.0	2624.6	14.25
October	901	19.8±0.45	17.0	1961.9	0.95
November	840	15.9±0.39	13.0	1636.7	−8.65
December	326	12.5±0.44	11.0	1341.7	−9.98
Overall	3861	20.9±0.28		1931.0	

Discussion

The observed seasonal variation in salinity is consistent with previous studies in the Dinh An estuary by Wolanksi et al. (1996), who showed that during the monsoon season the river mouth is stratified, with freshwater to a depth of 5 m. As the floor of the estuary in front of the study area is very gently sloping, with charted water depth of 1 m or less extending for at least 1 km offshore and with mean spring high tides of 3.2 m, persistent freshwater conditions are to be expected in the mangal through all tidal cycles at that time of year. Keenan et al. (1998) suggested that the distributions of the four *Scylla* species may be determined by their salinity tolerances, either at the larval or post-settlement stages. They point out that the most widely distributed species, *Scylla serrata,* is dominant in oceanic environments where salinity is greater than 34 ppt. The other species are distributed in seas where salinity is generally below 33 ppt and in mangal and estuarine habitats in which periods of low salinity occur seasonally. This is consistent with the present study, in which *S. paramamosain* was found to be the predominant mud crab species in an estuarine habitat with marked seasonal variation in salinity, including prolonged freshwater conditions.

Despite the large seasonal salinity variation, the CPUE data indicates persistence of populations and continuous recruitment of juvenile *S. paramamosain*, through periods of extreme low salinity. This is not typical for mud crab populations studied elsewhere, possibly reflecting species differences. Chandrasekaran & Natarajan (1994) followed the seasonal abundance of juvenile *Scylla* (species now uncertain) in Southeast India, in a mangrove-lagoon system subject to similar seasonal salinity variation. Using cast-net sampling, they found that juveniles were absent in October, when the salinity dropped to 1.5–2 ppt. Hill (1979) reported that juvenile *S. serrata* in South Africa could survive at salinities as low as 2 ppt. Although adults could survive freshwater conditions for several months, episodes of freshwater flooding were observed to cause mass mortalities (Hill, 1975). Davenport & Wong (1987) showed that adult *Scylla* (species now uncertain) from Penang, Malaysia were very effective osmoregulators at low salinities and could survive freshwater conditions for several hours under laboratory conditions. This is consistent with the persistence of *Scylla paramamosain* populations during the rainy season observed in the present study.

The difference in size-composition of crabs collected in the mangal and the estuary, and the continuous loss of large crabs from the intertidal population, indicates that the mangal is acting as a nursery area, with larger crabs living more subtidally. A similar size distribution with depth has been reported in *S. serrata* in Australia by Hill et al. (1982). However, it is also possible that fishing mortality is greater in the more accessible intertidal population. The CPUE analysis for small juvenile crabs (3–4 cm CW) during 1998 indicates a peak in abundance at the beginning of the monsoon season, in August–September, when salinity has already dropped to close to zero. These animals are likely to be about 2–3 months post-settlement (Ong, 1966), indicating a period of high recruitment during the period of higher salinity in April–May, with a significant decline during the rainy season resulting in significantly lower abundance of juveniles in November–December. However, the overall relatively narrow range of mean and median monthly CPUE values for juveniles through the year and the lack of modal progression in the size-frequency data, together indicate that recruitment is continuous through the wet and dry seasons. This is consistent with the composition of other mud crab populations in tropical regions (Quinn & Kojis, 1987; Poovachiranon, 1992), as is the presence of mature females throughout the year with seasonal peaks associated with the rainy season (Arriola, 1940; Quinn & Kojis, 1987; Poovachiranon, 1992; Kathirvel & Srinivasagam, 1992; Macintosh et al., 1991).

Conclusions

The consistent, non-selective, hand-fishing methods used in the intertidal study area and the availability of large data sets for each month provide a good baseline of relative crab abundance throughout the year, against which future changes in crab populations can be monitored. As the mangal is acting as a nursery area, intertidal CPUE data should be a particularly sensitive measure of long-term variation in recruitment to the estuary. The narrow range in relative abundance of adult and juvenile *S. paramamosain* despite long periods of freshwater inundation confirms the adaptation of this species to highly variable estuarine conditions. Although there appears to have been a peak in recruitment during the dry season, recruitment continued during periods of very low salinity. It is unlikely that the CPUE data presented here will be

directly comparable to values from other fisheries, due to the potential variation in fishing effort and gear used (Angell, 1992). However, in trap fisheries, a CPUE approach to estimating seasonal and longer-term variation in relative crab abundance may also be possible, but will require quantification of trap saturation and temperature effects (Robertson, 1989). In the present study area, a priority for future work will be to estimate crab population density using a combination of mark-recapture and trap sampling, which should allow comparison of CPUE values to actual abundance.

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Major claws make male fiddler crabs more conspicuous to visual predators: a test using human observers

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Abstract

One of the possible costs of the male fiddler crabs enlarged claw can be conspicuousness to predators. This hypothesis was tested using human observers as a model of visual predators. In the European fiddler crab, *Uca tangeri* Eydoux, the males' major claw is white contrasting with the orange-brownish colour of the carapace and of the feeding claw, and the mudflat background. The following morphotypes were created from close-up photographs taken in nature using an image processing software: male, male without claw, female, female with enlarged claw, male with enlarged claw of the same colour of the feeding claw, male with 75% sized claw, male with 50% sized claw. These morphotypes were then presented in a randomised order to students, using a psychology test software, which allows the measurement of response time in msec. The subjects were allowed to look at the images for an unlimited amount of time, until they detected the individual or until they decided to pass on to another image. Backgrounds (i.e. mudflat picture) without individuals were also presented as a control. Male crabs were detected significantly sooner than females. When we compared males with the claw removed with females with an enlarged claw added, the pattern is reversed and the latter are detected significantly faster. Thus, the enlarged claw seems to be the key feature that makes the individuals more conspicuous. Size and colour seem to be the main aspects of the claw's conspicuousness. The data of these experiments support the initial prediction of males being more conspicuous than females because of their enlarged claw. The possible costs and benefits of this trait, related to predation, are discussed.

Introduction

Advertising traits are assumed to be costly to produce or maintain in order to be considered 'honest' signals. Hypothesised costs include the energetic demands of trait development, maintenance and production (e.g. Vehrencamp et al., 1989), attraction of parasites (e.g. Cade, 1975), higher risk of mortality due to violent fights (Clinton & Le Boeuf, 1993), among others. By far, the most frequently demonstrated cost related to traits that enhance male's attractiveness to females is conspicuousness to predators. In the guppy (*Poecilia reticulata*), for example, more colourful males are preferred by females (Houde, 1997) but are also more susceptible to predators (Endler, 1987). Populations where predator density is high are composed of much duller males than populations where predation risk is low (Endler, *op. cit.*). Similar situations are reported for other species (for a review see Andersson, 1994).

Fiddler crabs are well known for their high degree of sexual dimorphism (Crane, 1975). Females have two isomorphic feeding claws, whereas males possess one feeding claw and one highly enlarged claw reaching up to 40% of the individual's total body weight (Rosenberg, 1997). In the European fiddler crab *U. tangeri*, the major claw of the males is used both in fighting and threatening displays (intra-sexual selection) and in a waving display that attracts females to the male's burrow for mating (inter-sexual selection). Thus, in this species, the major claw plays a dual role of an armament and an ornament.

Larger claws are, generally, preferred by females. Experimental data by Oliveira & Custódio (1998) shows that female *U. tangeri* spend more time near

models of males with larger claws in binary choice tests. This female preference is confirmed by field observational data which shows that females visit males with larger claws more often (Latruffe et al., 1999). There is also observational evidence of this fact for other species. Backwell & Passmore (1996) showed that females of the species *Uca annulipes* mate with males with claw size larger than the population average. Greenspan (1980) demonstrated a significant association between the rate of attracting females and male major claw length for *Uca rapax.* In *Uca pugilator*, Christy (1983) observed that males with larger claws had a mating advantage that, however, could be on the account of the fact that larger males built better burrows, and Hyatt (1977) also found a female preference for larger males. On the other hand, no preference for males with larger claws was found in *Uca vocans vocans* (Salmon, 1984) and *Uca beebei* (Christy, 1987). Males with larger claws also have an advantage in male-male competition (Crane, 1967, 1975).

Apart from the benefits of mate attraction and male–male competition, larger claws also involve a cost for the male. The male's enlarged claw is not used in feeding and thus, feeding in males is restricted to one minor claw. Although, different species have different mechanisms to cope with this disadvantage. *Uca panacea* (Caravello & Cameron, 1987) and *U. vocans vocans* (Murai et al., 1983) males spend the same amount of time as females in feeding activities but the rate of the feeding claw's movement is significantly higher. In both *Uca pugnax* (Valiela et al., 1974) and *U. tangeri* (Faria, 1994), males spend more time feeding than females.

Another potential cost of the male fiddler crab's enlarged claw is its apparent conspicuousness due to its size and colour, contrasting with the rest of their orange-brownish coloured body and the mudflat background, suggesting that males may be more easily detected by predators than females.

In the present study, we intend to (a) test the hypothesis, using humans as a model of visual predators, that the enlarged claws of male fiddler crabs makes them more conspicuous to visual predators and, if so, (b) whether its effect depends on size or colour of the enlarged claw, and then, (c) discuss the costs and possible benefits of this trait in relation to predation.

Methods

Several morphotypes, all of the same size, of the fiddler crab *U. tangeri* were created from close-up photographs taken in nature (Parque Natural da Ria Formosa, Algarve, Portugal) using the image-processing software Adobe Photoshop 4.0: a right-handed male (M) with the major claw in the resting (horizontal) position and an identical male with no major claw (MNC); a female (F) with two feeding claws and an identical female with an enlarged claw (identical to M's) added (FWC); a male identical to M with the major claw of the same colour (orange-brownish) of the feeding claw (MOC); and two males, identical to M, but with smaller claws (M75–75% sized claw, and M50–50% sized claw).

Using the software SuperLab 1.04, these morphotypes were pasted in a random position on a background picture of their natural habitat (i.e. mudflat) and were presented in a randomised order to students, who were asked to detect them. The average age of the students was 24 years old; 18 males and 71 females, a sex-bias that reflects the student's population registered in that year at the Instituto Superior de Psicologia Aplicada (Lisbon): 82% females and 18% males. We looked for possible differences in detection abilities between female and male students, which could bias our results, using a two-way ANOVA, but we found none ($F_{1,6}$=2.07; P=0.163). The subjects were allowed to look at the images for an unlimited amount of time, until they detected the individual or until they decided to pass on to another image (non-detection). Backgrounds without individuals were also presented as a control. Response time was registered in msec by the psychology test software SuperLab 1.04.

The following *a priori* planned comparisons were made: (a) to test for the importance of possessing a claw, we compared M with F and FWC with MNC; (b) to test for claw colour, we compared M with MOC; (c) to test for claw size, we compared M with M75 and M50, and M75 with M50; (d) to test for the importance of claw colour versus claw size, we compared MOC with M75 and M50. To avoid the assumptions of parametric statistics we used the non-parametric Wilcoxon matched pairs test. Since there is a different number of non-detections for each morphotype, the N value of the matched pairs differs for each comparison.

To keep the joint level of significance at the desired value (0.05), the level of significance of each individual test was reduced using the Dunn–Bonferroni procedure. Individual levels of significance for each

Table 1. Differences between percentages of non-detections for all morphotypes

Comparison	Percentage of non-detections		p
M vs. F	M=2.25	F=17.05	< 0.001 *
FWC vs. MNC	FWC=2.25	MWC=39.33	< 0.001 *
M vs. MOC	M=2.25	MOC=28.09	< 0.001 *
M vs. M75	M=2.25	M75=3.41	0.322
M vs. M50	M=2.25	M50=6.74	0.075
M75 vs. M50	M75=3.41	M50=6.74	0.157
MOC vs. M75	MOC=28.09	M75=3.41	< 0.001 *
MOC vs. M50	MOC=28.09	M50=6.74	< 0.001 *

* Indicates significant differences.

comparison are as follows: M vs. F, p=0.010; FWC vs. MNC, p=0.025; M vs. MOC, p=0.007; M vs. M75, p=0.007; M vs. M50, p=0.007; M75 vs. M50, p=0.008; MOC vs. M75, p=0.008; MOC vs. M50, p=0.008.

The occurrence of order effects was controlled *a posteriori* by testing the order of the presented *stimuli* (Wilcoxon matched pairs test). No significant differences were found.

Comparisons of the percentage of non-detections of each morphotype were also used to assess the claw's conspicuousness using a significance test for differences between two percentages (Statistica for Windows 5.0).

Results

Presence of major claw

Male fiddler crabs of *U. tangeri* (M) were detected significantly sooner than females (F), (Wilcoxon matched pairs test; N=71, Z=3.770, P<0.001). When we compared males with the claw removed (MNC) with females with an enlarged claw added (FWC), the pattern was reversed and the latter were detected significantly sooner (Wilcoxon matched pairs test; N=53, Z=5.838, P<0.001), (see Fig. 1).

Colour

The subjects took significantly more time to detect males with the enlarged claw artificially coloured with the same colour as the feeding claw (MOC) when compared to males with claw of its natural colour (M), (Wilcoxon matched pairs test; N=62, Z=5.809, P<0.001), (see Fig. 2).

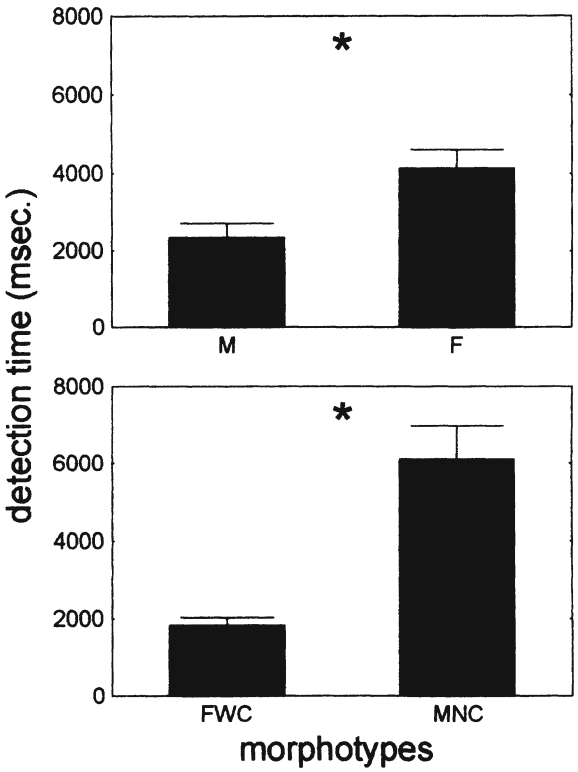

Figure 1. Detection time of morphotypes with major claw (M and FWC) and without major claw (F and MNC). Values are means±se (msec). * indicates significant differences.

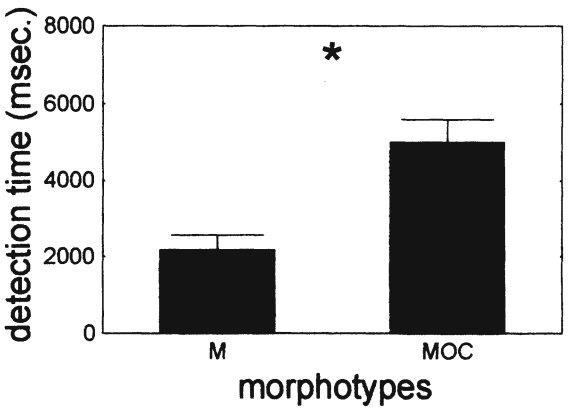

Figure 2. Detection time of morphotypes with chela of the same colour as feeding claw (MOC) and with natural coloured chela (M). Values are means±se (msec). * indicates significant differences.

Size

Crabs with smaller claws took longer to be detected but there were only significant differences when the relative size difference is of 50% (M vs. M50), (Wilcoxon matched pairs test; M vs. M75: N=83, Z=1.689,

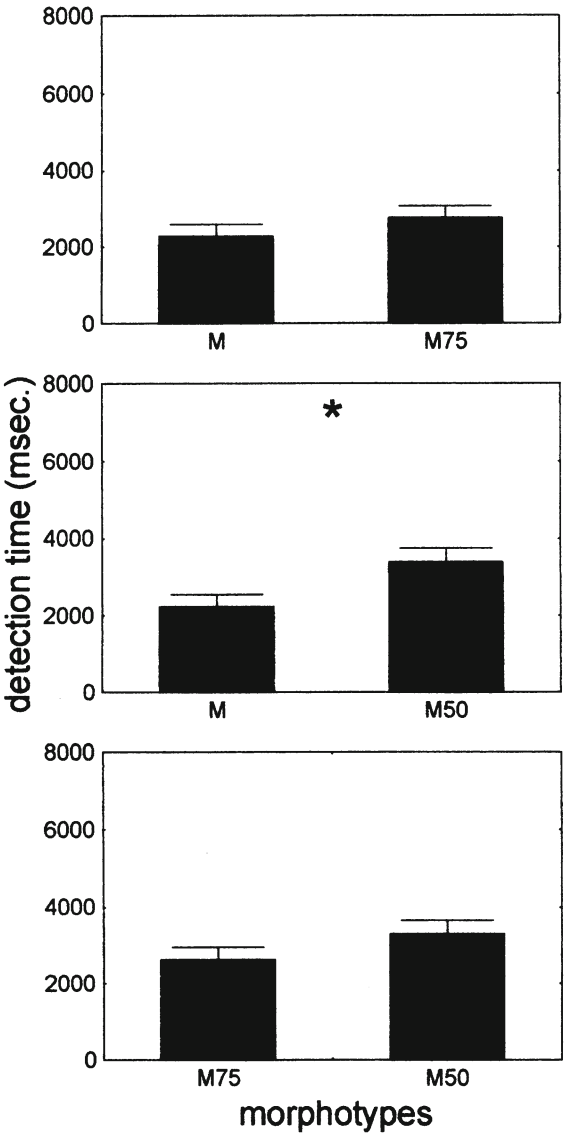

Figure 3. Detection time of morphotypes with different sized claws. Values are means±se (msec). ∗ indicates significant differences.

P=0.091; M vs. M50: N=82, Z=4.052, P<0.001; M75 vs. M50: N=79, Z=2.353, PN=0.019) (see Fig. 3).

Colour vs. size

To assess the relative importance of claw colour and size for its conspicuousness, we compared the male with the coloured claw (MOC) with males with smaller claws of their natural colour (M75 and M50). Males with natural coloured claw were still detected significantly sooner than males with a claw of the same colour of the feeding claw, even when it is 50% smaller (Wilcoxon matched pairs test; MOC vs. M75: N=61, Z=4.593, P<0.00). As relative claw size increases, differences continue to be significant (Wilcoxon matched pairs test; MOC vs. M50: N=60, Z=3.147, P=0.002), (see Fig. 4).

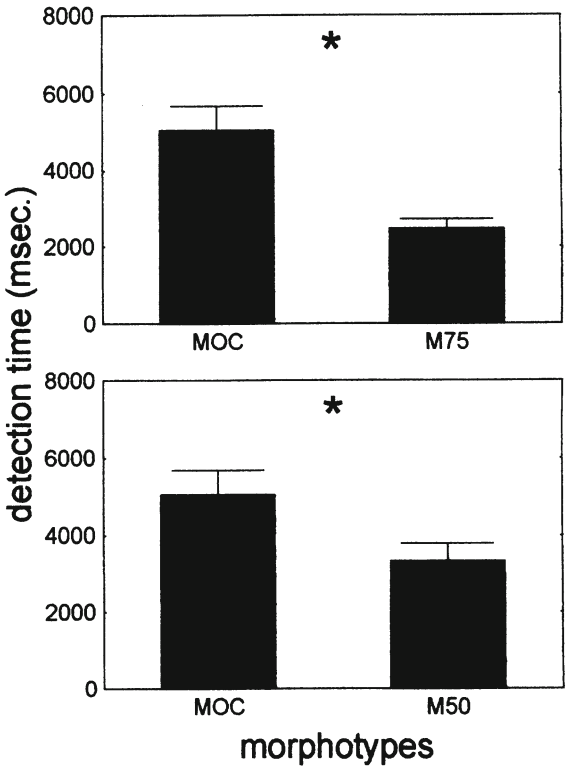

Figure 4. Detection time of morphotypes with chela of the same colour as feeding claw (MOC) and natural coloured claws of smaller sizes (M75 and M50). Values are means±se (msec). ∗ indicates significant differences.

Non-detections

Percentages of non-detections (as shown in Table 1) are consistent with the previous results. Individuals with no major claw (F and MNC) had a significantly higher percentage of non-detections than crabs with major claw (M and FWC, respectively), and crabs with the major claw of the same colour as the feeding claw were significantly less detected compared to crabs with major claw of its natural colour, independently of its size. However, there were no significant differences in the percentage of non-detected crabs with different claw size, even when the relative size difference was of 50%.

Discussion

Our data suggests that, as hypothesised, the enlarged claw of male fiddler crabs is a very conspicuous trait. Males with larger claws had a non-significant tendency to be detected sooner when relative claw size difference was of 25% and there was a significant difference when the claw was 50% larger. However, claw colour seems to be the main aspect that makes males easier to detect. Males with an orange-brownish coloured claw were detected significantly slower compared to males possessing a white major claw, even when it was 50% smaller.

An evolution towards cryptic coloration in fiddler crabs is suggested by the fact that chromatophores of five species of the genus *Uca* (*U. subcylindrica, U. panacea, U. spinicarpa, U. longisignalis* and *U. rapax*) exhibit dispersion patterns indicating statistically significant background adaptation (Thurman, 1990). Although, despite the fact that many species of fiddler crabs are cryptically coloured when the animals are feeding or constructing burrows, when social activities become more frequent the large claw, and sometimes even the carapace and other appendages, become brighter in some species (Weygoldt, 1977). This is not an intriguing fact, given the social signalling function of the major claw.

From an evolutionary perspective, a signal is constrained by the properties of the sensory system of the receivers (Endler, 1992). Thus, the bright colour of the male's major claw should be the result of a selection pressure imposed both by conspecifics and predators. Waving the major claw is the main mechanism of visual communication among fiddler crabs and a conspicuous claw could make it more effective.

However, from a conspecific point of view, the background for a waving claw is the sky, not the mudflat (like we used in this experiment), unless the male is displaying in an area with a steep slope (e.g. creek) or against salt marsh vegetation background. So our results may not apply for a flat environment, and the claw-background contrast is yet to be evaluated in those circumstances. Also, the eyes of fiddler crabs appear to be especially well designed to resolve objects in the vertical plane. Zeil et al. (1986) have shown that two species of *Uca* have narrow vertical corneal pseudopupils on each eye equator, yelding an acute zone of high vertical resolving power. They also use their visual horizon to distinguish between predators and conspecifics. If an object's image does not extend above the horizon, it is smaller than the receiver which perceives it as a conspecific. If an object image extends above the horizon it is perceived as a predator (Zeil et al., 1986; Land & Layne, 1995; Layne et al., 1997). When a male waves its claw it does intrude into the space above the horizon, but not very high. This may capture the attention of another fiddler crab but not evoke a full escape response (Layne et al., 1997). Thus, size more than colour, seems to be main feature involved in signalling to conspecifics. Nevertheless, colour discrimination has been described for two species of fiddler crabs (Hyatt, 1975), but only one pigment has been found in the rhabdoms using microspectophotometry (Scott & Mote, 1974). This apparent contradiction can be solved by the finding that in another brachyuran crab, *Scylla serrata*, colour discrimination occurs with a single visual pigment due to the existence of different colour filters in different photoreceptors (Leggett, 1979). In *Uca*, photoreceptors screening pigments have also been described (Cronin & Forward, 1988) which may account for the behavioural evidence for colour discrimination. An alternative causal explanation for colour discrimination in *Uca* would be the existence of a violet/UV receptor in the smaller 8th retinular cell, like the one described for crayfish and other brachyuran crabs (Cummins & Goldsmith, 1981; Martin & Mote, 1982). Despite which is the causal mechanism underlying colour discrimination in fiddler crabs, colour could amplify the effect of size on conspecific detection of the male enlarged claw.

From the predators point of view, our results suggest that colour would be the key feature that makes the claw conspicuous. But we should be conservative when applying our results to natural predators of fiddler crabs since colour is not property of an object, but the result of the nervous system that perceives it (Bennett et al., 1994), and human vision is different from other organism's. The main natural predators of *U. tangeri* African populations are sea birds (Ens et al., 1993). The only described predator in Ria Formosa was the ashy curlew *Numenius arquata* (Von Hagen, 1962) but indirect evidence of predation by seagulls (*Larus* spp.), whimbrels (*Numenius phaeopus*), turnstone (*Arenaraia interpres*) and rats also exists (Von Hagen, *op. cit.;* Faria, 1994; pers. obs.).

To what extent the results present here are relevant for the natural predators is a major issue.

Humans have three colour channels associated with different receptors that absorb maximal light at different wave lengths. This way, accordingly to which receptor is being stimulated, there are three primary

colour sensations: blue, green and red. If all cones are stimulated at the same time, the sensation of white is produced (Bennett et al., 1994; Finger & Burkhardt, 1994). Most birds, except maybe some nocturnal species (Bennett & Cuthill, 1994), possess another cone which absorbs UV light, thus producing another primary colour, and a system of oil droplets that filter the light entering individual cones (Bennett et al., 1994; Finger & Burkhardt, 1994). Thus, most probably, fiddler crab colours would look different to humans and to sea birds. However, the efficiency of a visual signal depends critically on the difference in radiance between the object and the background in a range of wavelengths (Dusenbery, 1992). Thus, contrast more than colour is the more relevant feature for increasing detectability. The fact that neither the white colour present in the claw nor the mudflat reflect UV (M.C. Cummings & R. F. Oliveira, unpublished data) suggests that at least brightness contrast (for the measurement of brightness contrast see Bradbury & Vehrencamp, 1998) between the white claw and the mudflat background could be equally perceived both by humans and by birds, and thus the present result could be extrapolated, with caution, to natural predators. Nevertheless, further experimental studies with conditioned birds in captivity are being planned to test the present hypothesis with a closer model to the natural predators.

Although, using humans as models for visual predators was not inappropriate since the main fiddler crab predators in the Algarve population are fishermen that remove the male's major claw, used in local gastronomy. The males are then released again in the mudflat to regenerate their claws (see Oliveira et al., 2000). In this particular population there is a sex-biased 'predation' and the conspicuousness of the claw is, indeed, a cost for male *U. tangeri*.

In spite of the possible disadvantage of making males easier to detect by visual predators, it must be taken into account that the enlarged claw is also a powerful weapon used in defence and might function as a deterrent for predators. Formanowicz & Brodie (1988) reported that only males adopt a threatening behaviour in the presence of a potential predator. This advantage may compensate for the conspicuousness cost imposed by the claw. In fact, Bildstein et al. (1989) demonstrated that when given a choice between intact males and females or between intact males and males with the major claw removed to a natural predator, white ibis (*Eudocimus albus*), they chose the individuals without the major claw. When they chose the male with the major claw, on many occasions, the crab grasped the ibis bill. Situations like this result on a much longer handling time for the predator, risk of injury and risk of loosing its prey in case the crab autotomizes its claw.

If the major claw makes males such unprofitable prey, then it would be expected that they would be less exposed to predation pressure in the field than females, and be passed over by predators. Despite that, Backwell et al. (1998) did not find a selective predation pressure in either sex for *U. stenodactylus* and *U. princeps*. We suggest that the benefit of easy detection of male fiddler crabs in the mudflat may compensate for the costs involved in handling the prey. This way, the conspicuousness of the claw may not increase the risk of predation of males compared to females, but it may be sufficient to overweight the benefit of its anti-predator function.

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Feeding activity of *Callinectes ornatus* Ordway, 1863 and *Callinectes danae* Smith, 1869 (Crustacea, Brachyura, Portunidae) in Ubatuba, SP, Brazil

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Key words: Portunidae, *Callinectes*, diel rhythms, feeding activity, swimming crabs

Abstract

The feeding activity along the day cycle and the time consumed for extracellular digestion were evaluated in the portunids *C. ornatus* and *C. danae*. Swimming crabs were obtained from trawling in Ubatuba bay, São Paulo, Brazil, during both the rainy and dry seasons. In each season, daily scheduled samples were taken at dawn (±6 h), noon (±12 h), dusk (±18 h) and midnight (±24 h). All individuals were dissected and the degree of stomach replenishment was recorded. In order to estimate the time elapsed for extracellular digestion, crabs were fed, and groups were dissected at 30 min intervals to check the conditions of their stomachs. In general, both species show a higher feeding activity during periods of lower light intensity, as evidenced by an increased percentage of full stomachs in dusk and midnight samples. The obtained results support higher feeding activity at night in these species and indicate short time for extracellular digestion, not exceeding 8 h. Nevertheless, full stomachs were recorded in all sampling schedules. In this case, it should be considered that elimination of certain food items such as fish bones, mollusk shells and carapace fragments of crustaceans could take more time than other items. Additionally, some crab species could require a cycle of cell replacement in the midgut gland epithelium until they can take their next meal.

Introduction

Studies on the feeding habits of brachyuran crabs living in the marine ecosystem are of great importance for the understanding of biotic relationships among such organisms. Brachyuran crabs are known to affect the distribution and abundance of their prey populations (Kitching et al., 1959; Muntz et al., 1965; Seed, 1980; Lipcius & Hines, 1986; Perez & Belwood, 1988).

Determining the diel feeding patterns is a major issue in studies concerning the feeding ecology of benthic predators (Fernández et al., 1991a,b). The activity rhythms of these organisms may be related to those patterns. The estimation of the feeding period within the day cycle has been achieved by means of classifying the degree of stomach replenishment, together with an examination of its contents, in crab samples taken along the 24 h period (Stevens et al., 1982; Mori, 1986; Wassemberg & Hill, 1987; Freire et al., 1991).

Gathering information on the time elapsed for digestion is important for the understanding of foraging activity because it is an indicator of the period when they are actually feeding. Hill (1976) estimated in 12 h the time taken for digestion in *Scylla serrata* (Forskål), while Abbas (1985) found that 12–36 h are needed to evacuate the stomach of *Carcinus maenas* (Linnaeus), Choy (1986) verified that digestion in *Liocarcinus puber* (Linnaeus) and *Liocarcinus holsatus* (Fabricius) is relatively fast, however, certain food items such as shell fragments and algae are not digested and, therefore, their elimination rate is slower.

According to Pires (1992) and Fransozo et al. (1992), the most abundant swimming crabs of the genus *Callinectes* occurring in the Brazilian south-

eastern coast are *Callinectes ornatus* Ordway and *Callinectes danae* Smith. These species are commonly found living in shallow water areas and have a similar diet (Reigada, 1999).

In this study, the diel feeding activity of *C. ornatus* and *C. danae* was determined and compared between the rainy and dry seasons.The time elapsed for each species to receive another meal was also determined.

Description of the study area

Ubatuba bay is located at 23° 20′, 23° 35′ S and 44° 50′, 45° 14′ W, in São Paulo State, Brazil. According to Pires (1992), three water masses are present on the continental shelf off southeastern Brazil with different distributional patterns in summer and winter. These water masses are the South Atlantic Central Current, the Tropical Current and the Coastal Current. As stated by Pires (op. cit.), such water masses greatly differ concerning temperature, salinity and nutrient composition.

This is a sheltered area with an average depth of 3.41±0.61 m, and bottom sediments mainly composed of very fine sand, with 7.99±2.21% of organic matter content.

Other environmental factors characterizing Ubatuba bay are described in Negreiros-Fransozo et al. (1999).

Materials and methods

Swimming crabs were collected with a shrimp fishery boat, supplied with trawling 'otter trawl' nets. Daily trawls conducted over a 30′ period were carried out during four pre-scheduled occasions according to different light conditions, i.e. dawn (±6 h), noon (±12 h), dusk (±18 h) and midnight (±24 h). Samples were taken during the rainy (from January to March) and the dry (from June to August) season of 1996.

All swimming crabs were dissected and the degree of stomach replenishment recorded as follows: empty = low food amount (0–25%); partially filled = food amount occupying from 25 to 50% of the stomach capacity; $\frac{1}{2}$ full = 50–75% of total stomach replenishment; full = from 75 to 100%. The influence of diel variations on stomach replenishment was tested by means of a chi-square test applied to contingency tables (Zar, 1999) and complemented by a Goodman test for multiple comparison of proportions (Goodman, 1964, 1965). The significant level considered for all tests was 0.05.

Table 1. C. ornatus Proportions of stomach replenishment degrees at different daily schedules during the rainy and dry seasons. Results are outcomes of Goodman's tests

Rainy	Dawn	Noon	Dusk	Midnight
Empty	0.388	0.252	0.161	0.198
	D	C	A	B
Partially Filled	0.317	0.187	0.211	0.284
	B	A	A	B
1/2 Full	0.253	0.177	0.213	0.357
	C	A	B	D
Full	0.196	0.170	0.282	0.352
	A	A	B	C
Dry				
Empty	0.259	0.217	0.294	0.231
	AB	A	B	AB
Partially Filled	0.260	0.169	0.299	0.273
	B	A	B	B
1/2 Full	0.180	0.193	0.333	0.295
	A	A	B	B
Full	0.187	0.180	0.375	0.258
	A	A	C	B

Note: Comparisons are valid within the same season and proportions sharing the same letter did not differ among schedules in the same line ($p>0.05$).

Only adult intermolt males were used to estimate the extracellular digestion time. A total of 56 *C. ornatus* specimens from 54.2 to 74.2 mm CW, and 44 *C. danae* from 71.5 to 91.8 mm, were used in the experiment. Crabs were initially placed in separate aquaria and kept unfed for 48–56 h as to ensure complete stomach clearance. After this period, each specimen was provided with the same quantity of shrimp muscle pieces and two individuals were dissected at each 30′ interval. Their degree of stomach replenishment was recorded until all dissected crabs had empty stomachs.

Results

Stomach replenishment

A total of 8522 and 2198 *C. ornatus* individuals were used in the rainy and dry seasons, respectively. In the case of *C. danae*, 1068 and 543 specimens were examined in the same order.

The replenishment degree of stomachs followed a significant diel variation ($p<0.05$) for both species. Results of Goodman tests for proportion contrasts are presented in Tables 1 and 2. Non-empty stomachs

Table 2. C. danae Proportions of stomach replenishment degrees at different daily schedules during the rainy and dry seasons. Results are outcomes of Goodman's tests

Rainy	Dawn	Noon	Dusk	Midnight
Empty	0.266	0.178	0.230	0.326
	AB	A	AB	B
Partially Filled	0.249	0.181	0.214	0.356
	AB	A	A	B
1/2 Full	0.201	0.136	0.299	0.364
	A	A	B	B
Full	0.205	0.096	0.288	0.411
	B	A	C	D
Dry				
Empty	0.314	0.255	0.245	0.186
	B	AB	AB	A
Partially Filled	0.283	0.233	0.289	0.194
	A	A	A	A
1/2 Full	0.221	0.210	0.255	0.314
	A	A	A	A
Full	0.225	0.159A	0.357B	0.258ab
	A	A	B	AB

Note: Comparisons are valid within the same season and proportions sharing the same letter did not differ among schedules in the same line ($p>0.05$).

were found in all studied schedules for both species, but full stomachs were significantly higher in periods of lower light intensity and empty stomachs in periods of higher light intensity. This pattern was found for both species, independently of season.

Time for extracellular digestion

Extracellular digestion in adult intermolt males takes from 5 to 8 h in *C. ornatus*, and from 5 h 30′ to 8 h in *C. danae*.

Discussion

Analyzing feeding rhythms in crustaceans by means of examining stomach replenishment has rendered strikingly different results. Stevens et al. (1982) concluded that higher activity occurs in dark conditions; Brethês et al. (1984) recorded a bimodal diel pattern with a nocturnal major peak and a second smaller one during the day; finally, Lawton (1987) observed that such activity is restricted to the night. Otherwise, Bernard's (1979) results indicated that feeding activity is taking place during the day, from 8 to 18 h; Wassemberg & Hill (1987) suggested that foraging would occur at dawn, and Fernández et al. (1991a) did not find a diel pattern of such activity.

Feeding in *C. ornatus* and *C. danae* is mostly a nocturnal activity, as indicated by the higher incidence of full stomachs during dusk and night, but foraging during the day can not be discarded in both species. Similar results were obtained by Branco (1996), in his study on *C. danae*, in which he did not verified diurnal resting periods but a higher nocturnal activity. Uninterrupted feeding was also found in *Thalamita crenata* (Latreille, 1829), but in this case the percentage of full stomachs was significantly higher during the day (Cannicci et al., 1996).

In those studies, the prevalence of full stomachs during the day may be due to the ingestion of certain items that remain preserved overnight, such as shell fragments, fish bones and carapace pieces of other crabs (Reigada, 1999). Additionally, some crab species could require a cycle of cell replacement in the midgut gland epithelium until they can take their next meal. Diurnal feeding, however, may actually occur when potential food items are abundant, thus evoking feeding behavior independently of the period of the day.

Stevens et al. (1984) observed that higher feeding activity in *Cancer magister* Dana is elicited by the presence of juveniles of *Crangon* spp. Fabricius. Cannicci et al. (1996) verified that nocturnal activity of the cuttlefish *Sepia* sp. directly affects feeding behavior in *S. serrata* and *Portunus pelagicus* (Linnaeus).

Time spent for digestion is known to be relatively short for many crab species, especially in the case of animal tissues. Hill (1976) estimated in 12 h the maximum time length of digestion for *S. serrata*; Paul (1981) verified that stomach clearance do not take longer than 6 h for *Callinectes arcuatus* Ordway; Choy (1986) estimated that 50% of the stomach content is processed in 5 h in *L. puber* and *L. holsatus* with the rest being digested in 20 h. The digestion in *P. pelagicus* takes approximately 6 h, but fragments of shells and bones may take 24 h to be assimilated (Wassemberg & Hill, 1987).

Temperature is an important factor influencing the duration of extracellular digestion. Wear & Haddon (1987) found for *Ovalipes catharus* (White) that digesting a given food item may take from 5 h33′ at 19.5 °C to 14 h5′ at 9.5 °C. Metabolic rates in tropical and subtropical species are known to be higher than in temperate ones, thus affecting *a priori* the results of this kind of experiments (Choy, 1986). In *C. ornatus*

and *C. danae*, extracellular digestion is relatively fast, not exceeding 8 h for total stomach clearance. The presence of hard material such as shell fragments may, however, delay this process. By obtaining samples at 6 h-intervals, the results herein obtained confirm the nocturnal feeding activity in these species.

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A histochemical and ultrastructural study of oogenesis in *Aristaeomorpha foliacea* (Risso, 1827)

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Abstract

Ovaries from mature giant red shrimp *Aristaeomorpha foliacea* were investigated histochemically and ultrastructurally. Four growing stages of the oocytes were distinguished: premeiosis stage, previtellogenetic stage, early vitellogenic stage and late vitellogenic stage. In addition, occasional resorptive oocytes were found. Oogonia and premeiotic oocytes were found in germinative zones. Previtellogenic and vitellogenic oocytes were localized in maturative zones. As vitellogenesis proceeded, oocytes showed a progressive development in the number of lipid droplets as well as in the extension of RER, constituted of dilated cisternae, uniformely scattered throughout the cytoplasm. The RER produced yolk granules and a lampbrush-like substance. The latter was released under the oolemma and constituted a characteristic cortical zone. The oolemma did not develop microvilli or micropinocytotic vesicles to incorporate yolk precursors. Thus, the protein yolk appeared to be of endogenous origin. Few somatic cells were found around the oocytes, but they never gave place to a continuous epithelial layer around oocytes, thus it is not possible to speak of ovarian follicle. The cytoplasm of these mesodermal-oocyte associated cells (MOAC) was characterized by a typical steroidogenic apparatus. Few resorptive immature oocytes were found inside late vitellogenic oocytes. Since the ovaries were packed with late vitellogenic oocytes and the few immature oocytes were hardly detectable, oocyte maturation occurred in a synchronous way.

Introduction

Aristaeomorpha foliacea (Risso, 1827) represents an important biological and commercial resource of the Mediterranean Sea, living at depths between 300 and 700 m. Many studies have been carried out on the general biology and population ecology of this shrimp (Brian, 1931; Holthuis, 1980; Cau et al., 1982; Cau et al., 1987; Ragonese, 1993; Ragonese et al., 1994; Spedicato et al., 1994; Matarrese et al., 1995, 1997; D'Onghia et al., 1998).

Knowledge of reproductive biology of female *Aristaeomorpha foliacea* has come mainly from studies on sexual maturity by macroscopical investigations (Cau et al., 1982; Levi & Vacchi, 1988; Mura et al., 1992; Mori et al., 1994; Ragonese & Bianchini, 1995; D'Onghia et al., 1998). To our knowledge, oogenesis has been studied only with histological methods by Orsi Relini & Semeria (1983) and Levi & Vacchi (1988).

Ultrastructural studies of vitellogenesis have been carried out in many Decapoda (Beams & Kessel, 1963, 1980; Hinsch & Cone, 1969; Ganion & Kessel, 1972; Eurenius, 1973; Zerbib, 1973, 1979, 1980; Dhainaut & De Leersnyder, 1976; Erribabu et al., 1978), whereas they are lacking in Aristeidae. The aim of the present study was to investigate oocyte development by histochemical and ultrastructural approaches to improve understanding of oogenesis and management of this shrimp.

Materials and methods

Ovary fragments from five mature females of *Aristaeomorpha foliacea* (carapace length >60 mm),

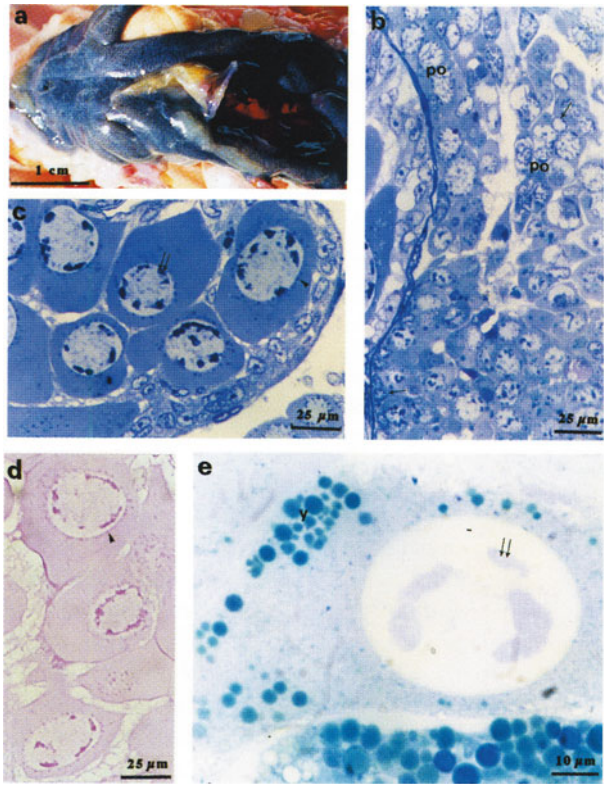

Figure 1. Light micrographs of ovaries of *Aristaeomorpha foliacea*. (a) Dorsal view of mature ovaries after removal of carapace. (b) Germinal zone. (c,d) Previtellogenic oocytes. (e) Early vitellogenic oocytes. po: premeiotic oocytes; y: yolk granules; arrow: oogonium; double arrows: nucleolus; arrowhead: perinuclear granules; *: mesodermal cells. (b,c,e) Toluidine blue staining. (d) PAS reaction.

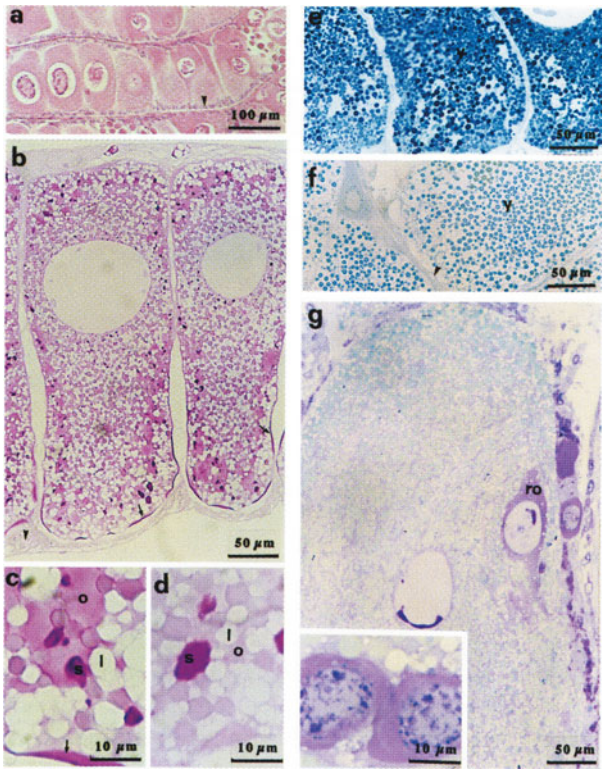

Figure 2. Light micrographs of late vitellogenic oocytes. (a) Paraffin section showing late vitellogenic oocytes piled up inside tubular structures. (b,c,d,e,f) Semithin sections. The presence of glycogen is evidenced by the comparison between PAS (c) and diastase-PAS stainings (d). (g) Oosorption phase. The inset shows two abortive premeiotic oocytes inside a late vitellogenic oocyte. l: lipid droplets; o: ooplasm; ro: resorptive oocyte; s: ooplasmic PAS positive spots; y: yolk granules; arrow: cortical zone; arrowhead: mesodermal cell. (a) Hematoxylin - Eosin. (b,c) PAS staining. (d) Diastase-PAS staining; (e,g) Toluidine blue. (f) Mercury - bromphenol blue staining.

stage 4 of macroscopic scale of maturity (see Fig. 1a) (Levi & Vacchi, 1988), collected by commercial bottom-trawl gear in the northwestern Ionian Sea in July, were fixed immediately after capture in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) diluted in sea water for 4 h at 4 °C. Then, the ovaries were thoroughly rinsed in 0.1 M cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 in the same buffer. After dehydration in a graded ethanol series and propylene oxide, the blocks were infiltrated and embedded in Araldite.

Semithin sections (1 μm thick) were stained with toluidine blue, PAS, diastase-PAS, and mercury-bromphenol blue (Pearse,1968). Diameter of oocytes and their nuclei were measured using the image analyzer Optilab/PRO 2.5.1,Graftek (France) software program. Image acquisition was made by means of a Neotech Image Grabber/24 (U.K.), located in a Power Macintosh (7100/66 AV) computer. Mean and standard deviation (S.D.) were calculated.

Ultrathin sections were placed on 200-mesh Formvar-coated copper grids and stained with uranyl acetate and lead citrate. The grids were then observed with a Zeiss Electron Microscope 109.

Results

Light microscopy

Based on the changes in size, tinctorial and cytological characteristics, four growing stages of the oocyte have been distinguished (as shown in Table 1): premeiotic stage, previtellogenetic stage, early vitellogenic

Table 1. Characteristic features identifying oocyte stages in mature female of *Aristaeomorpha foliacea* collected in July. −: no staining; ±: faintly visible staining; +: moderate staining; + +: strong staining; p: ooplasmatic patch; pg: perinuclear granules; y: yolk granules

Zone	Stage	Oocytes length (μm)	Nucleus diameter (μm)	Toluidine blue	PAS	Diastase PAS	Mercury – bromphenol blue	Lipid droplets
Germinative	Oogonia ($\bar{x} \pm$ s.d.)	10±0.11	7±0.28	±	±	±		
	Premeiosis	15–25	11–15	+	±	±		
Maturative	Previtellogenesis	25–90	19–45	+ + / pg	+ / pg	±		
	Early vitellogenesis	90–120	48–58	± / y	+ / y	±	y	
	Late vitellogenesis ($\bar{x} \pm$ s.d.)	335±15.31	86±11.11	± / y	+ / p / y	± / py	y	+

stage, late vitellogenic stage. In addition, occasional resorptive oocytes were found.

The ovarian parenchyma consisted of germinal and maturative zones. The germinal zone contained oogonia and premeiotic oocytes (Fig. 1b). The other oocyte stages were present in the maturative zone (Figs. 1c and 2a).

The oogonia (diameter 10±0.11 μm), round in shape, had a large nucleus with one nucleolus and peripheral heterochromatin. The limited amount of cytoplasm was slightly basophilic (Fig. 1b) and PAS positive.

The premeiotic oocytes (diameter 15–25 μm), comprehending leptotene, zygotene and pachytene phases, were polygonal in shape, basophilic (Fig. 1b) and slightly PAS positive.

The previtellogenic oocytes (diameter 25–90 μm) (see Fig. 1c,d) showed ameboid properties and radiated out from the germinative zone as their size increased. The cytoplasm, more basophilic than in the premeiotic stage, was characterized by toluidine blue and PAS positive perinuclear granules (1 μm maximum in diameter) (Fig. 1c,d), and glycogen, displayed by decrease of PAS staining after diastase treatment. The nucleus was vesicular and contained numerous basophilic and PAS positive nucleoli.

The early vitellogenic oocytes (Fig. 1e) (diameter 90–120 μm) were polymorphic in shape and showed large and randomly yolk granules (3.5 μ maximum in diameter). These were stained by toluidine blue, PAS and mercury-bromphenol blue methods. The nucleus was spherical and medial.

The late vitellogenic oocytes (Fig. 2a,b) were rectangular in sections (335±15.31 μm in maximum length). These oocytes were much more numerous than early vitellogenic oocytes and they were piled up in a single row inside tubular structures delimited by mesodermal cells (Fig. 2a,b). The oocytes were packed with glycogen (Fig. 2c,d), lipid droplets and yolk granules (5 μm maximum in diameter). The latter were toluidine blue, PAS and mercury-bromphenol blue positive (Fig. 2b,e,f). Throughout the cytoplasm PAS positive spots were distributed; sometimes they were in continuity with cortical PAS positive areas (Fig. 2b,c).

Occasionally, the largest late vitellogenic oocytes contained one or more premeiotic or previtellogenic oocytes in their cytoplasm (Fig. 2g). In comparison with the previtellogenic oocytes described above, the nucleus of these abortive oocytes showed no evident changes. The cytoplasm contained vacuoles, displayed metachromasia with toluidine blue, and seemed in continuity with the ooplasm of the host oocyte.

Electron microscopy

The cytoplasm of previtellogenic oocytes (Fig. 3a) contained numerous polysomes which determined its electron-dense aspect. Scattered in the cytoplasm there were small cisternae of rough endoplasmic reticulum (RER), flattened- or vesicular in shape, and numerous elongated mitochondria (2.5 μm × 0.5 μm). Small electron-opaque granules (1 μm maximum in diameter) were placed next to nucleus. The nucleus (Fig. 3b) showed dispersed chromatin and contained electron dense nucleoli characterized by

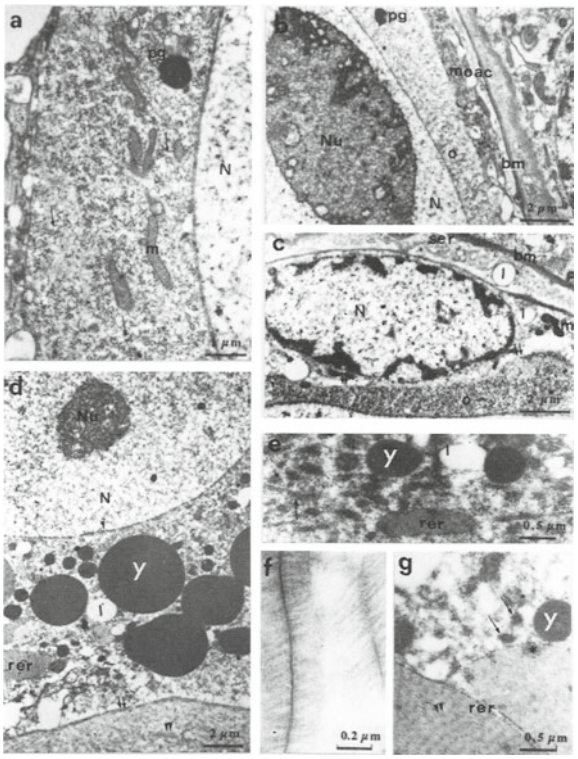

Figure 3. Electron micrographs of oocytes in *Aristaeomorpha foliacea*. (a,b) Previtellogenic oocyte. (c) mesodermal-oocyte associated cell. (d,e,g) Early vitellogenic oocytes. (f) Lampbrush-like substance. bm: basement membrane; l: lipid droplet; m: mitochondrium; moac: mesodermal-oocyte associated cell; N: nucleus; Nu: nucleolus; o: ooplasm; pg: perinuclear granules; rer: rough endoplasmic reticulum; ser: smooth endoplasmic reticulum; y: yolk granules; arrow: flattened- or vesicular RER; double arrows: oolemma; arrowhead: nuclear pore; double arrowheads: lampbrush-like substance; *: intracisternal granules. Uranyl acetate-lead citrate.

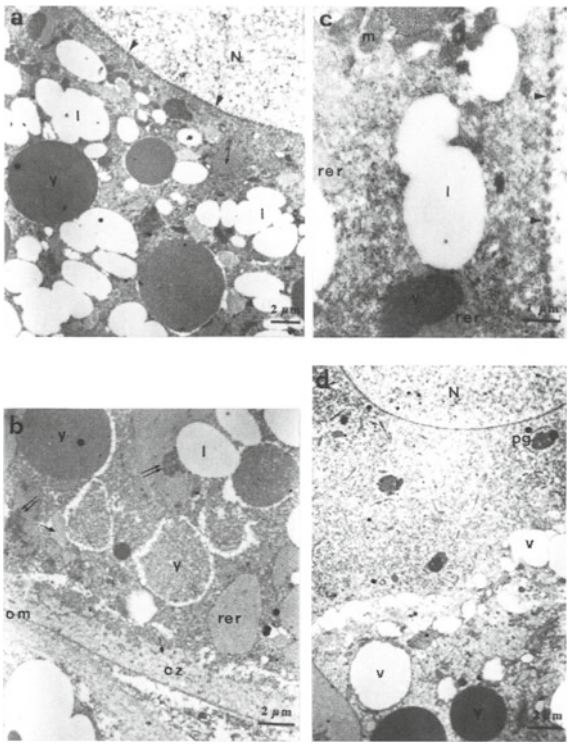

Figure 4. Electron micrographs of late vitellogenic oocytes (a,b,c) and a resorptive oocyte (d). cz: cortical zone; l: lipid droplet; m: mitochondrium; N: nucleus; om: oolemma; pg: perinuclear granules; rer: rough endoplasmic reticulum; v: vesicle; y: yolk granule; arrow: lampbrush-like substance; arrowhead: nuclear pore; double arrows: intracisternal granules.

electron-lucent inner zones connected with the nucleoloplasma surrounded by an electron-opaque matter. The oolemma was smooth and exhibited no particular morphological specialization. Few mesodermal cells (Fig. 3c) were found close to the oocyte, but they did not constitute the classic ovarian follicle. These cells were flattened and showed an oval-shaped nucleus containing clumps of peripheral heterochromatin. The cytoplasm was characterized by electron dense mitochondria, smooth endoplasmic reticulum (SER) and lipid droplets.

The early vitellogenic oocytes (Fig. 3d) showed a much more extensive lamellar RER (Fig. 3e) which began to dilate and to devolop large cisternae containing a lampbrush-like substance (Fig. 3d,f,g). This material consisted of a dense central core (10 nm thick) where lateral filaments (150 nm length) were inserted (Fig. 3f). The cisternae of RER sometimes contained small and electron-opaque granules (Fig. 3g) that seemed to originate from the fusion with vesicles from lamellar RER. These electron-dense granules (diameter 70–80 nm) showed an electron-lucent central zone. Other particularly striking features of this maturative stage were the presence of numerous electron dense granules in different stage of condensation (Fig. 3d,e) interpreted as yolk granules (the largest of them reached 3 μm) and lipid droplets. The nuclear envelope showed increase in number of pores (Fig. 3d). This was sign of increased nuclear-ooplasmic flow.

As vitellogenesis proceeded, the number of lipid droplets, yolk granules and the extension of the RER increased in the oocytes (Fig. 4a,b). At the end of vitellogenesis, the oocytes were packed with lipid droplets (some of them were coalescent), and with yolk granules (diameter 5 μm) at different maturative stages (Fig. 4b) as demonstrated by their content that had flocculent or homogeneous aspect (Fig. 4b,c).

The RER was uniformely scattered throughout the cytoplasm. It was constituted of dilated cisternae containing intracisternal granules and lampbrush-like substance (Fig. 4a,b). The latter was released under the oolemma and constituted a characteristic cortical zone (Fig. 4b). The oolemma was smooth without microvilli or micropinocytotic vesicles (Fig. 4b). The few somatic cells found around the oocytes showed features similar to ones described above.

The absorptive oocytes (Fig. 4d) showed an extensive lamellar RER containing electron-dense material, dense bodies (presumably lysosomes) and few mitochondria. The nucleus did not display structural impairments. These oocytes were surrounded with large vesicles. In addition, cytoplasmic continuity features between the oocytes were seen.

Discussion

This investigation on oogenesis in *Aristaeomorpha foliacea* mature females, collected in July, permitted to distinguish four growing stages that match in general the description found in the literature for other crustaceans (Adiyodi & Subramonian, 1983).

Oocytes growth depends on initial ribosome production followed by progressive increase of vitellogenesis. In the previtellogenic stage of *Aristaeomorpha foliacea*, an increase in size and number of nucleoli, as well as nucleolar vacuolization and perinuclear granules appearance occurred in the oocytes. Similar features with transfer of nuclear materials to the ooplasm have been found in *Paratelphusa hydrodromus* (Adiyodi, 1969) and *Libinia emarginata* (Hinsch, 1970). The passage of nuclear matter from nucleus to the cytoplasm takes place in young oocytes of other Decapoda (Kessel & Beams, 1968; Dhainaut & De Leersnyder, 1976; Beams & Kessel, 1980; Zerbib, 1980). Nuclear emission from young oocytes are common in autosynthetic oocytes and the transfer of nuclear material to the ooplasm is a prelude to protein yolk synthesis in various animal groups (Adiyodi & Subramoniam, 1983). In *Aristaeomorpha foliacea,* as the growth of oocyte goes on, the development of RER in dilated cisternae occurs and the vitellogenesis starts. In this stage, the RER carries out the main role among the organelles. The RER is the biochemical machinery to synthesize the massive stores of proteinaceous yolk present at the end of oogenesis. In *Aristaeomorpha foliacea*, the production of yolk components sems to have intraoocytic origin, since oolemma is lacking in microvilli and the micropinocytosis vesicles are rare. An exclusively endogenous synthesis of yolk by RER (alone or in association with Golgi apparatus) has been found only in a few species of crustaceans (Beams & Kessel, 1963; Kessel, 1968; Ganion & Kessel, 1972). In oocytes of crayfish, radioautograph investigations demonstrated that micropinocytosis plays little, if any role in the deposition of proteinaceous yolk. Instead, RER plays an active role in forming protein yolk which is not shuttled to the Golgi region (Ganion & Kessel, 1972). In crustaceans, considerable variability exists in the origin of yolk precursors. In most crustacean species, ultrastructural investigations have demonstrated that protein yolk globules are of mixed origin (Beams & Kessel, 1963; Kessel, 1968; Hinsch & Cone, 1969; Wolin et al., 1973, Dhainaut & De Leersnyder, 1976; Beams & Kessel, 1980; Schade & Shivers, 1980; Zerbib, 1980; Souty, 1980, 1983). In many Decapoda, there is evidence that yolk protein synthesis depends on extraoocytic sequestration of vitellogenin, an haemolymph protein fraction specific of vitellogenic females (Meusy, 1980; Han & Bae, 1992).

The synthesis of a large amount of a PAS positive and lampbrush-like substance takes place in the well developed RER of vitellogenic oocytes. This material is released at the periphery of an oocyte, constituting a peculiar cortical region. To our knowledge, no macromolecule with this or similar ultrastructural features has previously been described in any of animal cell types. Therefore, its function is unknown, although on the basis of their histochemical characteristics as well as localization, it is possible to hypothesize a role similar to cortical granules which are present in Malacostraca (Zerbib, 1975; Demestre & Fortuño, 1992).

In *Aristaeomorpha foliacea*, the somatic (mesodermal) cells never gave rise to a continuous epithelial layer around oocytes, thus it is not possible to speak of an ovarian follicle. We call these somatic cells mesodermal-oocyte associated cells (MOAC). In higher crustaceans, follicular cells facilitate vitellogenic activities representing a prerequisite for the uptake of yolk protein from outside (Charniaux-Cotton, 1975). We did not find morphological structures, as microvilli or micropinocytosic vesicles, indicative of any funtional relation between mesodermal-oocyte associated cells and oocytes. It has also been hypothesized that the follicular cells have some endocrine functions (Lachaise & Hoffmann, 1977; Rateau & Zerbib, 1978; Charniaux-Cotton, 1980; Lachaise et al., 1981; Arcier & Brehelin, 1982). The MOAC

of *Aristaeomorpha foliacea* contain SER, mitochondria and lipid droplets representing a typical apparatus related with steroidogenesis. Follicular cells have steroidogenic (chiefly ecdysone) activity in *Carcinus maenas* (Lachaise & Hoffmann, 1977; Lachaise et al., 1981).

Oosorption is a common event in crustaceans occurring under starvation, hormone deprivation, or lack of mating conditions (Adiyodi & Subramonian, 1983). In oosorption, the involvement of follicular cells as well as haemocytes is well known (Carayon, 1941; Hort-Legrand et al., 1974). In the Aristeaidae *Aristeus antennatus*, the non-functional oocytes become irregular in shape and they gradually decrease in size, and finally they undergo phagocytosis (Demestre & Fortuño, 1992). In *Aristaeomorpha foliacea*, oosorption involves immature oocytes that are in maturative retardation. They are eliminated by dissolution inside late vitellogenic oocytes. In oosorption, the involvement of follicular cells as well as haemocytes is well known (Carayon, 1941; Hort-Legrand et al., 1974). The abortive oocytes of *Aristaeomorpha foliacea* might have a behaviour similar to nurse cells, considered abortive oocytes, found in some non-malacostracans (Trentini & Sabelli Scanabissi, 1978; Zaffagnini, 1987; Zeni & Zaffagnini, 1989).

The ovaries were packed with late vitellogenic oocytes and the few immature oocytes were hardly detectable. This induces one to suppose that in *Aristaeomorpha foliacea* oocyte maturation occurs in a synchronous way.

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Prevalence of bacteria in the spermathecae of female snow crab, *Chionoecetes opilio* (Brachyura: Majidae)

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Key words: bacteria, seminal fluid, spermatheca, snow crab, *Chionoecetes opilio*

Abstract

The spermathecal contents of primiparous, multiparous and barren female snow crabs were observed by a scanning electron microscope. Bacteria colonies were observed with a significantly higher frequency in the spermathecae of primiparous and old barren females. Bacteria infect the spermathecae and destroy the spermatophores and spermatozoa inside. These observations suggest that bacteria in the spermathecae do not exclude opportunistic microbes by modifying pH of the medium as suggested in the literature. The prevalence of bacteria in primiparous and old barren females suggests that they infect individuals with a weak anti-microbial protection. The absence of bacteria in the highly acidic seminal fluid derived from males upon copulation suggests that it may provide anti-microbial protection. Bacteria do not seem to be able to survive in an anaerobic environment for a long period.

Introduction

In some brachyurans, the spermathecal contents form a hard body inside spermatheca, extends into the vagina and even protrudes from the vulva forming as a sperm plug (Hartnoll, 1969). The role of male seminal fluid in the spermathecae has not been well understood in decapod crustaceans. Johnson (1980) suggested that sperm plugs of blue crab (*Callinectes sapidus*) could prevent loss of sperm and provide a mechanical or chemical barrier to deterious material. Sasikala & Subramoniam (1987) suggested that the acid mucopolysaccharide of spermatophores in shrimp (*Monodon monoceros*) might act as a cementing agent or to prevent dehydration or as an anti-microbial agent. Subramoniam (1991) also suggested that nutrient rich seminal plasma may help maintain spermatozoa during storage. Diesel (1990,1991) reported that seminal plasma fills the ventral part of the receptacle of female ghost spider crab (*Inachus phalangium*) and a sperm packet is placed at the lowermost position in the seminal plasma which prevents rivals' sperm from fertilizing oocytes.

In snow crab (*Chionoecetes opilio*), Benhalima & Moriyasu (2000) reported that male crab transfer two different types of seminal fluid into the spermathecae upon copulation: (1) one derived from the median vas deferens (MVD) in which numerous spermatophores are lodged and, (2) another of highly acidic nature derived from the posterior vas deferens (PVD). Colonies of bacteria have often been observed in the spermathecae of mature snow crab (Beninger et al., 1993). In this study, the prevalence of bacteria in different types of mature females and the morphology of bacteria and their presence in the spermathecae were examined by light microscope and scanning electron microscope.

Materials and methods

Female and male snow crab, *Chionoecetes opilio* (O. Fabricius, 1758), were collected in the southern Gulf of St. Lawrence, Canada, by a bottom trawl between July and November in 1997 and 1998. The carapace width (CW) and chela height (for male only) were

measured to the nearest 1/10th mm using a caliper. The carapace condition (new, intermediate, old and very old), based on the degree of epibiont fouling on the carapace (Benhalima et al., 1998), and the color of external egg mass for females (Moriyasu & Lanteigne, 1998) were noted.

A pair of the first pleopods from 50 males (88.5–140.7 mm CW) and paired spermathecae from 177 females (26 immatures, 60 primiparous, 46 multiparous and 45 barren females: 36.5–72.7 mm CW) were dissected with sterilized surgical scissors. Primiparous females are ones that moulted to maturity and mated, then extruded fertilized eggs for the first time. Their carapace is new without accumulation of epibionts. Multiparous females are carrying their second or later batch of eggs with older carapace and pre-copulatory embrace marks on their walking legs. Barren females are at the end of their reproductive life and have undeveloped or degenerating ovaries without external eggs.

For histological preparation, the right side of the spermathecae was preserved in Bouin's solution for 24 h and the fixed tissues dehydrated in a series of graded ethanol solutions (50, 70, 95 and 100%), cleared in xylene agent, then embedded using paraffin wax. Paraffin blocks were sectioned serially at 5–7 μm on a rotary microtome. Serial sections were mounted on glass slides and stained with modified Masson's trichrome (Gabe, 1968), then dehydrated with ethanol and cleared with xylene. Light microscopic examinations were done with a compound light microscope.

The left spermatheca was cut sagittally into two portions with a sterilized fine surgical scalpel. One side of the spermatheca was used for microbiological study and Gram stain was applied to fresh smears of spermathecal contents. The other portion was immersed in liquid nitrogen, then fractured with sterilized fine surgical tweezers. Cryofractured pieces of the spermatheca and a pair of the first pleopods were fixed for 1 h at 4 °C with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and rinsed in cold 0.175 M cacodylate buffer at pH 7.2 (Moriyasu & Benhalima, 1998). Specimens were post-fixed with 2% osmium tetroxide in 0.31 M cacodylate buffer at pH 7.2 for 1 h, and rinsed in the same buffer (Moriyasu & Benhalima, 1998). Following dehydration (15 min each) through increasing concentration (up to 100%) of ethanol. The cryofractured spermatheca pieces and the first pleopods were placed in a Polaron CPD750 critical-point dryer and infiltrated with carbon dioxide

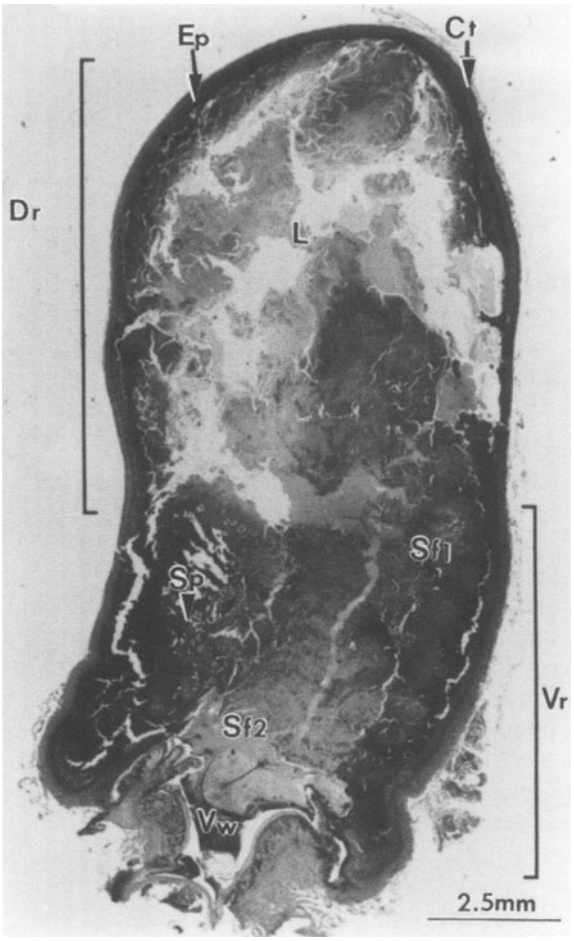

Figure 1. Light micrograph of the sagittal section of a spermatheca of a primiparous female snow crab *Chionoecetes opilio* (CW = 64.3 mm) showing two different types of the seminal fluid in the lumen of the ventral region. Ct: connective tissue, Dr: dorsal region, Ep: epithelium, L: lumen, Sf1: seminal fluid (Type-1), Sf2: seminal fluid (Type-2), Sp: spermatophores, Vr: ventral region, Vw: vaginal wall.

for 1 h. The specimens were mounted on aluminum stubs, then coated with gold-palladium. The specimens were viewed with a JEOL JSM 6400 SEM at 10 kV acceleration voltage.

The dependency of infection rate on the female type was examined by a contingency table test, and the proportion of individuals infected by bacteria was compared between two female types at a time by the Fisher exact test (Zar, 1999).

Results

Microscopic observations showed the epithelial lining of the spermathecae consisted of layers of inner

glandular epithelium and outer flexible connective tissue (Fig. 1). The ejaculate was clearly distinguishable in the ventralmost region. Numerous spermatophores occupied the lumen of the ventralmost region and were embedded in a dense seminal fluid, stained in dark red with Masson's trichrome (type-I). This seminal fluid was often observed along the spermathecal wall (Fig. 1). In the center of the ventralmost region, the other type of seminal fluid (type-II), stained in gray with Masson's trichrome, was observed (Fig. 1).

SEM observations of the content in the lumen of the spermathecae of multiparous females showed densely aggregated spermatophores embedded in a homogenous seminal fluid (Fig. 2A). Two morphological types of spermataphoric wall (smooth or wrinkled) were observed (Fig. 2B). Inside the spermatophoric wall, numerous spermatozoa stellate in shape with several arms radiating from the body were observed and their acrosome was globular (Fig. 2C).

Long slender rod shape bacteria were found in the type-I seminal fluid and covering the surface of the spermatophoric walls most frequently in primiparous females (Fig. 2D). At the moderate infection stage, large colonies of bacteria were found on the spermatophores causing numerous perforations on the spermatophoric wall (Fig. 2E). At advanced infection stage, the bacteria penetrated into the spermatophore and attacked the spermatozoa (Fig. 2F). In infected spermathecae, bacteria colonies were not found in the type-II seminal fluid in the dorsal region (Fig. 1B), whereas bacteria were often observed in the type-I seminal fluid in the ventralmost region (Fig. 1B). In some females, proliferating bacteria were also observed on the cuticular surface near the spermatheca entrance (Figs. 3A, B). However, no damage of the cuticular structure was observed (Fig. 3B). Microbiological analysis of fresh spermathecal content smears from infected females revealed that bacteria were Gram-positive, whereas the spermathecal content of uninfected females were Gram-negative. The presence of a colony of rod-shaped bacteria was also observed in the opening of the apical tip of the first pleopod (Figs. 3C, D) in 22.2% of males observed.

The infection rate (Table 1) was not independent of the female categories (contingency table test: χ^2=22.2 with DF=2, $P< 0.0001$). The infection rate was significantly higher in primiparous than multiparous females (Fisher's exact test: $P<0.0001$), and higher in barren than multiparous females (Fisher's exact test: P=0.009). The infection rate was not significantly different between primiparous and barren females (Fisher's exact test: P=0.10).

Table 1. Number of normal and infected spermathecae by bacteria in three different types of mature female snow crabs, *Chionoecetes opilio*

	Primiparous	Multiparous	Barren
Normal	38	56	44
Infected	22	1	12
Total observed	60	57	56
Infection rate	36.7%	1.8%	21.4%

Discussion

Beninger et al. (1993) first reported the presence of bacterial colonies in snow crab spermathecae. Beninger et al. (1993) and Elner & Beninger (1995) suggested that a homogeneous bacterial colony is maintained by spermathecal secretion and male insemination fluid. Their metabolic by-products could render the spermatophore pellicle more susceptible to dehiscence and a dense homogeneous bacterial population may inhibit the growth of other potentially harmful microbes. In this study, the presence of bacteria in the spermathecae was confirmed although our interpretation of their role in the spermathecae differs from Beninger et al. (1993) and Elner & Beninger (1995).

Benhalima & Moriyasu (2000) reported that the seminal fluid of a different chemical nature is stored in specific regions of the vasa deferentia; one in the MVD with acid mucopolysaccharide (stained in dark red with Masson's trichorome), and the other in the lower region of the PVD with a highly acidic mucosubstance (stained in gray with Masson's trichrome). Two types of seminal fluid found in the spermathecae in the present study correspond to that of MVD-origin and PVD-origin, respectively, based on the texture of the fluid observed by SEM and their chemical nature. MVD-origin seminal fluid corresponds to the amorphous matter reported by Urbani et al. (1998). Benhalima & Moriyasu (2000) described that two types of seminal fluid are placed in the different parts of the spermatheca upon copulation, and may play different roles. The authors also reported that MVD-origin seminal fluid serve as nutrient for spermatozoa. The PVD-origin seminal fluid serve as an anti-microbial agent as well as a catalytic agent, for the dehiscence of

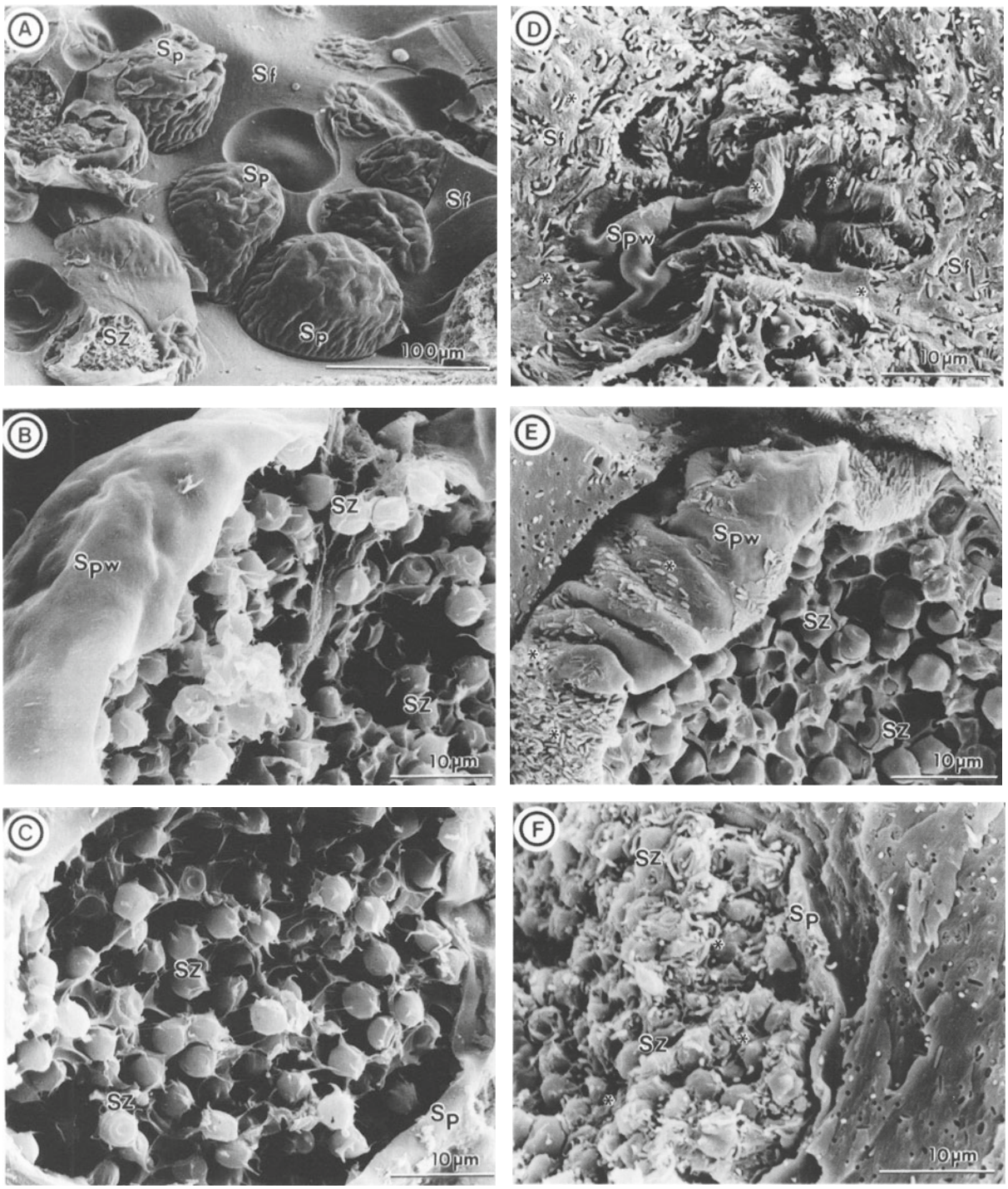

Figure 2. Scanning electron micrographs of the cryofractured pieces of the spermathecal contents. (A) Non-infected spermatophores (Sp) embedded in homogenous seminal fluid (Sf). Some fractured spermatophores are shown exposing numerous spermatozoa (Sz) in a multiparous female. (B) Normal condition of fractured spermatophoric wall (Spw) containing numerous intact spermatozoa (Sz) in a multiparous female. (C) Intact spermatozoa (Sz) lodged in a spermatophore (Sp) of a multiparous female. (D) Colony of the rod-shaped bacteria (asterisks) present on the spermatophoric wall (Spw) and in the surrounding seminal fluid (Sf) of a primiparous female. (E) Rod-shaped bacteria (asterisks) perforating the spermatophoric wall (Spw) in a primiparous female. Spermatozoa (Sz) lodged inside are not infected. (F) Rod-shaped bacteria (asterisks) invaded the inside the spermatophore (Sp) and infecting spermatozoa (Sz) in a primiparous female.

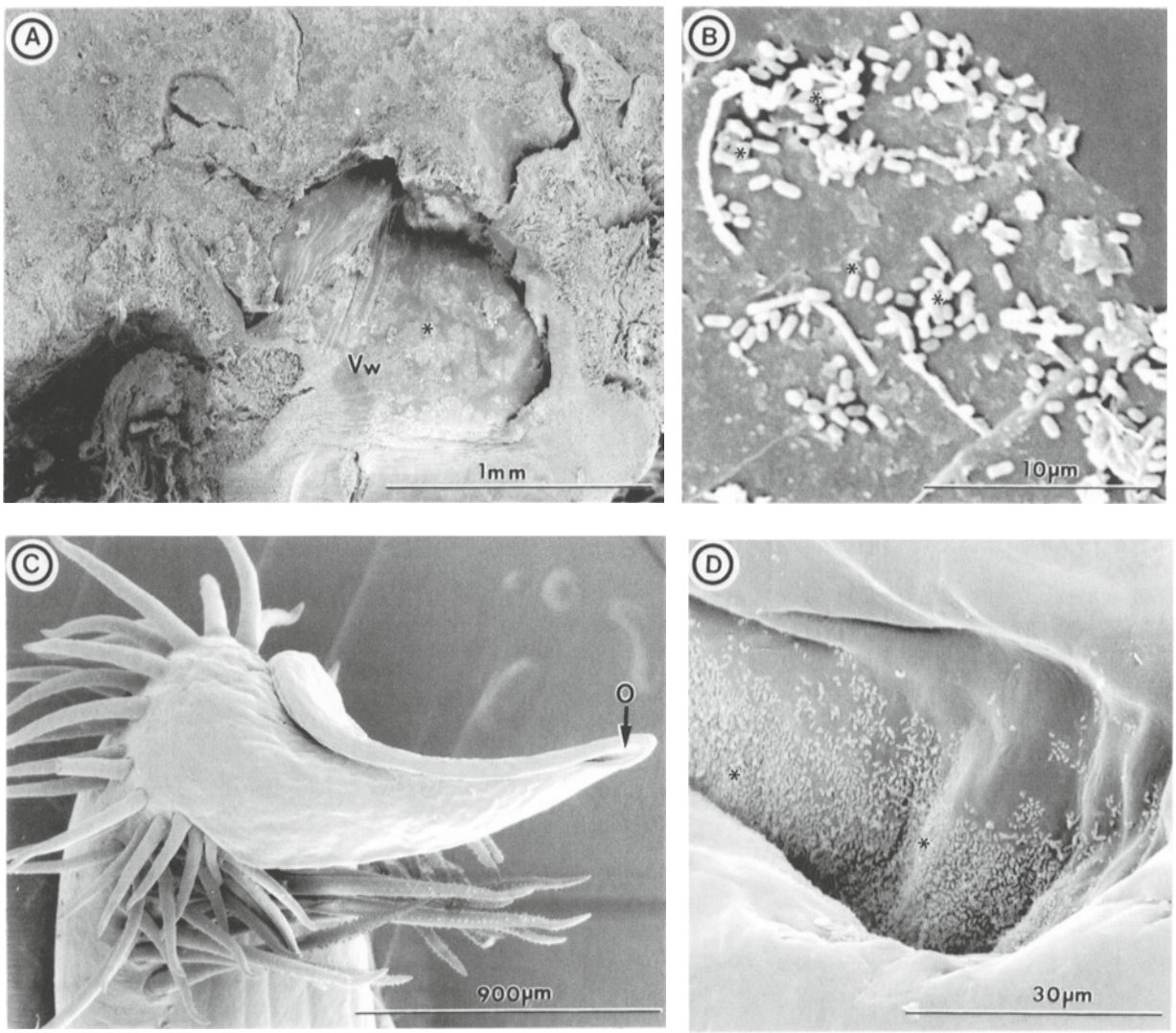

Figure 3. (A) Scanning electron micrographs of the ventralmost region of the spermatheca showing the presence of a bacteria colony (asterisk) on the vaginal wall (Vw). (B) A higher magnification of the vaginal wall region showing the presence of a colony of rod-shaped bacteria (asterisks). (C) General view of the first pleopod showing the opening (O) at the apical tip. (D) A higher magnification of the opening of the apical tip of the first pleopod showing the presence of a colony of rod-shaped bacteria (asterisks).

spermatophores in the subsequent mating season. The presence of bacterial colonies in the MVD-origin seminal fluid and their absence in the PVD-origin seminal fluid support Sasikala & Subramoniam's (1987) hypothesis that the PVD-origin seminal fluid plays the role of an anti-microbial agent in the spermathecae. Moriyasu & Lanteigne (1998) reported that the duration of embryonic development of multiparous females is 2 years and they hatch their brood 3 times at most in their reproductive life. Multiparous females that mated after having hatched their previous brood may be protected with new sperm plugs with a highly acidic muco-substance received from the male partner. In contrast, individuals fertilizing their brood without mating are more susceptible to the attack by bacteria and other micro-organisms because of using spermatophores stored in the spermathecae. However, Conan & Comeau (1986) reported that mating of multiparous females occurs in their natural environment and the use of stored sperm in the spermathecae seem to be a safe guard in case that no male partner is available.

Primiparous female mates for the first time after moulting to maturity. Upon the insertion of the first pleopods into female's spermathecae, they introduce bacteria present in their apical openings together with seminal fluid and spermatophores. Observations of

the spermathecae of barren females showed that the spermathecal content is disintegrated and PVD-origin seminal fluid is loosing its highly acidic chemical nature. In addition, unidentified worms are also found in the spermathecae of old barren female (M. Moriyasu & K. Benhalima, unpubl. obs.). The prevalence of bacteria in the spermathecae of both primiparous and old barren females and the quasi-absence in multiparous females provide an indirect evidence that bacteria are only present in the spermatheca of females having a weak anti-microbial system.

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Discards of the Algarve (southern Portugal) crustacean trawl fishery

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Key words: crustacean fisheries, by-catch, discards, ecological impact

Abstract

The crustacean trawl fishery off the Algarve coast (southern Portugal) takes place on the lower continental shelf and upper continental slope at depths between 150 and 600 m. This is a multi-species fishery targeting the shrimps *Parapenaeus longirostris* and *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus*, with the latter two species the most important in the landings. The fishery is characterised by significant by-catch and discarding of a large number of species. As part of a study on the fate of trawl fishery discards, this component of the study focused on the quantification of the by-catch and discards of crustacean trawlers. Sampling took place on board seven commercial trawlers from June 1998 to October 1999. Data was collected from 48 tows in 22 fishing trips. The observers collected all of the catch that was discarded by the crew during the sorting operation and samples were taken to the laboratory for identification, weighing and measuring. The quantities of target species were recorded along with the presence of retained by-catch. Commercially valuable species that were retained included *Plesiopenaeus edwardsianus*, *Aristeomorpha foliacea*, *Plesionika* sp., and the fishes *Lophius piscatorius* and *Merluccius merluccius*. However, most of the species had no or little commercial value and were almost always discarded to the sea (90%). A total of 91 species were identified, 47 vertebrates and 44 invertebrates corresponding to 65 families. The Teleostei (78% and 68%) were the dominant group, both in number and weight. The species *Micromesistius poutassou* (34%), *Gadiculus argenteus* (10%) and *Hoplostethus mediterraneus* (8%) were the most important in weight. Ten species represented more than 82% of all discards in weight. *Gadiculus argenteus* (29%), *Hoplostethus mediterraneus* (21%) and *Nezumia sclerorhynchus* (10%) accounted for 60% of all discards in numbers. Data on the landed species composition is also presented.

Introduction

The crustacean trawl fishery that takes place off the Algarve coast (South of Portugal) is of considerable importance due to the quantity and value of the landings. The most important crustacean fishing grounds in Portugal are located off this coast, contributing an annual crustacean catch of 984.3 tons in 1998 (D.G.P.P., 1999). This is a multispecies fishery targeting the shrimp *Parapenaeus longirostris*, the red shrimp *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus*. In Portuguese waters, these species are found in areas on the continental shelf and upper slope between 80 and 700 m (S.E.P., 1984). Although their distributions overlap, *P. longirostris* prefers sandy mud or muddy sand bottoms between 200 and 400 m, while *A. antennatus* is more commonly found in muddy areas between 300 and 600 m (Cascalho, 1992). *N. norvegicus* has an irregular distribution between 200 and 700 m (S.E.P., 1984), depending on bottom topography and sediment type which is a limiting factor due to its burrowing behaviour (Figueiredo & Viriato, 1992).

In the Algarve, this fishery takes place at depths greater than 150 m (S.E.P., 1984) following the depth distribution of each species and also in part because of the limitations imposed by legislation that prohibits trawling in areas within 6 miles from the shore. The species are targeted according to a number of factors including their availability, market demand and price (Arrobas, 1982).

As in others crustacean trawl fisheries around the world (Saila, 1983; Gray et al., 1990; Harris & Poiner, 1990; Wassenberg & Hill, 1990; Alverson et al., 1994;

Borges et al., 1998; Merella et al., 1998), many other species to which effort is not directed are also caught (by-catch) since trawls are not very selective (Dayton et al., 1995; Kennelly, 1995). Globally, it has been estimated that 2.7 million t of by-catch were caught during prawn trawling in 1978, of which 1.4 million were discarded (Saila, 1983). According to Alverson et al., (1994), tropical shrimp trawl fisheries generate more discards than any other fishery and account for just over one-third of the global total. Borges et al. (1998) reported that the discards from the crustacean trawl fisheries in the Algarve ranged from 26% to 91% of the catch in weight, with an average of 70%.

A contributing factor to the by-catch problem in this fishery is the long tow duration, which is characteristic of this fishery. This leads to decreasing net size selectivity as the catch accumulates in the codend (Murawski, 1993). Part of the by-catch consists of crustaceans, fishes and molluscs with high commercial value and is landed. However, as in other crustacean fisheries most of the by-catch is composed of species of low or no commercial value and is discarded to the sea (Borges et al., 1997).

The difficulties of managing this fishery are largely due to its multi-species nature and to the lack of information on this developing fishery. Thus, there is at the moment no sustainable management scheme for the commercial species taking into account the by-catch. The by-catch of non-target species and their discarding may have negative consequences for non-commercial as well as commercial species due to influences on species interactions and consequent cascading effects throughout the trophic web (Hongskul, 1979; Saila, 1983; Harris & Poiner, 1990; Hill & Wassenberg, 1990; Alverson et al., 1994; McAllister & Spiller, 1994; Kennelly, 1995; Yamamura, 1997). However, as Gulland (1972) pointed out, the effects of non-sustainable fishing may not be obvious for some years. Determination of the nature and extent of the ecological interactions between commercially valuable species and less valuable bottom species is important in managing shrimp fisheries and in exploiting demersal fish resources (Sheridan et al., 1984; Kennelly, 1995). Therefore, in an attempt at establishing a multi-species or ecosystem-based management approach to the crustacean trawl fishery of the Algarve, the quantification of by-catch composition and rates is a necessary pre-requisite. A sampling programme aboard commercial trawlers can be a way to characterise the biological diversity of the fishing grounds. Although Borges et al. (1997) have previously studied the discards of crustacean trawlers, the need for further and more in-depth studies was recognised.

Between June 1998 and October 1999, observers went on board Algarve trawlers. Their main goal was to quantify trawl discards and to assess their fate and importance to marine communities. This study reports the results concerning the species composition and quantities of discards in this fishery.

Material and methods

Trawl catches can vary within the same area, between areas and over time (Saila, 1983). Since this study was carried out on board commercial trawlers, it was not possible to select the sampling areas beforehand or to randomly stratify the sampling. Therefore, it was not possible to cover all the fishing grounds as the sampling was decided by the trawl skippers who based their decisions on economic considerations. We attempted to sample the greatest possible number of vessels given the available means.

The regular operation pattern aboard a crustacean trawler consists in an immediate separation on deck of commercially valuable species and the throwing back to sea of all the catch that will not be landed (discards). Since by definition discards are never landed, it was necessary to have observers on board the trawls. However, as a prerequisite, a sampling strategy was chosen that minimised interference with normal fishing operations. Observers collected all discards during the sorting process. The volume of fish discarded was estimated by placing discards in plastic 0.03 m^3 fish boxes. Sub-samples were taken that ranged from at least a third of a box to a full box. The sub-samples were taken to laboratories where they were sorted into six main groups of species: Teleosts, Chondrichthyes, Crustacea, Cephalopoda, Gastropoda and Others. The latter group consisted mainly of benthic invertebrates. In the case of relatively small amounts of discards, the entire catch was brought to the laboratory. Although it was not possible to sample the discards of every tow, the main characteristics of each tow were recorded. For some tows, the discard samples were sorted and identified to the species level, and all the individuals measured and weighed.

The composition of the discard samples was used to estimate the total discard composition for each tow. The catch of crustacean target species per tow was estimated by counting the number of baskets of each species. Based on information provided by the skip-

pers, the following average values per basket were used:

1. *Nephrops norvegicus:* 5 kg,
2. *Plesiopenaeus edwardsianus:* 5 kg,
3. *Aristeomorpha foliacea*: 5 kg,
4. *Parapenaeus longirostris:* 7 kg,
5. *Aristeus antennatus:* 7 kg,
6. *Plesionika* sp: 7 kg.

Catches of *P. edwardsianus* and *A. foliacea* were estimated jointly because these species are not separated and are landed and sold together. It was not possible to obtain reliable data concerning the quantities of retained non-target fish and mollusc species per tow. This means that discard rate values were overestimated, because only landed crustaceans and discards were considered: (discard rate = discards (kg) / (discards (kg) + commercial crustaceans (kg)). However, the presence of by-catch species was noted. For each sampled tow, geographic co-ordinates and depth were recorded at the beginning and at the end of the tow. Towing speed and duration were also recorded.

Analysis of variance was used to study the discard variation according to the above six groups of species. The arc-sin of the square root was used to transform the proportion index of each group prior to analysis of variance (SAS Institute Inc., 1988).

Results

Sampling and study area

The observers went on 22 fishing trips during which 48 tows were sampled from seven trawlers. Most of the trawlers sampled were from the Port of Olhão; one of the most important in the Algarve and in Portugal, with the advantage of being located near the University where the samples were analysed (Fig.1). On two occasions, the fishing trips were carried out on the West Coast on board trawlers from the Port of Sines (Fig. 1). The seven trawlers that we sampled worked mainly in these two areas of the Portuguese coast.

As can be seen in Figure 1, fishing took place near the above mentioned ports in fairly limited areas and depths. The characteristics of the fishing operations are summarised in Table 1. This fishery is characterised by tows of considerable duration, with a mean towing time of 5 h and 46 min (sd = 123 min). The longest tow recorded was almost 12 h (Table 1). Trawling took place at a mean towing speed of 2.9 Knots (sd = 0.3 Knots) (Table 1) and at a mean depth of 433 m (sd = 172 m), with a range from 95 to 657 m.

Table 1. Towing duration, towing speed and fishing depth descriptive statistics. The depth values are from the beginning and the end of each tow

	Mean	Minimum	Maximum	sd	n
Towing (h: min)	5:46	2:55	11:37	2:0 3	48
Towing speed (Knots)	2.9	2.5	3.8	0.3	46
Depth (m)	433	95	657	172	96

Discard catch composition

Due to the conditions aboard the trawlers, it was not possible to collect discard samples from all 48 tows. For some of the tows, observers were limited to making observations and collecting some data on the catches. Discards from a total of 25 tows were sorted and weighed according to the six groups of species. The contribution by weight of each group for all 25 tows pooled together is shown in Figure 2.

As can be seen, Teleosts (68%) and Chondrichthyes (14%) dominated the by-catch by weight. Analysis of variance showed that the proportions between tows of the different groups were significantly different ($p < 0.01$). However, all the groups were always represented in the discard catch. Mean discards per tow were: 45.0 kg (sd = 40.5) Teleosts, 6.9 kg (sd = 11.1) Chondrichthyes, 4.2 kg (sd = 7.2) Others, 2.7 kg (sd = 5.0) Cephalopoda, 2.5 kg (sd = 2.8) Crustacea and 1.4 kg (sd = 2.3) Gastropoda. Teleosts (78%) and Crustacea (11%) dominated the discards in numbers in 14 tows where the numbers of each species were recorded (Fig. 3).

A total of 91 species were identified, 47 vertebrates and 44 invertebrates corresponding to 65 families. The families identified are distributed among the main groups of species as follows: Teleosts 29 (44.6%), Crustaceans 18 (27.7%), Others 8 (12.3%), Chondrichthyes 4 (6.2%), Cephalopoda 4 (6.2%) and Gastropoda 2 (3.1%) (Tables 2 and 3).

The 10 most important species in number and weight are listed in Tables 4 and 5. As can be seen in Table 4, 9 fish and 1 crustacean species accounted for more than 82% of all the discarded biomass. The species *Micromesistius poutassou* (34%), *Gadiculus argenteus* (10%) and *Hoplostethus mediterraneus* (8%) were the three most important species

Table 2. Invertebrate discard species composition by number and weight

Species	Family	Species group	Percent of total weight (16 tows)	Percent of total number (14 tows)
Actinauge richardi	Hormathiidae	Others	1.1834	1.2203
Anseropoda placenta	Asterinidae	Others	0.0002	0.0032
Aphrodite aculeata	Aphroditidae	Others	0.0166	0.0097
Argobuccinum olearium	Cymatiidae	Gastropoda	1.4961	0.3485
Aristeus antennatus	Aristeidae	Crustacea	0.0915	0.4516
Astropecten aranciacus	Astropectinidae	Others	0.0030	0.0258
Bathynectes maravigna	Portunidae	Crustacea	1.9126	4.2961
Calappa granulata	Calappidae	Crustacea	0.3416	0.1224
Calliactis parasitica	Hormathiidae	Others	0.1223	0.3477
Cassidaria tyrrhena	Cassidae	Gastropoda	0.4980	0.4089
Charonia lampas	Cymatiidae	Gastropoda	0.0507	0.0032
Dardanus arrosor	Diogenidae	Crustacea	0.1368	0.1996
Echinus acutus	Echinidae	Others	0.0158	0.0773
Eledone moschata	Octopodidae	Cephalopoda	0.4154	0.0773
Geryon longipes	Geryonidae	Crustacea	0.2788	0.0869
Goneplax rhomboides	Goneplacidae	Crustacea	0.0018	0.0193
Homola barbata	Homolidae	Crustacea	0.0149	0.0547
Illex coindetii	Ommastrephidae	Cephalopoda	0.5064	0.0676
Liocarcinus depurator	Portunidae	Crustacea	0.1829	0.4701
Macropipus tuberculatus	Portunidae	Crustacea	0.0893	0.4194
Monodaeus couchii	Xanthidae	Crustacea	0.0009	0.0032
Munida rugosa	Galatheidae	Crustacea	0.0485	0.1449
Neorossia caroli	Sepiolidae	Cephalopoda	0.1432	0.1964
Nephrops norvegicus	Nephropidae	Crustacea	0.0932	0.0869
Octopus salutii	Octopodidae	Cephalopoda	0.0116	0.0064
Ophiura texturata	Ophiuridae	Others	0.0580	0.5023
Pagurus alatus	Paguridae	Crustacea	0.0183	0.1642
Pagurus sp.	Paguridae	Crustacea	0.0130	0.0853
Parapenaeus longirostris	Penaeidae	Crustacea	0.2810	0.7124
Parthenope macrochelos	Parthenopidae	Crustacea	0.0021	0.0032
Pasiphaea sivado	Pasiphaeidae	Crustacea	0.0004	0.0129
Plesionika ensis	Pandalidae	Crustacea	0.1437	0.8726
Polybius henslowii	Portunidae	Crustacea	0.7009	2.2459
Polycheles typhlops	Polychelidae	Crustacea	0.1390	0.3027
Pontocaris lacazei	Crangonidae	Crustacea	0.0002	0.0032
Rossia macrosoma	Sepiolidae	Cephalopoda	0.3250	0.1256
Scaeurgus unicirrhus	Octopodidae	Cephalopoda	0.0110	0.0097
Scalpellum scalpellum	Scalpellidae	Crustacea	0.0020	0.0064
Sepia elegans	Sepiidae	Cephalopoda	0.0556	0.0869
Sepia orbignyana	Sepiidae	Cephalopoda	0.0033	0.0032
Sepietta oweniana	Sepiolidae	Cephalopoda	0.0029	0.0193
Sphaerechinus granularis	Toxopneustidae	Others	0.0113	0.1811
Stichopus regalis	Stichopodidae	Others	0.0653	0.0483
Todaropsis eblanae	Ommastrephidae	Cephalopoda	0.7491	0.1288

Table 3. Vertebrate discard species composition by weight and number

Species	Family	Species group	Percent of total weight (16 tows)	Percent of total number (14 tows)
Antonogadus megalokynodon	Gadidae	Teleost	0.0393	0.0998
Breviraja sp.	Rajidae	Chondrichthyes	0.0137	0.0386
Capros aper	Caproidae	Teleost	0.0100	0.0129
Chaunax pictus	Chaunacidae	Teleost	0.0218	0.0386
Chimaera monstrosa	Chimaeridae	Chondrichthyes	0.8093	0.0773
Chlorophthalmus agassizii	Chlorophthalmidae	Teleost	0.0390	0.0483
Citharus linguatula	Citharidae	Teleost	0.0250	0.0161
Coelorhynchus coelorhynchus	Macrouridae	Teleost	0.8938	0.6536
Conger conger	Congridae	Teleost	3.5381	0.6222
Dalatias licha	Squalidae	Chondrichthyes	0.0362	0.0032
Deania calceus	Squalidae	Chondrichthyes	0.5008	0.2165
Dicologoglossa cuneata	Soleidae	Teleost	0.0012	0.0072
Diretmoides parini	Diretmidae	Teleost	0.0033	0.0032
Etmopterus pusillus	Squalidae	Chondrichthyes	1.6802	0.6061
Etmopterus spinax	Squalidae	Chondrichthyes	4.8522	2.3763
Facciolella oxyrhyncha	Nettastomatidae	Teleost	0.0321	0.0354
Gadiculus argenteus	Gadidae	Teleost	9.8148	29.4038
Galeus melastomus	Scyliorhinidae	Chondrichthyes	5.2697	3.4597
Helicolenus dactylopterus	Scorpaenidae	Teleost	0.6209	0.1642
Hoplostethus mediterraneus	Trachichthyidae	Teleost	8.1397	20.7697
Lepidopus caudatus	Trachiuridae	Teleost	0.5007	0.1803
Lepidorhombus boscii	Scophthalmidae	Teleost	0.2300	0.1127
Lophius piscatorius	Lophiidae	Teleost	0.1572	0.0773
Macroramphosus scolopax	Macroramphosidae	Teleost	0.1367	0.2286
Malacocephalus laevis	Macrouridae	Teleost	3.3191	1.1664
Merluccius merluccius	Merlucciidae	Teleost	0.5369	0.5611
Micromesistius poutassou	Gadidae	Teleost	33.9307	9.7803
Mora moro	Moridae	Teleost	0.0470	0.1352
Myctophidae	Myctophidae	Teleost	0.0006	0.0097
Nemichthys scolopaceus	Nemichthyidae	Teleost	0.0046	0.0386
Nezumia sclerorhynchus	Macrouridae	Teleost	7.8355	10.1047
Notacanthus bonapartei	Notacanthidae	Teleost	0.0114	0.0169
Notacanthus chemnitzii	Notacanthidae	Teleost	0.0059	0.0072
Ophisurus serpens	Ophichthidae	Teleost	0.0093	0.0032
Peristedion cataphractum	Peristediidae	Teleost	0.0093	0.0097
Phycis blennoides	Gadidae	Teleost	3.7271	2.1758
Polymetme corythaeola	Photichthyidae	Teleost	0.6793	1.4618
Raja oxyrinchus	Rajidae	Chondrichthyes	0.0079	0.0097
Raja sp.	Rajidae	Chondrichthyes	0.0657	0.0676
Scomber scombrus	Scombridae	Teleost	0.0942	0.0451
Scyliorhinus canicula	Scyliorhinidae	Chondrichthyes	1.4490	0.1030
Serranus hepatus	Serranidae	Teleost	0.0101	0.0322
Symphurus ligulatus	Cynoglossidae	Teleost	0.0067	0.0274
Synaphobranchus kaupi	Synaphobranchidae	Teleost	0.0377	0.1884
Trachurus picturatus	Carangidae	Teleost	0.3621	0.0773
Trachurus trachurus	Carangidae	Teleost	0.2381	0.0362
Venefica proboscidea	Nettastomatidae	Teleost	0.0090	0.0290

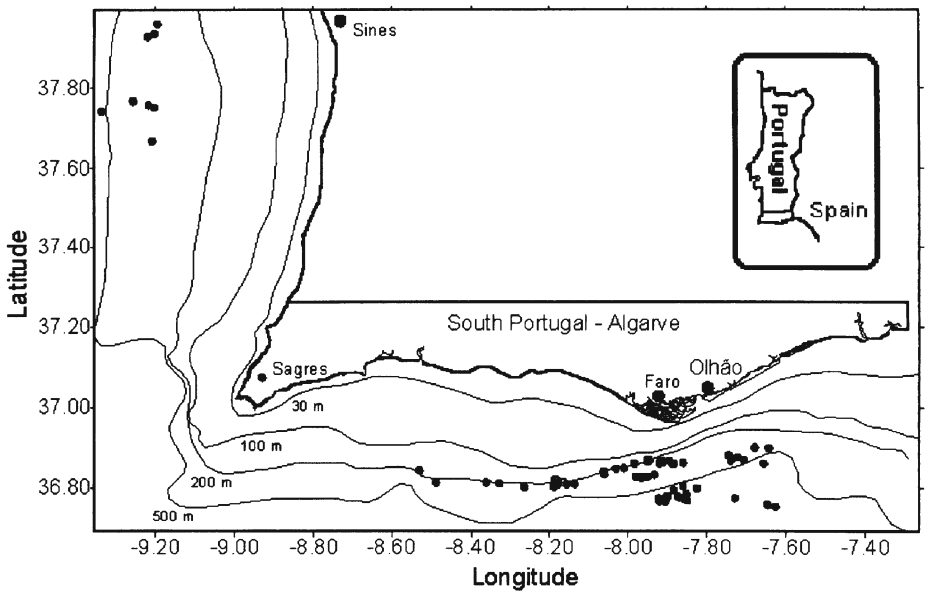

Figure 1. Geographic location of each tow. Each point marks the beginning or end of a tow.

Table 4. Percentage of total weight of top 10 main discard species (16 tows)

Species	Species group	Percent
Micromesistius poutassou	Teleost	33.93
Gadiculus argenteus	Teleost	9.82
Hoplostethus mediterraneus	Teleost	8.14
Nezumia sclerorhynchus	Teleost	7.84
Galeus melastomus	Chondrichthyes	5.27
Etmopterus spinax	Chondrichthyes	4.85
Phycis blennoides	Teleost	3.73
Conger conger	Teleost	3.54
Malacocephalus laevis	Teleost	3.32
Bathynectes maravigna	Crustacea	1.91
Total		**82.35**

Table 5. Percentage of total number of top 10 main discard species (14 tows)

Species	Species group	Percent
Gadiculus argenteus	Teleost	29.40
Hoplostethus mediterraneus	Teleost	20.77
Nezumia sclerorhynchus	Teleost	10.11
Micromesistius poutassou	Teleost	9.78
Bathynectes maravigna	Crustacea	4.30
Galeus melastomus	Chondrichthyes	3.46
Etmopterus spinax	Chondrichthyes	2.38
Polybius henslowii	Crustacea	2.25
Phycis blennoides	Teleost	2.18
Polymetme corythaeola	Teleost	1.46
Total		**86.07**

in weight. However, species that were important in weight were not necessarily as important in discard numbers. As is shown in Table 5, *Gadiculus argenteus* (29%), *Hoplostethus mediterraneus* (21%) and *Nezumia sclerorhynchus* (10%) accounted over 60% of all discards in numbers.

As seen in Tables 4 and 5 the major discard species, in number and weight, are essentially all Teleosts and Chondrichthyes. The only two species from the other major groups were *Bathynectes maravigna* (Crustacea) and *Polybius henslowii* (Crustacea).

Target crustaceans identified in the discards essentially consisted of specimen parts and were relatively unimportant in the discards. This component of the discards resulted from damage in the net and during the sorting process. *Merluccius merluccius*, an important commercial species, was discarded quite frequently, with most discards consisting of individuals below the minimum landing size of 27 cm total length. Commercial fish species were also occasionally discarded because of legislation limiting the percentage

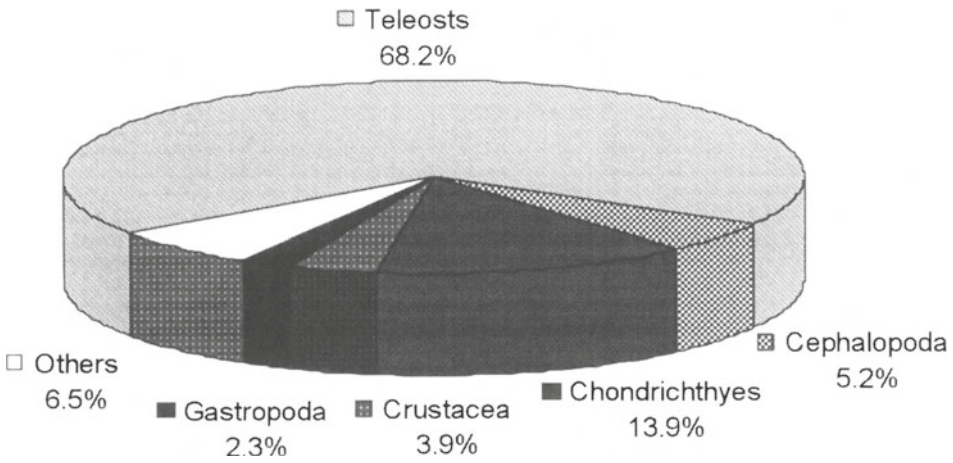

Figure 2. Discard composition by main species groups in weight. Pooled information from 25 tows.

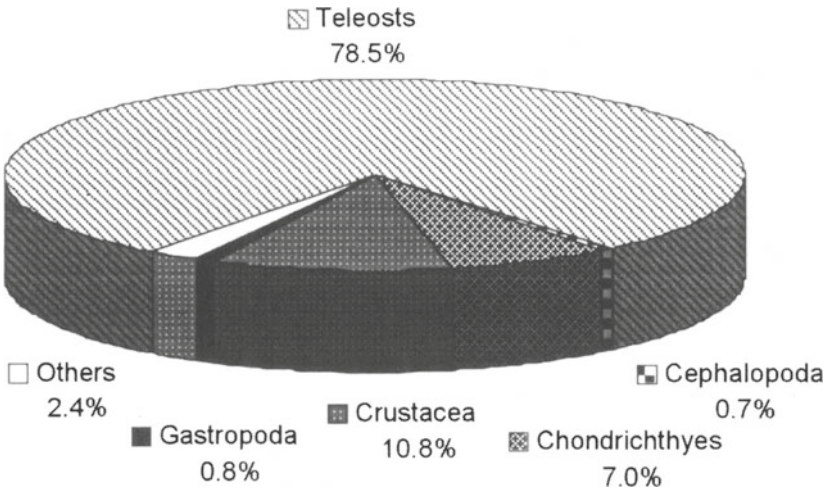

Figure 3. Discard composition by main species groups in number. Pooled information from 14 tows.

of fish by-catch that can be landed in this crustacean trawl fishery.

Landed catch and discard rate

The mean commercial crustacean catch per tow was 97 kg, ranging from a minimum of 10 kg to a maximum of 248 kg (Table 6). Discarded species varied from 6 kg to 170 kg per tow, with a mean of 58 kg per tow (Table 6). The mean discard rate per tow was 37% (sd = 21) ranging from 5% to 76% (Table 6).

As expected, the most frequent species were the three major species of this fishery: the shrimp *P. longirostris*, the red shrimp *A. antennatus* and the Norway lobster *N. norvegicus*. Figure 4 shows the frequency of occurrence in the sampled tows of the commercial crustacean species. *Parapenaeus longirostris* was the most frequently caught with 97% occurrence. The Norway lobster and red shrimp had similar frequencies, respectively, 56% and 45%. Three other crustacean species, *A. foliacea*, *P. edwardsianus* and *Plesionika* sp., having high commercial value were also landed but less frequently caught. Figure 5 shows the mean catches in weight, per tow, of each of the commercial crustacean species.

In addition to the above commercial crustaceans, 21 species of fishes and molluscs of commercial value were also caught as by-catch (Fig. 6). However, only two species (hake, *Merluccius merluccius* and angler fish *Lophius piscatorius*) occurred frequently as landed by-catch with 91% and 70% of frequency of presence, which, due to their high value, made

Table 6. Mean catches of landed crustacean and discard species per tow in the trawl fishery

	Mean	Minimum	Maximum	sd	*n*
Discard species (Kg)	58.1	6.2	169.1	45.6	28
Landed crustacean species (Kg)	96.9	9.6	248.5	57.9	37
Discard rate (%)	37.5	5.4	76.1	21.0	23

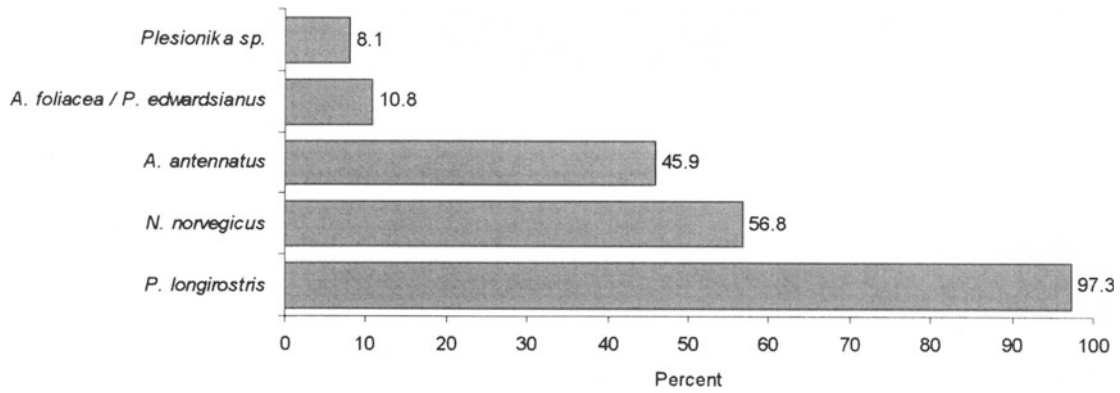

Figure 4. Frequency of presence of main target crustacean species. Pooled information from 37 tows.

important contributions to the value of the landings (Fig. 6). The remaining species were occasional in the landed catch and occurred in less than 50% of the tows (Fig. 6). They were generally discarded due to low value and/or small quantities caught.

Discussion

Long tows along with the non-selective characteristics of the trawl net used lead to the capture of considerable quantities of by-catch per tow. Discard quantities and rates in this study were high but less than those reported by Borges et al. (1997) for the same fishery. The discarded proportion of the catch in the latter study ranged from 26 to 91% of the catch in weight, with an average of 70%. The difference in results is even higher if we consider that in the present study, the biomass comprising landed by-catch was not taken into consideration in the calculation of discard rates. These differences between the findings of the two studies can only be explained by inter-annual variability, with marked differences in the abundance of some of the key Teleost discard species from year to year. As in the previous study (Borges et al., 1997), discards were dominated by a few vertebrate and invertebrate species. These two groups had almost the same specific richness in the catch; 47 vertebrate and 44 invertebrate discard species were identified, respectively. However, there were significant differences in terms of biomass and numbers, with Teleosts and Chondrichthyes accounting for 82% and 85% of the total discards in weight and number. Although catches varied considerably in terms of specific composition from tow to tow, the relative importance of each of the six main species groups was fairly constant.

Discarded by-catches were dominated by 10 species of which the most important by number and weight were *M. poutassou, G. argenteus, H. mediterraneus* and *N. sclerorhynchus*. These four species represented about 60 and 70% of all discards in this study, respectively, by number and weight. These results are somewhat different from the ones obtained in the study by Borges et al. (1997), where a greater number of species were reported. There was a significant difference in terms of specific composition between the two studies. Whereas, for example, in the current study *M. poutassou* (34%) was the dominant species in terms of weight, in the previous study it was the sixth most important species, behind *Torpedo nobeliana* (14.8%), *S. canicula* (14.6%), *C. conger* (10.4%), *C. aper* (6.0%) and *M. merluccius* (4.8%). *T. nobeliana* was not caught at all in the present study.

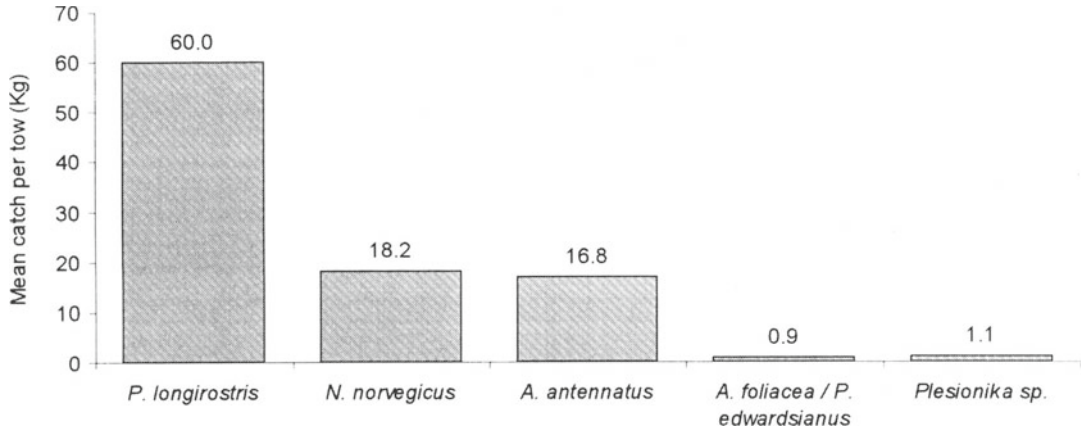

Figure 5. Mean catch in kilograms, per tow, of the main target crustacean species. Pooled information from 33 tows.

As noted above, these differences are probably due to inter-annual variability and changes in abundance, in particular of small, schooling species such as *M. poutassou* and *C. aper.*

Only a few other studies have examined discards in southern European or nearby waters. Balguerías (1997) studied the discards of the cephalopod trawl fishery on the continental shelf off the Saharan coast (21°N–26°N) and reported a mean discard rate of 66% of the total catch with discards consisting of at least 60 fish species. Invertebrates were a significant component of the discards and the fish discards consisted mainly of small pelagics and under-sized sea breams (Sparidae).

Tursi (1994) sampled two trawlers once a month in the Mediterranean (Ionian Sea), and found that a total of 64 fish species were discarded. Of these, 29 had no commercial value while 36 were discarded because the fish were under-sized. The two trawlers discarded 47% and 45% of the catch in weight.

The first comprehensive study of trawl discards in the western Mediterranean (Spain and Italy) was carried out with sampling on board commercial trawlers from June 1995 to June 1996 (Carbonell, 1997). Stratification was by port (7) and depth (A:< 150 m, B:151–350 m, and C:> 350 m). Additionally, trawl type (2) and horse power (<, > 150 h.p.) were also used to stratify the sampling in two of the seven ports. A total of 609 species in 14 major taxonomic groups were caught, of which only 20% were commercially exploited and landed. Fish dominated the discards (Osteichthyes: 128 species and Chondrychthyes: 24 species). Discarding rates were highly variable, with means ranging from 13.1% to 52.5% of the total catch among the different ports. Depth was an important factor influencing commercial fraction, discard rates, discard composition and reasons for discarding. The discarded biomass ranged from a low of 2.2 kg/h at depths greater than 350 m to 118.3 kg/h in the lower depth stratum. Invertebrates and algae contributed significantly to the discards in stratum A, while potentially commercial species were particularly important in stratum B and non-commercial species dominated at the greater depths.

More information is needed to improve our understanding of the impact of discarding on multi-species fisheries such as the one studied here. The exploited species are interdependent through competition and predator–prey relationships. In addition to the direct impact on populations of discard related mortality, there are ecosystem and trophic level effects which are poorly understood. Any effect on one stock, population or species may produce a change in another, resulting in readjustment in both populations (Hongskul, 1979; Saila, 1983; Alverson et al., 1994; McAllister & Spiller, 1994; Kennelly, 1995).

There is some evidence that the introduction of food in the form of discards to scavengers can lead to significant ecological changes (Harris & Poiner, 1990; Wassenberg & Hill, 1990; Alverson et al., 1994; Kennelly, 1995; Yamamura, 1997). Discards represent an introduction of more or less localised but important quantities of energy and may constitute a perturbation of the trophic system, resulting in significant non-selective predation and scavenging. This energy is often directly available to the higher trophic levels, namely marine birds and large demersal scavengers such as sharks. Pauly & Christensen (1995) reported

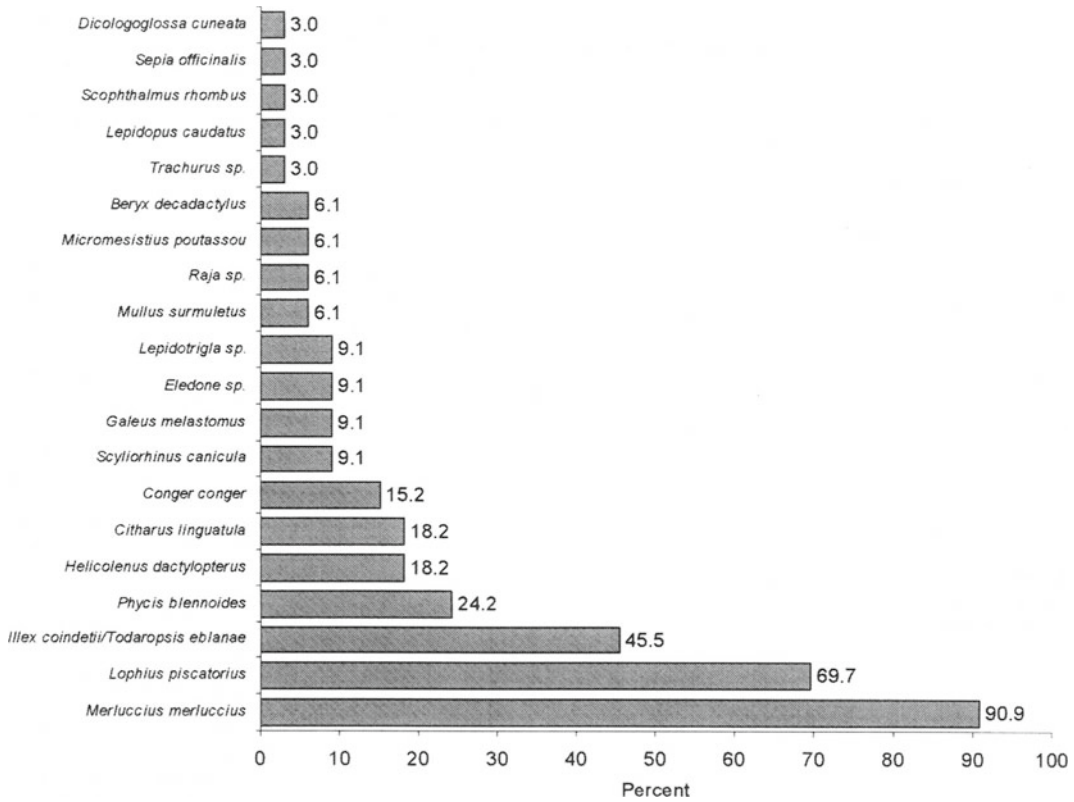

Figure 6. Landed by-catch species' frequency of presence. Pooled information from 33 tows.

that the contribution of recycled discards to the energy budget may be significant in some marine ecosystems and discards were shown to contribute a significant part of the diet of many species of sea birds in areas such as the North Sea (Garthe et al., 1996).

Future studies must focus on understanding the fate of discards, their impact on the marine ecosystem and on mitigation. From an economic perspective, there are possibilities of making a better use of some discarded species, thereby possibly reducing the pressure on target species. Technical measures such as the use of square meshes and grids can be used to significantly reduce by-catch in trawl fisheries (Kennelly, 1995). The critical fact is that sustainable management of fishing resources must take place in the ecosystem context, with a good understanding of all the possible effects of fishing activities.

Acknowledgements

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Effect of codend mesh size on the performance of the deep-water bottom trawl used in the red shrimp fishery in the Strait of Sicily (Mediterranean Sea)

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Key words: bottom trawl, codend retention, deep-water shrimp, *Aristaeomorpha foliacea*, Mediterranean Sea

Abstract

The influence of different codend nets mounting diamond-shaped (16, 20, 24, 28 and 45 mm, mesh side) in the performance of the commercial bottom trawl presently in use in the Strait of Sicily (Central Mediterranean Sea) to catch deep-water red shrimps, *Aristaeomorpha foliacea* and *Aristeus antennatus*, is analyzed. In two surveys carried out in fishing grounds in spring and summer 1993, 93 daylight hauls (of 3 hours each) based on the covered codend (cover of 14 mm mesh side) method were carried out. The composition and quantity of the combined, retained and escaped catch for the most relevant species and mixed categories of the different gear configurations (codends + cover) were analyzed and compared by applying both univariate (ANOVA) and multivariate (clustering and multidimensional scaling) techniques. The reduction of by-catch, the economic loss and the economic efficiency were used as indexes of the retention performance of the different codends. The overall catch was 6398 t (23 kg/h); *Aristaeomorpha foliacea* accounts for most of the catch in number, weight (48% of the total) and value (87% of the total value). Bony fishes, cartilaginous fishes and other crustaceans are the by-catch, representing, respectively, 26%, 16% and 9% of the catch in weight, the rest belonging to cephalopods. The use of the different codends does not influence the overall performance of the gear. No saturation was detected, with the escapement ratio uninfluenced by the amount retained. Considering the retention characteristics, results indicate that the 16 mm and 20 mm mesh codends are not selective, whereas the 24/14 and 28/14 configurations produce increased escaping ability. The 45 mm mesh sieves out 79% of specimens, indicating that no masking effect was induced by the cover. Regarding economic efficiency, the 20/14 configuration provides values comparable to those obtained with the 24/14, whereas the 16/14 configuration is as efficient as the 28/14. The lowest efficiency was obtained using the 45/14 configuration. Multidimensional scaling and clustering techniques (performed on 20, 24 and 28 mm codends) allow to discriminate the escapement ability of the 28/14 gear configuration only. Present results support the introduction of the 28 mm mesh codend in the deep-water red shrimp fisheries in the view of a precautionary management of the resources.

Introduction

The Mediterranean bottom trawl fisheries are traditionally characterized by the use of very small diamond-shape mesh sizes in the codends (about 14–16 mm side), which practically tend to retain almost all the animals encountered by the gear (Ragonese et al., 1994; Stergiou et al., 1997). Fishery scientists and regulatory agencies have demanded an increase in the mesh size as a management tool to reduce the fishing pressure on juveniles and to improve the state of the resources (Caddy, 1990). At the moment, both Italian and European legislation require a minimum of 20 mm mesh side (European Union, 1994), but fishermen are reluctant to adopt such new mesh, given the necessity to exploit a mixture of species which show higher growth rates, and shorter life span than the Atlantic counterpart on one hand and the aim to catch some

small-size species or species which reach the maximum value in the juvenile stage (Caddy, 1993) on the other.

However, given that no recruitment failure has been detected for most important demersal species, mainly as a consequence of the partial unavailability to the trawl of the spawning stock (Caddy, 1990; Abella et al., 1997), a mesh size of 20 mm side is generally considered a reasonable compromise for the Mediterranean fisheries traditionally exerted on the shelf and upper slope.

Conversely, there is some doubt about the plausibility of this mesh size to catch the deep-water red shrimps, *Aristaeomorpha foliacea*, Risso 1827 and *Aristeus antennatus*, Risso 1816. Such fishery pursues large-size shrimps (carapace length, CL, greater than 30–40 mm), which are priced 3–4 times the smaller ones. As a matter of fact, this fishery can be generally considered as monospecific since no economically important species are caught in the same time in such a quantity to represent a sought-after prey for fishermen (Bianchini & Ragonese, 1994).

At the moment, the use of mesh size smaller than 20 mm in these deep-water fisheries lead to the capture of by-catch species both of commercial value for the shelf and upper slope fisheries (such as the rockfish *Helicolenus dactylopterus dactylopterus*) and species that are not marketable at all (such as the small-size shark *Galeus melastomus*). The by-catch is almost entirely discarded in the Strait of Sicily given the limited storage capability of the fishing vessels, which stay at sea for two or more weeks.

In 1993, a research program funded by the European Union was started with the aim of analyzing the retention of the commercial bottom trawl for the red shrimp fishing, using the covered codend method.

The basic assumptions in such studies are that the different codends do not affect the overall performance of the gear (Pope et al., 1975), that the mesh size of the cover is completely non-selective for the investigate species and that the observed differences in selectivity are significant (Bergh et al., 1990).

In the present paper, these basic assumptions were tested by analyzing the overall performance of different gear configurations (GC= codend + cover) and by comparing the retention efficiency (in term of by-catch reduction, economic loss and economic return of the catch retained in the codends, taking also into account the amount of the filtered component), with both univariate and multivariate techniques.

Materials and methods

The area of study belongs to the central basin of the Mediterranean Sea (FAO fishing zone 37.2.2), known as the Strait of Sicily, an area routinely monitored by the Institute of Mazara (Levi et al., 1998). In this area, four major deep-water red shrimp fishing grounds are found (Fig. 1): close to Marettimo (Egadi Islands; MA), North-West (NW), South-East (SE) and North-East (NE) of Linosa (Pelagie Islands).

Data were collected within two periods, during spring (12–27 May 1993) and summer (22 July–8 August 1993), i.e. for 14 and 16 days, respectively. The technically difficult MA zone, however, was not sampled in summer. Data were obtained in daytime (when red shrimps are most vulnerable to the gear; Bianchini et al., 1998a) by sampling commercial locations and emulating the professional procedures. The duration of the hauls (3 h each) was chosen as a compromise between the fishermen habits (from 1–2 up to 6 hours) and the requirement to increase the sample size. The hauls were distributed within 500–700 m depth, which is the bathymetric range preferred by the fishermen (Ragonese, 1995), since trawling for red shrimp outside this range yields low catches or presents technical difficulties.

Sampling was conducted with the commercial stern trawler 'Sant'Anna' of 197 gross registered tonnage and powered by a 1012 HP engine. A commercial nylon and polyamide trawl net was used (Fiorentini & Cosimi, 1987); the length of the head and lead ropes of the gear was 66 m and 67 m, respectively. Steel and polyamide lines ('calamenti', 231 m long) linked the gear to the steel otter boards (oval shaped, 2.3×1.1 m, weighing 400 kg each). The otter boards were attached to the boat by a steel cable ($\varnothing$14.5 mm).

The performance of five different codends with diamond-shaped, cast, non-knotted mesh size of 16, 20, 24, 28 and 45 mm (knot to knot; nominal size) was assessed by using the covered codend method, the elective choice in selectivity studies (Pope et al., 1975; Mac Lennan, 1992; Millar & Walsh, 1992). Each codend resembled a cylinder of about 5 m length, with a frontal mesh number of 380, 260, 210, 180 and 150 for the 16, 20, 24, 28 and 45 mm, respectively. Each codend was covered with a single type of cover, of 14 mm mesh, about 10 m long, larger than the codend and with a frontal mesh number of 420; plastic balls at the top of the cover prevented it from collapsing. The correspondence between nom-

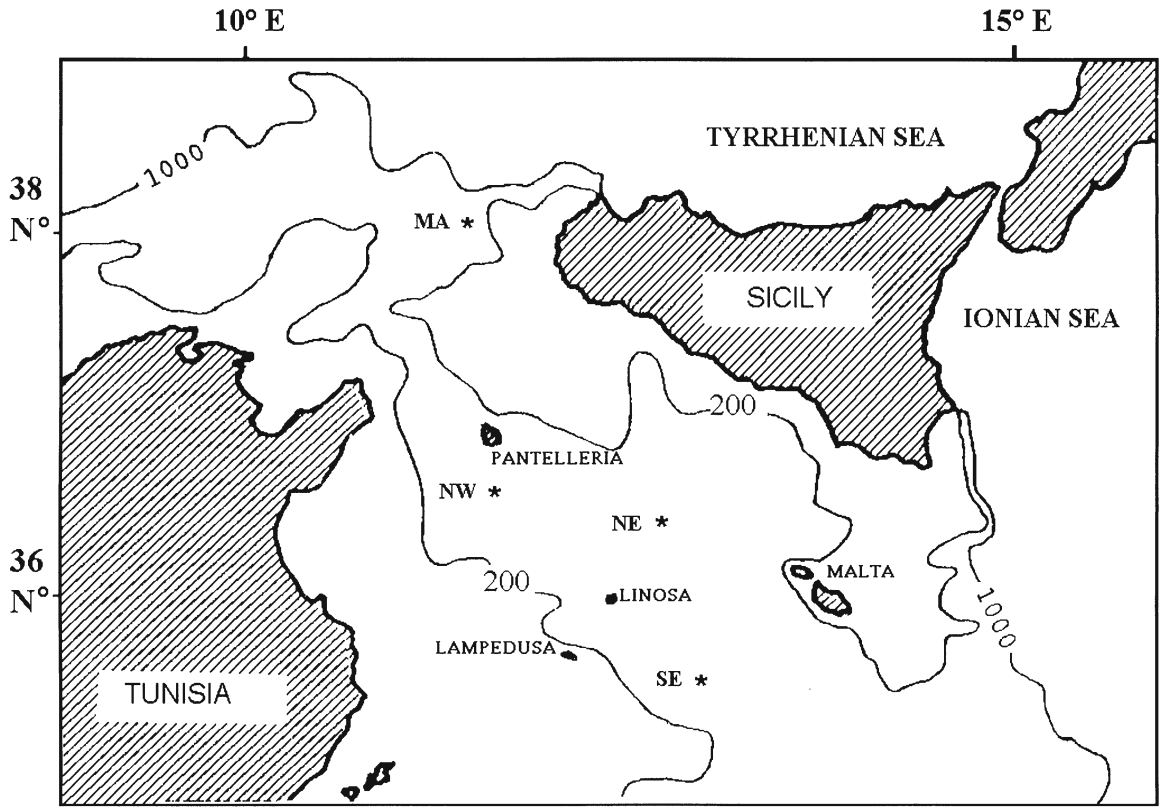

Figure 1. Location of the deep-water red shrimp fishing grounds within the Strait of Sicily (Mediterranean Sea). NW, NE and SE: North-West, North-East and South-East of Linosa Island; MA: Marettimo (depth contours in meters).

inal and effective mesh sizes was tested according to the standard methodology (UNI rule no 8783; 1985).

For each valid haul and gear configuration, both the retained (IN= inside the codend) and escaped (OUT= inside the cover) catch in number and weight (kg) were recorded for selected species and categories, IN+OUT giving the total catch (TOT). A complete systematic classification was beyond the aim of this research, but a list of species was obtained by sub-sampling the catch.

The escapement (selection) proportion, i.e. the ratio of the escaped vs. the total catch (ER= OUT/TOT;%) was computed for both the catch in number (ERn) and weight (ERw) for each gear configuration (GC). This proportion represents an index of the ability of certain species to escape from a given codend (Liu et al., 1985).

Three market codes ($= high value, *= medium-low value and §= discard) and an average value per kilogram in Euro (price €/kg; where 1 € is about 1 U.S.$) were assigned to each species/category of the catch. Medium-low market codes do not mean necessarily a commercial value since some species/categories, although sold by small-scale fisheries, are normally discarded (or used for internal consumption) in the deep-water red shrimp trawl fisheries. In order to take into account this 'technical interaction', a rough value was assigned also to the categories which include these commercial/discarded species.

As concerns the economic performance of the different codends, the loss in economic value (EL= €.OUT/€.TOT;%) and the economic efficiency (EE= €.IN/kg.TOT) were computed and compared. The first parameter is fisherman-oriented, since it considers only the immediate monetary loss which could be expected by enlarging the codend mesh size. The second parameter is more ethically-oriented, taking into account the total catch in terms of biomass which would be captured (and mostly wasted) by employing the cover mesh size (14 mm).

The packages PRIMER (Clarke & Warwick, 1994) and Systat 8.0 (Systat, 1998) were used for computations. Differences were statistically tested (by assuming p= 0.05 as significant level) only for the overall

escapement ratio and catch of the gear configurations. All statistical analyses were conducted on transformed data ($\sqrt{x}$, $\log_e x+1$ and $2*\text{arcsinh}*\sqrt{x}$, respectively, for numbers, weights and ratios; Neter et al., 1985). For the escapement ratio of the pooled catch, the means per haul and standard deviations by gear configuration were obtained by retransformation of the corresponding statistics estimated on $\text{arcsinh}*\sqrt{x}$ values. A sub-sample of the data, corresponding to only one selected fishing ground and season (SE; summer), was analyzed (ANOVA) to compare the overall catch (i.e., independently from the species composition) obtained with three of the codends employed (20, 24 and 28 mm mesh) and to test the independence between the escapement ratio and the amount of the overall retained catch (simple linear regression). A comparison of the three selected gear configurations based on the corresponding catch compositions was carried out using multivariate analyses (clustering and multidimensional scaling methods) according to a procedure already employed in similar cases (Stergiou et al., 1997; Hobday et al., 1999).

Distance matrices of similarity between all pairs of station/gear configuration were obtained following the Bray–Curtis ordination (Bray & Curtis, 1957), and thereafter submitted to both clustering (dendrograms employing group average linking) and multidimensional scaling (MDS) techniques. The MDS results are presented as two-dimensional 'maps' of station/codend with overlaid clustering lines: the more similar (as concerns the catch composition) the stations are, the closer they are on the map; the configuration is arbitrarily oriented and scaled. The goodness of fit of each of MDS plot was evaluated on the basis of its stress coefficient (Field et al., 1982; Clarke & Warwick, 1994). Differences between gear configurations can be considered as real only when the two approaches agree (Clarke & Green, 1988).

Results

A total of 132 taxa were identified during the study, distributed among bony fish (61), cartilaginous fish (20), cephalopods (26) and crustaceans (25). The scientific or vernacular names of the considered taxa/categories, the frequency of occurrence in the samples, the relative contribution to weight and value of the catch are presented in Table 1 (species compositions of the mixed categories are listed as footnotes).

Considering the 93 valid hauls, the overall catch was 410 148 specimens and 6398 kg (23 kg/h), for an economic value of 40 287 €. Crustaceans were the most abundant group (57% in weight), yielding also

Table 1. Species and categories for spring, summer and seasons combined, in the Sicilian red shrimp fishery. Market code: $ high, * medium-low, § discard-price: average rough commercial price in €/kg - freq%: prevalence in the hauls – weight%: percentage of the overall catch in weight – value%: percentage of the total commercial value. Actual absolute numbers denote the total number of hauls, the overall catch (in kg) and its total value (in €). Lists of taxa identified in the catch and included within the mixed categories as footnotes

Species/category	Market code	Price €/kg	Spring			summer			Seasons combined		
			freq%	weight%	value%	freq%	weight%	value%	freq%	weight%	value%
Crustaceans											
Aristaeomorpha foliacea	$	11.5	100.0	37.0	79.9	100.0	57.5	92.2	100.0	47.7	87.2
Aristeus antennatus	$	9	82.0	3.0	5.0	90.7	1.0	1.3	87.1	2.0	2.8
Nephrops norvegicus	$	11.5	94.9	2.7	5.8	94.4	0.9	1.5	94.6	1.8	3.2
Parapenaeus longirostris	$	7	59.0	1.3	1.8	96.3	1.1	1.1	80.6	1.2	1.4
Mixed crustaceans	*/§	0.25	100.0	5.0	0.2	100.0	4.3	0.1	100.0	4.7	0.2
Total crustaceans	$/*/§		100.0	49.0	92.7	100.0	65.0	96.2	100.0	57.4	94.8
Cephalopods											
Todarodes sagittatus	*	0.5	76.9	1.1	0.1	53.7	0.9	0.1	63.4	1.0	0.1
Mixed octopuses	*	3.75	84.6	0.2	0.1	66.7	0.1	0.1	74.2	0.2	0.1
Mixed squids and cuttlefishes	*/§	1	41.0	0.3	0.0	83.3	0.4	0.1	65.6	0.3	0.0
Total cephalopods	*/§		94.9	1.5	0.3	96.3	1.4	0.2	95.7	1.5	0.2

Continued on p. 283

Table 1. continued

Species/category	Market code	Price €/kg	Spring			summer			Seasons combined		
			freq%	weight%	value%	freq%	weight%	value%	freq%	weight%	value%
Bony fishes											
Conger conger	*	0	89.7	1.7	0.0	68.5	0.6	0.0	77.4	1.1	0.00
Helicolenus d. dactylopterus	*	0.5	64.1	1.2	0.1	88.9	0.7	0.0	78.5	0.9	0.1
Lepiaorhombus boscii	*	3.5	56.4	0.3	0.2	61.1	0.2	0.1	59.1	0.3	0.1
Merluccius merluccius	$	4	59.0	3.1	2.3	88.9	3.0	1.6	76.3	3.0	1.9
Micromesistius poutassou	*	0.5	53.8	1.2	0.1	94.4	1.5	0.1	77.4	1.3	0.1
Mullus surmuletus	$	5	51.3	0.6	0.6	57.4	0.1	0.1	54.8	0.4	0.3
Nettastoma melanurum	§	0	84.6	0.4	0.0	61.1	0.2	0.0	71.0	0.3	0.0
Phycis blennoides	*	0.5	100.0	6.3	0.6	100.0	4.3	0.3	100.0	5.2	0.4
Mixed anglerfishes	*	4	43.6	1.7	1.3	31.5	0.4	0.2	36.6	1.0	0.6
Mixed macrurid grenadiers	§	0	38.5	1.6	0.0	31.5	0.6	0.0	34.4	1.1	0.0
Mixed bony fishes	*/§	0.5	100.0	12.3	1.1	100.0	9.6	0.7	100.0	10.9	0.9
Total bony fishes	$/*/§		100.0	30.6	6.4	100.0	21.2	3.2	100.0	25.7	4.5
Cartilaginous fishes											
Galeus melastomus	§	0	100.0	8.7	0.0	98.1	4.8	0.0	98.9	6.7	0.0
Etmopterus spinax	§	0	94.9	5.0	0.0	96.3	3.2	0.0	95.7	4.1	0.0
Chimaera monstrosa	§	0	69.2	0.5	0.0	22.2	0.2	0.0	41.9	0.3	0.0
Mixed skates and rays	*/§	0.5	38.5	2.0	0.2	38.9	1.5	0.1	38.7	1.7	0.1
Mixed sharks	*/§	0.75	46.1	2.6	0.4	37.0	2.7	0.3	40.9	2.7	0.3
Total cartilaginous fishes	*/§		100.0	18.8	0.5	100.0	12.4	0.4	100.0	15.5	0.4
Actual absolute numbers	$/*/§		**39**	**3063**	**16302**	**54**	**3335**	**23985**	**93**	**6398**	**40287**

Mixed crustaceans: *Acanthephyra* sp. §; *Anamanthia rissoana* §; *Bathynectes maravigna* §; *Calappa granulata* *; *Geryon longipes* *; *Goneplax rhomboides* §; *Macropipus tuberculatus* §; *Maja squinado* *; *Monodaeus couchii* §; *Munida* sp. §; *Paguridae* §; *Pandalidae**; *Parasquilla ferussaci* §; *Paromola cuvieri* §; *Parthenope macrochelos* §; *Pasiphaea multidentata* *; *Pasiphaea sivado* *; *Polycheles typhlops* §; *Pontocaris lacazei* §; *Sergestes* sp. §; *Solenocera membranacea* §.

Mixed octopuses: *Bathypolypus sponsalis* *; *Eledone cirrhosa* *; *Octopus salutii* *; *Pteroctopus tetracirrhus* *; *Scaeurgus unicirrhus* *

Mixed squids and cuttlefishes: *Abralia veranyi* §; *Ancistrocheirus lesueri* §; *Ancistroteuthis lichtensteini* §; *Argonauta argo* (fresh shells) §; *Chiroteuthis veranyi* §; *Heteroteuthis dispar* *; *Histioteuthis bonnelli* §; *Histioteuthis reversa* §; *Illex coindetii* *; *Loligo forbesi* *; *Neorossia caroli* *; *Octopoteuthis sicula* §; *Onychoteuthis banksi* §; *Pyroteuthis margaritifera* §; *Rossia macrosoma* *; *Sepia elegans* *; *Sepia orbignyana* *; *Sepietta oweniana* *; *Sepiola* sp. *; *Todaropsis eblanae* *

Mixed anglerfishes: *Lophius budegassa* *; *Lophius piscatorius* *

Mixed macrurid grenadiers: *Coelorinchus coelorincus* §; *Nezumia sclerorhynchus* §

Mixed bony fishes: *Acantholabrus palloni* §; *Alepocephalus rostratus* §; *Argentina sphyraena* *; *Argyropelecus hemigymnus* §; *Bathypterois mediterraneus* §; *Bathysolea profundicola* §; *Boops boops* *; *Brama brama* *; *Capros aper* §; *Centrolophus niger* §; *Ceratoscopelus maderensis* §; *Chauliodus sloanei* §; *Chlorophthalmus agassizi* *; *Cubiceps gracilis* §; *Diaphus metopoclampus* §; *Diaphus rafinesquei* §; *Dysomma brevirostre* §; *Epigonus constanciae* §; *Epigonus denticulatus* §; *Evermanella balbo* §; *Gadella maraldi* §; *Gadiculus argenteus argenteus* §; *Gaidropsarus megalokynodon* §; *Gonostoma denudatum* §; *Hoplostethus mediterraneus* §; *Hymenocephalus italicus* §; *Lampanyctus crocodilus* §; *Lepidopus caudatus* *; *Lepidotrigla cavillone* §; *Lestidiops jayakari* §; *Lobianchia dofleini* §; *Molva dipterygia macrophthalma* §; *Molva molva* §; *Mora moro* §; *Mullus barbatus* *; *Myctophum puncatum* §; *Notacanthus bonapartei* §; *Notoscopelus elongatus* §; *Pagellus bogaraveo* *; *Peristedion cataphractum* *; *Polyprion americanus* *; *Scomber scombrus* *; *Scorpaena elongata* *; *Scorpaena scrofa* *; *Stomias boa* §; *Sudis hyalina* §; *Symphurus ligulatus* §; *Symphurus nigrescens* §; *Synchiropus phaeton* §; *Trachurus trachurus* *

Mixed skates and rays: *Raja alba* *; *Raja clavata* *; *Raja fullonica* *; *Raja melitensis* *; *Raja oxyrinchus* *; *Torpedo marmorata* §; *Torpedo nobiliana* §

Mixed sharks: *Centrophorus granulosus* *; *Centrophorus uyato* §; *Dalatias licha* §; *Heptranchias perlo* *; *Hexanchus griseus* *; *Mustelus asterias* *; *Oxynotus centrina* §; *Scyliorhinus canicula* *; *Squalus acanthias* *; *Squalus blainvillei* *

the highest contribution in value (95%), followed by bony fishes (26% in weight) which, however, had only a marginal economic interest (5%). The cartilaginous fishes added an appreciable weight (16%), but almost no value (< 1%); cephalopods were the least abundant and valuable group.

The target red shrimp, *Aristaeomorpha foliacea*, was the dominant species being captured in 100% of the hauls and contributing 48% of the catch in weight. Its relevance is highlighted by the fact that it represented almost entirely the total value of the catch (87%). All the other taxa/categories shared the rest of the catch, but none was close to the yield of the red shrimps. The other most abundant species were the shark *Galeus melastomus* and the bony fish *Phycis blennoides*. The former species is discarded (Ragonese et al., 2000) and the latter has a very low value. The catch of other high-prized species (the hake *Merluccius merluccius* and the crustaceans *Aristeus antennatus, Nephrops norvegicus* and *Parapenaeus longirostris*) was quite low (2–3% of the weight) and consequently their contribution to the global value was also marginal (about 1–3%). All the other medium-low or discarded species/categories showed a reduced occurrence (30–70%) and captures lower than 1% (number and weight), with only few exceptions, such as the discarded shark *Etmopterus spinax*.

Taking into account the seasons, catches were of comparable level, with the exception of few spring hauls in the SE and NE zones that yielded above-average catches. Mean values per standard haul (3 h each) resulted slightly higher in spring than summer both in weight (79 vs. 62 kg) and number (4891 vs. 4063 specimens). On the contrary, the mean value per haul was lower in spring than summer (418 vs. 444 €). Considering species and seasons, there are remarkably lower catches of red shrimp in spring, especially in weight (37% vs. 57%, i.e. 29 ± 13.5 vs. 36 ± 15.2 kg/haul on average). The differential catch is, however, distributed more or less evenly among the other species/categories. Considering the catch in weight per haul, important relative variations between spring and summer were observed in *A. antennatus, N. norvegicus, P. blennoides*, the anglerfishes, the grenadiers and the three most abundant species of sharks (Table 1).

Such seasonal pattern, however, are not so much evident when the zones are considered; for example, the mean catch per haul of *A. foliacea* was higher in spring (29.5 kg) than summer (26.3 kg) in the NE zone. Evidence of some spatial preference (results not reported) was also observed in *P. longirostris*, *N. norvegicus*, *P. blennoides*, *M. merluccius* and *Chimaera monstrosa*. Nevertheless, these seasonal and geographical differences must be considered only as indicative given the reduced overall sample size, the large standard error associated to the means and the low frequency of occurrences of many of the captured

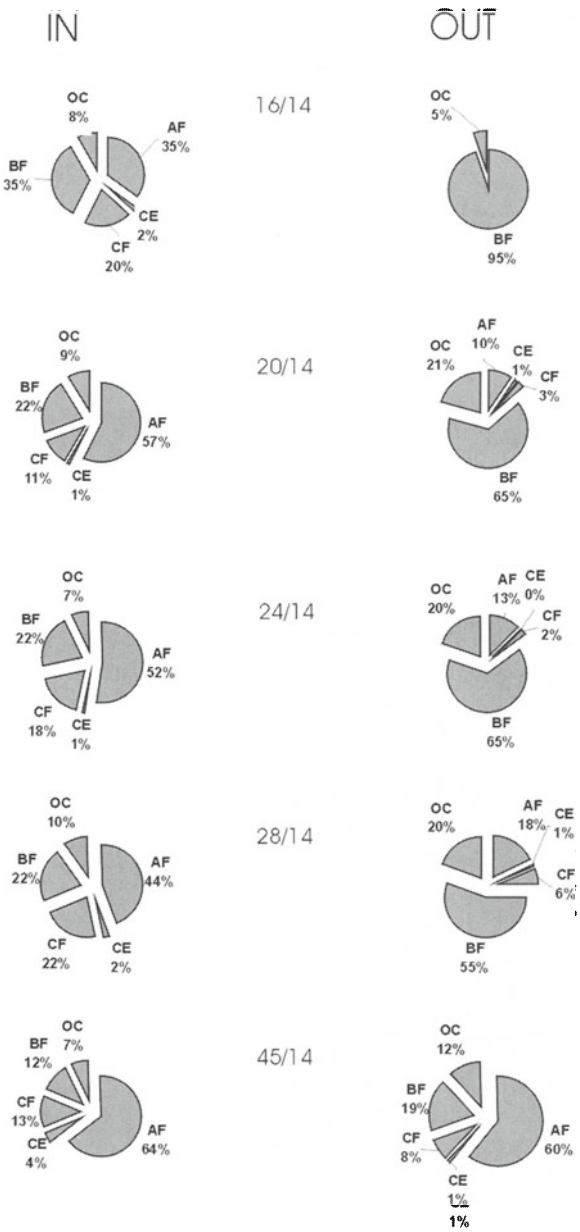

Figure 2. Overall (zones and seasons combined) percentage composition in weight of the retained (IN) and escaped (OUT) component by main categories with different gear configurations (GC; codend/cover, mesh side in mm). AF: red shrimp; BF: bony fishes; CF: cartilaginous fishes; CE: cephalopods; OC: other crustaceans.

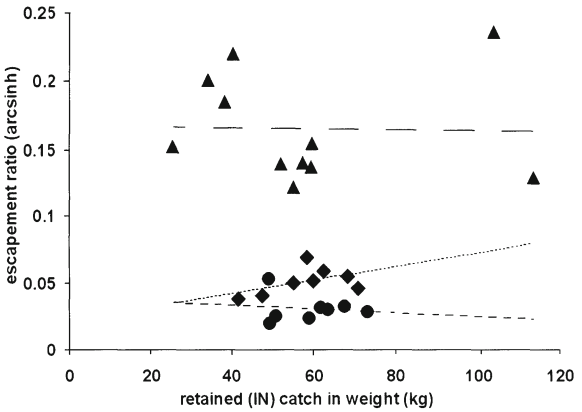

Figure 3. Haul escapement ratio vs. retained catch in weight with different gear configurations (●= 20/14, ♦= 24/14, ▲= 28/14 codend/cover, mesh side in mm) limited to the South-East (SE) and summer sub-set. The overlaid regression lines are not significant (considering a p level of 0.05).

species. Therefore, the related variability was included in the general sampling variability.

As concerns the performance of the gear configurations (GCs), the percentage composition by the various codends of the retained and escaped fractions of the pooled (zones and seasons) catch (in weight) by main groups is presented in Figure 2.

The 16/14 GC was not selective at all, allowing only small mixed bony fish (95% of the OUT catch) to escape. On the contrary, the 45/14 configuration was too selective, retaining only large specimens. Incidentally, this GC demonstrated the lack of any masking effect of the cover.

The other three gear configurations (20/14, 24/14 and 28/14) showed comparable catch compositions, both considering the retained and the escaped fraction. The highest percentage contributions to the retained (IN) catch in weight were given by red shrimp (57%, 52% and 44% for the 20/14, 24/14 and 28/14 GC, respectively), whereas the bulk of the escaped fraction (OUT) was represented by bony fishes (65%, 65% and 55%, respectively).

The global (zones, seasons and taxa/categories combined) mean values and corresponding coefficient of variation (c.v.%) of the retained and escaped fraction of the catches obtained by the different gear configurations, with also the economic statistics, are presented in Table 2.

Considering the differences among the mean retained catch by standard haul, beside the high coefficient of variation, it is evident a similarity between the 16/14, 20/14 and 24/14 gear configurations, a strong reduction in the 45/14 GC, and an intermediate value for the 28/14 GC.

The mean escapement ratios (ER%; retransformed data) increased constantly from 16/14 to 45/14 GC when numbers are considered (from 4.2 to 79.0%). In the case of the weight, the 16/14, 20/14 and 24/14 GC showed low values (<10%), slightly higher for the 28/14 (16.1%), and maximum with the 45/14 (64.2%).

Concerning the economic aspects (Table 2), it is worth noting the similarity of the value per kilogram of the retained catch among the GCs (7–8 €/kg), with the exception of the finest mesh (5.1 €/kg). The economic loss remained very low (<3%) for the 16/14, 20/14 and 24/14 GCs, but increases sharply with the 28/14 GC (9.2%). In spite of the comparable value per kilogram of the retained catch, the 45/14 GC would determine an unbearable immediate economic loss (62.3%).

The economic efficiency revealed a peculiar feature: it was quite comparable between the 20/14 and the 24/14 GCs (ca. 7 €/total kg) and between the 16/14 and the 28/14 GCs (ca. 5 €/total kg); the lowest efficiency (2.9 €/total kg) resulted in the 45/14 GC. However, it must be noted that the large variation generally associated to the mean values in Table 2 could mask the effective difference between the GCs.

The escapement ratios (overall values, presented in Table 3) showed that only the slender *Nettastoma melanurum* (39% of the total number), the powerful swimmer *Conger conger* (7%) and *P. blennoides* (5%) were able to escape from the 16 mm mesh codend; among the mixed categories, only macrurid grenadiers, mixed bony fish and mixed crustaceans were found in the OUT component. The lost weight fraction was negligible in all cases at the exception of *N. melanurum* (21%).

The 20 mm mesh codend lost a higher fraction (up to 72% in number for *N. melanurum*) of the previous species/categories, a low fraction (<5%) of other species (such as *A. foliacea* and *E. spinax*), and more appreciable values (8–23%) for species and categories, such as *M. merluccius*, mixed octopuses and squids and cuttlefishes. The loss in weight remained quite low (<10%), with the exception of *N. melanurum* (59%) and other mixed categories.

For the other GCs, beside the very high general escapement ratios of the 45/14 GC (only large or 'wide' species, such as *Centrophorus* sp. or skates and rays, were completely retained), the 24 mm and 28 mm mesh codends produced intermediate values, both considering numbers and weights. Considering

Table 2. Overall (zones and seasons pooled) observed descriptive statistics (mean by standard 3 h haul and associated coefficient of variation, c.v.%) for each gear configurations (GC). *n*: number of hauls; IN: retained catch; OUT: escaped catch; ER%: escapement ratio (OUT/TOT; reconverted after arcsinh transformation) in number and weight (TOT=IN+OUT). Economic loss as €.OUT/€.TOT (%); economic efficiency as €.IN/kg.TOT

GC		Number			Weight			Value		economic	economic
		IN	OUT	ER (%)	IN (kg)	OUT (kg)	ER (%)	IN (€/kg)	OUT (€/kg)	loss (%)	efficiency
16/14	mean	4316.3	202.9	4.2	60.3	0.7	1.2	5.1	0.7	0.2	5.1
n=6	c.v.%	44.3	66.8	25.2	30.0	56.3	17.1	42.2	117.6	100.0	41.9
20/14	mean	3724.2	735.4	13.7	63.2	2.3	3.1	7.3	1.6	0.8	7.0
n=27	c.v.%	41.2	82.5	6.1	38.2	74.7	7.0	27.4	100.0	115.4	28.1
24/14	mean	3839.0	1422.4	23.2	71.4	5.8	6.6	7.2	2.2	2.6	6.8
n=22	c.v.%	61.1	99.0	3.6	46.5	85.9	3.6	37.1	62.3	132.7	38.3
28/14	mean	2401.3	1808.4	40.7	59.0	12.1	16.1	6.5	3.5	9.4	5.4
n=27	c.v.%	48.9	68.5	1.4	43.2	72.8	2.4	36.0	56.2	45.6	37.6
45/14	mean	648.1	2371.0	79.0	21.2	37.6	64.2	8.0	7.3	62.3	2.9
n=11	c.v.%	51.2	31.8	0.1	32.6	23.7	0.1	23.8	23.0	6.7	26.7

species, the new findings in the OUT component of the 20/14 and 24/14 GCs were the small-size but valuable crustaceans (for example, 8–16% of *A. foliacea*) and bony fish (for example, 29–54% of *M. merluccius*). A squid, *Todarodes sagittatus*, and mixed sharks specimens were found with variable consistency (1–20%) in the OUT component of the 28/14 GC only. The loss in weight by species ranged between 2–27% (shrimps) and 0.2–81% (bony fish), the maximum values being presented with the 28/14 GC by *C. conger* (69%) and *N. melanurum* (81%). Considering the mixed categories, a large but variable amount (20–71% in number and 5–60% in weight, depending on the shape of the animals but also on the frequency of capture; cf. Table 1) was lost by the 24 mm and 28 mm mesh codends, with the exception of the mixed cartilaginous fishes, which were never able to escape.

Data relative to the performance comparison of the three most relevant (20/14, 24/14 and 28/14) GCs, limited only to the SE zone and summer sub-set, are presented in Table 4. Referring to the corresponding data in Table 2, it is worth noting the general reduction in the coefficients of variation (with exception of the retained catch of the 28/14 GC) and the similarity between the 20/14 and the 24/14 GCs for all the statistics considered. Furthermore, the higher catches and corresponding higher coefficient of variation of the 28/14 GC were a consequence of two hauls with extreme values. The ANOVA resulted in a non-significant difference in the global (IN+OUT) catch both considering the (transformed) numbers (p= 0.81) and weights (p= 0.91), i.e. the global performance was not affected by the different codends used or by the cover.

The (transformed) escapement ratios vs. weights of the retained catch (aimed at testing the codend saturation) by the different configurations is presented in Figure 3. The attempts to fit regression lines never resulted in significant relationships for the 20/14, 24/14 and 28/14 GCs (p= 0.89, 0.18 and 0.87, respectively). The same results were obtained by regressing the escapement ratios vs. numbers of the retained catch (p= 0.85, 0.21 and 0.57). Considering the comparison between the retained fraction of the catch, the mean value in number reduced slightly passing from the 20/14 to the 24/14 GCs and then strongly from the 24/14 to the 28/14 GCs, always with a high variance. The ANOVA evidenced a codend effect (p= 0.02), but a paired difference was detected only between the 20/14 and the 28/14 GCs (p= 0.04), whereas the 28/14 GC resulted not statistically different from the 24/14 GC (p= 0.17). On the contrary, no codend effect was detected on weight data (p= 0.78).

Considering the escapement ratio, the mean values in number indicated a similar pattern between the 20/14 and the 24/14 GCs, with the 28/14 GC departing

Table 3. Overall escapement ratios ($\sum$ OUT/$\sum$ TOT) in number (ERn%) and weight (ERw%) by species/category and different gear configurations (GC). Lists of taxa identified in the catch and included within the mixed categories as footnotes in Table 1

Species/category	16/14		20/14		24/14		28/14		45/14	
	ERn%	ERw%	ERn%	ERw%	ERn%	ERw%	ERn%	ERw%	ERn%	ERw%
Crustaceans										
Aristaeomorpha foliacea	1.0	0.0	3.2	0.6	8.5	2.1	15.6	7.7	68.3	61.1
Aristeus antennatus	0.3	0.0	0.7	0.1	10.1	3.1	17.7	9.9	80.4	71.3
Nephrops norvegicus	0.0	0.0	1.6	1.1	4.5	1.9	14.6	11.1	72.9	68.7
Parapenaeus longirostris	1.7	1.9	2.8	2.3	9.4	9.8	27.6	26.7	79.5	76.1
Mixed crustaceans	3.4	1.4	25.4	13.9	47.1	31.1	61.9	46.5	95.1	73.7
Total crustaceans	1.2	0.1	8.7	1.7	16.4	4.4	30.3	12.7	73.1	62.8
Cephalopods										
Todarodes sagittatus	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.2	15.3	13.1
Mixed octopuses	0.0	0.0	7.7	4.3	18.2	14.3	37.5	29.5	60.0	50.0
Mixed squids and cuttlefishes	0.0	0.0	13.3	3.3	30.0	8.2	25.7	4.8	35.3	17.2
Total cephalopods	0.0	0.0	8.4	1.5	18.7	2.7	19.2	5.4	32.7	17.5
Bony fishes										
Conger conger	6.7	2.2	30.8	4.1	60.3	27.6	83.6	68.7	96.8	58.0
Helicolenus d. dactylopterus	0.0	0.0	0.6	0.3	0.6	0.2	8.4	3.2	87.0	88.1
Lepidorhombus boscii	0.0	0.0	0.0	0.0	1.3	0.2	16.8	3.9	58.3	43.0
Merluccius merluccius	0.0	0.0	22.8	3.2	29.2	6.2	54.2	20.5	94.3	76.7
Micromesistius poutassou	0.0	0.0	0.0	0.0	7.6	5.8	40.5	32.7	84.1	86.3
Mullus surmuletus	0.0	0.0	0.0	0.0	0.8	0.6	3.6	1.4	1.0	1.0
Nettastoma melanurum	38.8	21.3	71.9	58.8	66.7	59.3	84.3	80.7	81.8	82.1
Phycis blennoides	5.2	0.2	3.7	0.6	2.6	1.0	19.1	7.0	77.0	68.6
Mixed anglerfishes	0.0	0.0	10.0	0.2	10.0	0.5	0.0	0.0	0.0	0.0
Mixed macrurid grenadiers	11.6	4.8	26.6	16.5	44.9	30.7	71.0	60.5	99.3	97.7
Mixed bony fishes	5.6	4.0	32.8	20.2	43.5	31.9	64.3	54.9	93.9	89.6
Total bony fishes	6.0	3.2	31.4	9.6	42.3	19.1	62.9	33.9	93.9	78.0
Cartilaginous fishes										
Galeus melastomus	0.0	0.0	0.6	0.4	3.0	0.9	10.9	4.0	84.3	85.3
Etmopterus spinax	0.0	0.0	2.3	1.7	4.5	1.4	20.9	16.6	88.2	88.5
Chimaera monstrosa	0.0	0.0	9.7	1.4	8.3	4.2	1.0	2.3	33.3	50.0
Mixed skates and rays	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mixed sharks	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.4	6.3
Total cartilaginous fishes	0.0	0.0	1.8	0.8	3.9	0.9	14.8	5.4	81.5	51.6

from the previous configurations. The ANOVA confirmed the overall differentiation of the codends ($p< 0.01$) but, again, the 20/14 and 24/14 GCs were not significantly different ($p= 0.38$). The separation of the different codends was complete when the weight ratios were considered. In this case, the 24 mm mesh resulted different from both the 20 mm ($p= 0.02$) and the 28 mm mesh codend ($p< 0.01$).

The multivariate techniques confirmed the above results. For the sake of simplicity, only one graphical output referred to the weight variable for the escaped (OUT) catch is presented in Figure 4 (analogous results were obtained with the number variable).

At least two main groups were identifiable in the MDS plot for the escaped catch (stress= 0.10), the first including all the 20/14 and almost all the 24/14 GCs, the second limited to the 28/14 GC. The cluster

Table 4. Overall observed descriptive statistics (mean by standard 3 h haul and associated coefficient of variation, c.v.%) for each gear configurations (GC), limited to the South-East (SE) and summer sub-set. *n*: number of hauls; IN: retained catch; OUT: escaped catch; ER%: escapement ratio (OUT/TOT; reconverted after arcsinh transformation) in number and weight. Economic loss as €.OUT/€.TOT (%); economic efficiency as €.IN/kg.TOT

GC		Number			Weight			Value		Economic	Economic
		IN	OUT	ER(%)	IN (kg)	OUT (kg)	ER (%)	IN (€/kg)	OUT (€/kg)	loss (%)	efficiency
20/14	mean	3911.4	623.1	13.6	59.1	1.9	3.0	9.7	2.0	0.6	9.4
n=8	c.v.%	20.6	25.3	0.5	15.1	30.9	19.5	7.5	34.5	24.6	8.3
24/14	mean	3418.5	696.0	16.1	58.0	3.2	5.1	10.2	3.4	1.7	9.7
n=8	c.v.%	21.2	38.8	1.4	17.1	30.0	0.9	3.3	39.1	38.2	4.0
28/14	mean	2618.2	1707.7	39.3	58.1	11.7	16.3	7.7	4.2	9.9	6.4
n=11	c.v.%	48.3	50.2	0.6	47.4	64.3	1.2	35.9	49.4	46.7	36.9

analysis, based on the Bray–Curtis ordination, allowed the identification of the same groups at 50% of similarity. Considering the total catch, the different hauls were clumped together by MDS no matter the gear configuration (stress= 0.08). Yet, five outliers, three of which belonging to the same day, were identifiable in the 28/14 GC. The Bray–Curtis group average clustering revealed a strong similarity (>70%) between hauls and no clear separation among GCs. The MDS allowed only a partial separation of the different GCs on the basis of the retained catch, but at least six hauls of 28/14 GC were found apart of their main group (stress= 0.09). The Bray–Curtis group average clustering separated the six 28/14 GC hauls previously evidenced by MDS, with no clear distinction of the remaining others.

Discussion and conclusions

In the present study, the retention of different codends (with mesh ranging from 16 mm to 45 mm, side), presently used or potentially usable in the bottom trawl fisheries of deep-water red shrimp in the Strait of Sicily, was assessed. The main aim was to evaluate their performance and quantify the by-catch incidence, which is a worldwide concern in shrimp fisheries (Andrew & Pepperel, 1992).

In general, the estimation of the shrimp/by-catch ratio is very difficult (IDRC, 1982; Gulland & Rothschild, 1984; Andrew & Pepperell, 1992). Such estimates are highly variable, falling in the range 0.1–72% (Sheridan et al., 1984). Usually, shrimps represent less than 20% (Alverson et al., 1994). For management, the choice between better utilization or minimization should depend on the general situation (IDRC, 1982). In developed countries, where by-catches of shrimp trawlers represent the target of other valuable fisheries or rare/protected species, the second option seems the most realistic to pursue (Andrew and Pepperell, 1992).

In the Mediterranean Sea, information about discards is scarce. Generally, more than 560 000 tons of rejects per year have been estimated (Alverson et al., 1994). A few local studies are available (Stergiou et al., 1997), but only rough comparisons can be inferred since the selectivity process is highly dependent on the biocenoses, the bottom features and the gear design (Mac Lennan, 1992; Reeves et al., 1992). Specific information for the Strait of Sicily is poor and limited to the shelf bottom trawl fisheries (Andaloro, 1985; Milazzo, 1988). For the upper (200–400 m) and medium (>400 m) slope fisheries, the estimated percentage of discard is around 42% (94% of which are fish).

Present results have shown the lower relevance of the by-catch in the red shrimp fishery and, in the same time, the inadequacy of the presently used codend mesh size. In fact, the accompanying fauna, although well represented in number of species or categories (132 taxa), does not contribute substantially to the bulk of the catch, and the impact against other commercial species such as hake or red mullet seems low when compared with the amount of juveniles captured by the direct fisheries. That not withstanding, the reduction of the by-catch in red shrimp fisheries should be pursued anyhow, for instance by increasing the mesh size (Caddy, 1982). There are at least two main reasons to achieve this goal: it is unlikely that the discard of the by-catch from the deck does increase red shrimp

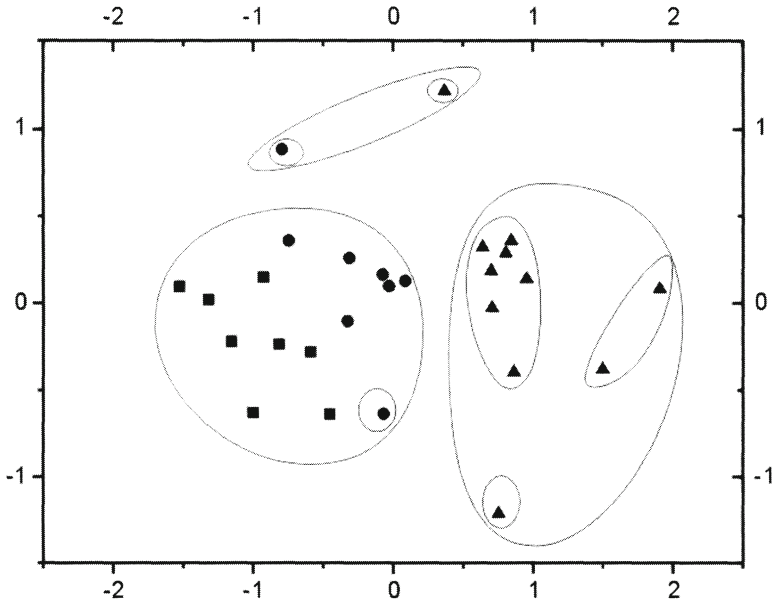

Figure 4. Multidimensional scaling plot based on Bray-Curtis similarities between hauls, considering the escaped fraction in weight with different gear configurations (●= 20/14, ♦= 24/14, ▲= 28/14 codend/cover, mesh side in mm), limited to the South-East (SE) and summer sub-set (stress= 0.10). MDS plot orientation and scale arbitrary. The overlaid lines identify the 50% (large circles) and 60% (small circles) similarity level resulting by group average clustering.

harvesting (a hypothesis quite realistic in other situations; Sheridan et al., 1984), and a better utilization of low-value species is practically unfeasible.

Although some conceptual problems arise in adapting univariate and multivariate techniques widely used in community studies to fisheries data, such procedures have been employed successfully to characterize fishing assemblages or areas (Murawsky et al., 1983; Stergiou & Pollard, 1994) or to compare gear performances (Stergiou et al., 1997). Even considering that inter- and intra-haul variations in yield and species composition is a well-known phenomenon, both univariate and multivariate techniques applied in the present study demonstrated that no substantial difference exists in the qualitative and quantitative overall performance of gear configurations (codend and cover) with comparable mesh size in the codends (from 20 to 28 mm mesh). This conclusion might not be true for gear configurations with substantially larger mesh in the codend (45 mm), which yield catches of different compositions and amounts.

Within the three most comparable gear configurations (GCs; 20/14, 24/14 and 28/14), both clustering and multidimensional methods lead to the conclusion that the escaped (OUT) component of the catch is the sole effective discriminant in assessing the codend performance. In fact, the haul variability (in terms of species composition, size diversity, catch in number and yield) fogs the expected differences inside the codend. Considering the retained (IN) component alone might not be sufficient, leading to spurious interpretations. Sometimes larger mesh codends are more efficient than narrow mesh codends, just because a large catch of larger specimens may occasionally occur. On the contrary, when the assemblages being fished are truly comparable in density-at-large and in the respective size spectra, then different yields and species compositions may be expected inside the various codends.

In the red shrimp fishery, nothing is known about the survival of the escaped fish. There are evidences that the probability of death for the injured fish which are able to escape from the codend reduces by increasing the mesh size (Chopin & Arimoto, 1995). Considering red shrimp, the rigid integument and the 'backward' escaping pattern (Bianchini et al., 1998b) should permit a better survival of escaped specimens. Moreover, while surviving hakes or mullets may be important for the shelf fisheries (Caddy, 1990), even the dead biomass, filtered out and left on the bathyal grounds, should enhance the nutrient budget of these oligotrophic bottoms.

Although with some caution (the significance of the differences was not tested for some of the invest-

igated parameters), the conclusions of this study can be summarized as follows: (1) Changing the codend may influence the quali-quantitative performance of the whole gear (codend plus cover) and therefore some preliminary tests must be performed before comparative analyses of codend selectivities. (2) The results indicate that the legal codend mesh size (20 mm) is non selective and that larger mesh sizes, falling between 24 and 28 mm, can be easily introduced in the fisheries, especially considering the minimal economic losses. In fact, neither the mesh size presently in use in the red shrimp fishery of the Strait of Sicily (16–18 mm) nor the legal mesh (20 mm, side) make any biological or economical sense. (3) In the 24/14 and 28/14 GCs, the economic loss is minimal, the economic efficiency is improved, and the amount of by-catch escaping is substantial. Since the adoption of the 28 mm mesh side would permit the escape mainly of small-size and low-value bony fish, crustaceans and cephalopods, the benefit for both the fishery, by means of minimizing the handling time and improving the catch quality, and the environment, by reducing the degree of 'impact stress', is evident. (4) The introduction of a codend with 28 mm mesh would result in a small reduction of the red shrimp catch in number (about 16%) and a minimal loss in weight (< 8%), almost exclusively referred to the young of the year. Since these small-size specimens (CL< 35 mm) command only a fraction of the unit value of the large-size (and still fully retained) specimens, only a negligible reduction in the fishermen immediate economic return should be expected. Moreover, such a loss would be recovered in the long term.

The control of the exploited sizes by mesh regulation is considered a necessary although not sufficient measure to be implemented in the management of any exploited resource. The usefulness of such kind of regulation, however, has been questioned for the penaeid shrimps (Gulland, 1972; p. 7) and for those fisheries based on shelf and upper slope multispecies assemblages (Caddy, 1990). In fact, even when studies have demonstrated that important economic benefits can be obtained by increasing the actual mesh size used to catch shrimps, doubts remain about the generalization of the results as a consequence of both short life span of the penaeids and the variety of the species exploited by the fisheries (Sobrino et al., 2000).

That not withstanding, there is a general agreement that the adoption of mesh size larger than those traditionally employed by fishermen "... could be beneficial to the economy as a whole irrespective of the effect on shrimps ..." (Gulland, 1984). It is perhaps a case that the mesh size fixed by the European Union (1994) for the Mediterranean Sea, 40 mm stretched, fall in the minimum range of increase (40–45 mm) envisaged by Gulland (1984). Given that deep-water red shrimps show longer life span and slower growth rate than their shelf and upper slope counterparts (Bianchini & Ragonese, 1994), constituting also the bulk of the catch, present results indicate that an intervention on mesh size is a plausible management tool which might improve the performance of these very valuable fisheries.

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Protein requirement of the prawn *Marsupenaeus japonicus* estimated by a factorial method

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Key words: protein requirement, prawn, *Marsupenaeus japonicus*, factorial method

Abstract

The protein requirement to give maximum body protein retention in the prawn *Marsupenaeus japonicus* was assessed by determining both daily protein needed for maintenance (M) and daily body protein increment (G) when the juvenile prawn was maintained on a diet containing high quality protein. The body protein increment was obtained by determining carcass nitrogen increment when the prawn was fed on casein-based diets. The protein required for maintenance was estimated by regressing weight gains of the prawn on the diets containing graded levels of casein. True daily increase or retention of body protein in the prawn corresponded to the sum of G and M, and it was 3.2 g protein per kg body weight per day. The dietary protein requirement of juvenile *M. japonicus* for maximum body protein retention was suggested to be about 10 g per kg body weight per day providing that the prawn was fed the casein-based diet containing 50% crude protein (net protein utilization = 32) at the feeding level of 2%.

Introduction

The prawn *Marsupenaeus japonicus* has been generally recognized to require more dietary protein for growth than other species of prawns (Kanazawa, 1990), because inclusion of high levels of protein in diets is necessary for good growth of this species (Deshimaru & Shigeno, 1972; Deshimaru & Yone, 1978). This postulates that protein utilization for growth by the prawn is lower than that of other prawns. In Japan, *M. japonicus* diets have been manufactured in the context of high protein requirement. Most studies on protein requirements of crustaceans like prawns and shrimp have demonstrated the optimum dietary protein levels for prawns by feeding trials using diets containing graded levels of test proteins (Deshimaru & Shigeno, 1972; Deshimaru & Yone, 1978; Kanazawa, 1990). Optimum dietary protein levels for penaeid prawns reported up to the present vary from 23% to 57% in diets (Kanazawa, 1985; Guillaume, 1997). In terrestrial animals, quantitative protein requirements are often expressed as the quantity of protein required daily for the maximum growth or body protein retention per body weight (BW). Optimum dietary protein levels for prawns, which meet true or absolute protein requirements (mg kg^{-1}BW d^{-1}), obviously fluctuate with feeding level and other factors like protein quality, dietary energy level, age, etc. Daily quantitative protein requirements for prawns in the above concept have not been estimated possibly due to the difficulty in measuring actual feed intake which changes with a degree of spill during mastication and the water stability and palatability of diets. Isocaloric test diets containing graded levels of a protein source may have different physico-chemical properties resulting in variation in actual feed intake. For better understanding protein nutrition in prawns from a viewpoint of comparative nutrition, it is desirable to determine daily protein requirement per unit BW.

On the other hand, Ogino (1980a, b) has shown that the protein requirements of carp *Cyprinus carpio* and rainbow trout *Salmo gairdneri* may be estimated by determining both daily protein needed for maintenance (M) and daily body protein increment (G) when these fish were fed nutritionally high quality proteins. His findings foresee the possibility of estimating quantitative protein requirement of prawns in a similar manner. The present study was planned to

evaluate protein requirement of prawn *M. japonicus* in terms of daily protein needed per unit body weight for maximal growth or body protein increase on the basis of both daily increase of carcass N and daily loss of endogenous N.

Materials and methods

Experimental design and diets

Two feeding trials were conducted to examine the effects of varying levels of protein on growth and body protein increment of juvenile *M. japonicus*. Test diets (Table 1) were formulated to contain 8.1–50.0% crude protein (CP = N · 6.25) by substituting dextrin for casein. Since digestible (DE) or metabolic (ME) energy values of these purified ingredients have not been determined with the prawn, energy content of test diets was calculated using mammalian physiological fuel values; protein (16.74 kJ g^{-1}), lipid (37.66 kJ g^{-1}) and carbohydrate (16.74 kJ g^{-1}). The energy values of test diets were about isocaloric using the above criteria. Procedures for preparation and storage of diets were the same as those previously described (Teshima et al., 1986). The juvenile prawns were obtained from Mitsui-Nohrin Co. Ltd., Kagoshima, Japan, and underwent a 1-week conditioning period to acclimate to the test diets and environmental conditions. Feeding trials were conducted in 54-l flow-through aquaria with a flow rate of approximately 900 ml min^{-1} under the conditions given in Table 2. All prawns in each group were weighed each week and the amount of diet given was adjusted accordingly. Initial mean body weights of the prawn in each feeding trial did not differ significantly ($p<0.05$) among experimental groups. In feeding trial-1, diets 6 and 7 containing 50% CP, an optimum dietary protein level for *M. japonicus* (Deshimaru & Yone, 1978), were fed to quadruplicate groups of the prawn for 20 or 40 days. In feeding trial-2, the prawns were reared with casein-based diets (diets 1–5) containing varying levels of CP from 8.1 to 29.0% in a similar manner for 40 days. Feeding trials were conducted with duplicate tanks for each test diet.

Increase of carcass nitrogen

Carcass nitrogen (N) content of test prawns before and after feeding trials was determined to assess increment of body protein. Daily protein retention (or true increment) (R) in the whole body of prawn was calculated by the following equation:

$$R = G + M,$$

where G = daily carcass-CP increment (g) in the prawn fed a diet containing high quality protein, M = daily obligatory CP loss. Carcass N increment data were obtained from carcass N of the prawns fed diets 6 and 7 in feeding trial-1. Obligatory N loss, corresponding to the loss of body N when animals are fed a protein free-diet, was estimated by extrapolation of the regression line between protein levels in diet and quantities of carcass N, as described by Gatlin et al. (1986). Five prawns were randomly sampled from each group at the start of each experimental period, weighed, and freeze-dried for subsequent proximate analysis of the prawn body. At the end of each feeding trial, all prawns were analyzed by the same methods as well. CP was determined by a Kjeldahl method with a Tecator Kjeltec System (1007 Digestion system, 1002 Distilling unit and Titration unit) using boric acid to trap ammonia. Moisture was determined on the freeze-dried samples. Weight gain and body N data were statistically analyzed by analysis of variance (ANOVA) at the significant level of 5%.

Estimation of net protein utilization

Net protein utilization (NPU;% body retention of ingested nitrogen) was estimated as follows. In the case of juvenile *M. japonicus*, it is difficult to measure correctly feed intake (or N intake) in feeding experiments because of the slow feeding habit resulting in leaching of some nutrients from diets into the water and scattering diets during mastication. Tracer experiments (Teshima & Kanazawa, 1987) showed that about half of a diet given was actually ingested by the prawn even if the diet seemed to be completely consumed. In the present study, therefore, true feed intake was provisionally calculated on the assumption that about 50% of given diets was ingested, and then the NPU of dietary protein (casein) at different dietary levels in the prawn was obtained.

Data of growth study and carcass analysis were analyzed using analysis of variance (ANOVA) and/or chi-square test at 5% level of significance (Steel & Torrie, 1960). Meaningful comparison of treatment means was conducted using Duncan's multiple range test (Duncan, 1955).

Table 1. Composition of diets (g/kg)

Ingredient	Diet No.						
	1	2	3	4	5	6	7
Casein	–	57	114	171	227	455	–
Fish meal (defatted)[a]	–	–	–	–	–	–	543
Squid protein[b]	–	–	–	–	–	44	44
Activated gluten	80	80	80	80	80	80	80
Dextrin	581	524	467	411	354	–	–
Pollack liver oil	60	60	60	60	60	40	40
Soybean oil	–	–	–	–	–	20	20
α-Cellulose	10	10	10	10	10	92	4
Other ingredient[c]	269	269	269	269	269	269	269
Crude protein (%)	8.1	12.0	18.6	24.0	29.0	50.0	50.0
GE (kJ g^{-1})[d]	17.78	17.74	18.32	18.61	18.99	17.69	17.69
Energy (kJ g^{-1})[e]	16.32	16.02	16.19	16.15	16.02	13.64	13.64

[a] A white-coloured fish meal produced from pollack, flatfishes, etc. on a factory-ship. The commercial products of fish meal were defatted with with solvents (chloroform-methanol, and then ethanol).
[b] Prepared from a squid muscle in our laboratory.
[c] Glucose, 10; sucrose, 10; α-starch, 80; glucosamine HCl, 8; Na citrate, 3; Na succinate, 3; soybean lecithin (Nakarai Chemicals Co., Japan), 30; cholesterol, 5; mineral mixture, 60; vitamins, 50; attractants (betain, 2; taurine, 1.3; inosine monophosphate, 0.7; glutathione, 0.7; Na glutamate 3.3; methionine, 2.0), 10.
[d] Gross energy (kcal g^{-1}) of diets (except for α-cellulose), was calculated based on: protein, 5.65; lipid, 9.45; carbohydrate, 4.10. Calories were transformed to joules using 1 calorie = 4.184 joules.
[e] Energy calculated based on mammalian physiological fuel value (kcal g^{-1}): protein, 4; lipid, 8; carbohydrate, 4.

Table 2. Rearing conditions in feeding trials

Condition	Feeding trial-1	Feeding trial-2
Prawn *M. japonicus*		
Body weight (BW)	1.69±0.1 g	0.70±0.05 g
Number of prawn $tank^{-1}$ (54 l)	10	11
Rearing and feeding methods		
Feeding period	20 and 40 days	40 days
Water temperature	28±1 °C	23±1 °C
pH of sea water	8.2	8.2
Water supply day^{-1}	400–500%	100–200%
Feeding frequency	Once a day	Once a day
Feeding level (% of BW d^{-1})	4%	4%

Results

Table 3 shows the growth and survival rates of the juvenile *M. japonicus* fed casein and fish meal-based diets containing 50.0% CP for 20 and 40 days in feeding trial-1. The casein-based diet (diet 6) gave higher weight gain than fish meal-based diet (diet 7) in both 20 day- and 40 day-feeding trials. Table 4 shows the carcass protein increments (mg $prawn^{-1}$) of the prawns fed diets 6 and 7 containing 50.0% CP. In the present study, we intended to estimate the protein requirement to give the maximum body protein increase in the juvenile *M. japonicus* on the basis of the protein needed daily for maintenance and body protein increment when the prawn was fed on a diet containing nutritionally high quality protein. Therefore, the body protein increments were estimated on the prawns fed on casein-based diets rather than fish meal-based diets. The daily carcass protein increment (mg $prawn^{-1}$ d^{-1}) was 5.08 when the prawn was fed on the casein-based diet for 40 days (Table 4).

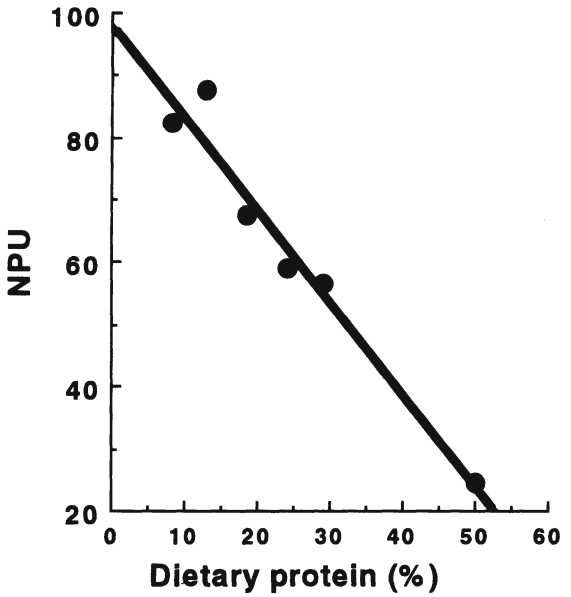

Figure 1. Net protein utilization (NPU) and dietary protein levels in the prawn *M. japonicus*.

Tables 5 and 6 and Figure 1 show the increments of weight gain and carcass protein (mg prawn^{-1}) of the prawns fed casein-based diets containing different protein levels for 40 days in feeding trial-2. The weight gain (Fig. 1) and carcass protein (Table 6) of the prawn increased with increasing dietary protein levels from 8.1 to 50.0%. Protein required for maintenance was estimated using data from feeding trial-2. When the prawn, weighing 700 mg in mean body weight, was fed on a protein-free diet, 31.9% weight loss was observed after 40 days (Fig. 2). The weight loss corresponded to the loss of 1.09 g protein kg^{-1}BW d^{-1} (see Table 7). Ogino (1980a) observed that obligatory N loss in carp and rainbow trout changed directly in proportion to body weight. The loss of body protein in the prawn receiving a protein-free diet can be regarded as the quantity of protein required daily for maintenance (*M*). Based on the data given in Table 4, the daily carcass protein increment (g^{-1} kg^{-1} BW d^{-1}), *G*, was estimated to be 2.11 (Table 7). The sum of *G* and *M* (3.2 g protein kg^{-1} BW d^{-1}) is probably comparable to the retention or true daily increment of body protein (*R*). The *R* is regarded to correspond to the quantity of protein needed daily for maximum body protein retention by this prawn. Table 8 shows the provisional NPU values of protein (casein) at different dietary protein levels in the prawn under the assumption that true daily feeding rate was 2% of BW. NPU values decreased with increasing dietary protein levels (Fig. 2).

Discussion

Protein for maintenance and body protein increment

Optimum dietary protein levels for crustaceans have been generally determined by examining the effect of varying protein levels on growth and survival rates (Deshimaru & Shigeno, 1972; Deshimaru & Yone, 1978; Kanazawa, 1990; Guillaume, 1997).But, there has been no approach to determine protein requirements of prawns and shrimp on the basis of daily protein needed for maintenance and growth, except for our previous work (Koshio et al., 1993). In the present study, the protein needed for maintenance of the prawn *M. japonicus* was determined to be 1.09 g protein (or 0.174 g N) kg^{-1} BW d^{-1} when casein was given as a major protein source. The N needed for maintenance (0.174 g N kg^{-1} BW d^{-1}) of the prawn was higher than those of the carp *C. carpio* (0.095 g) and rainbow trout *S. gairdneri* (0.14 g) determined in a similar manner by Ogino et al. (1976, 1980). This suggests that the juvenile *M. japonicus* possibly requires more protein for maintenance than fish.

The protein needed for body protein increment of the prawn *M. japonicus* determined in the present study was 2.11 g kg^{-1} BW d^{-1}. This value was lower than those for body protein increments (g kg^{-1} BW d^{-1}) in the carp (5.02 g kg^{-1} BW d^{-1}) and rainbow trout (5.09 g kg^{-1} BW day^{-1}) reported by Ogino (1980a). Also, the protein needed for maximum body protein retention of the prawn (3.2 g protein kg^{-1} BW d^{-1}) (Table 7) was lower than those of the carp and rainbow trout (about 5.8 g protein kg^{-1} BW d^{-1}) (Ogino, 1980a).

Protein requirements and optimum dietary protein levels

Reported optimum dietary protein levels for crustaceans range from 23.0 to 60.0% (Kanazawa,1985), apparently indicating species specificity. However, optimum dietary protein levels for prawns reported in earlier studies do not represent quantitative or true protein requirement, which is defined as a minimal quantity of protein needed daily per biomass or animal unless they are indicated together with net protein intake from diet.The optimum protein content in diet needed to meet daily protein requirement of the prawn varies with feeding level, spilling rate of diet, and NPU which possibly changes with dietary protein levels (Table 8). Ogino (1980a) has shown that optimum protein contents in diet for the carp and rainbow trout

Table 3. Growth of the prawn in feeding trial-1

Group[a]	Diet No.	Major protein	Period (days)	Av. BW[b] (g)		Av. BW gain[c]		Survival (%)[d]
				Initial	Final	(mg d^{-1})	Wt. gain (%)	
1	6	Casein	20	1.69±0.09	2.59±0.35	45.0±6.1	52	95
2	7	Fish meal	20	1.69±0.07	2.00±0.22	15.5±1.7	18	95
3	6	Casein	40	1.69±0.09	3.12±0.53	35.8±6.1	85	90
4	7	Fish meal	40	1.69±0.12	2.52±0.43	20.8±3.5	49	85

[a]For each group, the feeding trial was conducted using four replicated tanks. Initial mean body weight of the prawn among the groups (40 prawns per group) did not differ significantly ($p<0.05$). Data on body weight (BW) gain and survival are the mean values.
[b]Means ± S.D.
[c]Wt. gain (%) = 100(Final average BW - Initial average BW) · Initial average BW^{-1}. Analysis of variance showed that the daily weight gain and weight gain (%) at the end of the feeding period were significantly ($P<0.05$) different with two factors examined, the source and level of dietary proteins examined.
[d]Survival rates (%) of the prawns at the end of the feeding period were not significantly ($P>0.05$) different among the experimental groups.

Table 4. Carcass protein increment of the prawn in feeding trial-1

Diet No.	Feeding period (days)	Av. carcass protein (mg $prawn^{-1}$)			Increment of carcass protein (mg $prawn^{-1} d^{-1}$)[a]	
		Initial (A)	Final(B)	B-A	Protein	Nitrogen
6	20	231±14.1	348±31.3	117	5.58±0.51	0.94±0.08
7	20	231±15.1	274±38.6	43	2.15±0.3	0.34±0.048
6	40	231±15.3	434±33.1	203	5.08±0.41	0.81±0.064
7	40	231±15.1	333±25.2	102	2.55±0.2	0.41±0.032

[a]Data on increment of carcass protein are means±S.D. from four replicated tanks. Analysis of variance showed that the increment of carcass protein and nitrogen at the end of the feeding period were significantly ($P<0.05$) different with the source and level of proteins examined.

Table 5. Growth of the prawn in feeding trial-2

Diet No.[a]	CP(%) in diet	Period (days)	BW(g)[b]		Av. BW gain[c]		Survival (%)
			Initial	Final	(mg d^{-1})	(%)	
1	8.1	40	0.70±0.05	0.73±0.05	0.8±0.05	4.3	95
2	12.9	40	0.70±0.05	0.99±0.13	7.3±0.15	41	95
3	18.6	40	0.70±0.05	1.12±0.12	10.5±1.05	60	95
4	24.9	40	0.70±0.05	1.27±0.12	13.5±1.31	81	100
5	29.0	40	0.70±0.05	1.52±0.13	20.5±1.73	117	100

[a]The major protein source was casein. For each diet, the feeding trial was conducted using duplicated tanks.
[b]Means ± S.D.
[c] Initial mean body weight of the prawn among the groups (40 prawns per group) did not differ significantly ($p<0.05$). Data on average body weight (BW) gain and survival (see Table 3) are the mean values from duplicated tanks.

Table 6. Carcass protein increment of the prawn in feeding trial-2

Diet No.	CP(%) in diet	Feeding period (days)	Carcass protein[a] (mg prawn^{-1}) [b]			Increment of carcass protein[a] (mg prawn^{-1} d^{-1})	
			Initial (A)	Final (B)	B-A	Protein	Nitrogen
1	8.1	40	95.9	100	4.1	0.10±0.01	0.017
2	12.9	40	95.9	136	39.7	0.99±0.01	0.159
3	18.6	40	95.9	153	57.5	1.44±0.12	0.230
4	24.0	40	95.9	174	78.6	1.97±0.24	0.315
5	29.0	40	95.9	208	112.3	2.81±0.31	0.450

[a] Means from duplicated tanks.
[b] Feeding period; 40 days.

were variable from 35 to 50% under the various feeding levels, indicating that the protein requirements determined by carcass-N increment in the two fish species coincided with those based on N balance determinations. In the present study, optimum dietary protein levels for the maximum growth of the juvenile *M. japonicus* were assessed on the basis of daily protein requirement for maximum body protein retention. For the maximum body protein retention in the prawn, 3.2 g of protein per kg BW should be daily provided into the body. This means that the optimum dietary protein level for the prawn will increase with decreasing feeding levels of diets and lowering digestibility and NPU of dietary proteins. NPU of protein (casein) in the prawn decreased with increasing dietary protein levels (Table 8) as also observed in the rainbow trout (Tacon & Cowey, 1985). Provided that the NPU of dietary protein are 32 (see Table 8; NPU value of casein-squid protein at CP=50%), optimum protein content in diet needed to meet daily protein requirement of the prawn was suggested to be about 10 g kg BW^{-1} d^{-1} (Table 7) under the conditions that no diet is spilled over during feeding. This value was similar to the daily protein requirements of the carp and rainbow trout (12–13 g kg^{-1} BW d^{-1}) estimated by Ogino (1980b). This observation suggests that 'absolute' protein requirement of *M. japonicus* is not always higher than those of fish in terms of the protein needed daily for maximal body protein increase. In fish, several workers (Tacon & Cowey, 1985; Bowen, 1987; Wilson, 1993) have pointed out that the efficiency of protein utilization is similar among the species when several parameters relating protein intake to growth of fish. As for the juvenile *M. japonicus*, we noted that better estimation of dietary cholesterol requirements may be achieved by determining body cholesterol of prawn fed highly nutritive diets and calculating the quantity of cholesterol required daily per kg of prawn at maximum growth (Teshima et al., 1997). There has been no attempt to estimate protein requirements of this prawns and other crustaceans in the similar manner.

Table 7. Body protein increments and protein requirements of the prawn receiving casein-based diet (CP=50%) for 40 days

Item	Value (means±S.D.)
Carcass protein increased (mg prawn^{-1} d^{-1})[a]	5.08±0.41
(g kg^{-1} BW d^{-1}) ..... G	2.11±0.17
Protein for maintenance	
Weight loss (mg) in protein-free diet (mg prawn^{-1} d^{-1})[b]	5.58±0.62
(g kg^{-1} BW d^{-1})	7.97±0.89
Body protein loss (g kg^{-1} BW d^{-1}) .. M	1.09±0.16
True body protein retention (R) = G + M (g kg^{-1} BW d^{-1})	3.20±0.42
Dietary protein needed (g kg^{-1} BW d^{-1}) if NPU is 32 [c]	10.0±1.3

[a] Average body weight (BW) of the prawns during the feeding period: (BW at initial + BW at final) · 2^{-1} = 2.41 g.
[b] The percent body weight loss (31.9% of initial body weight) was obtained from Figure 2, and the weightloss (mg) was cauculated as follows: 700 mg · 0.319 · 40^{-1} = 5.58.
[c] NPU value when CP level in diet is 50% (refer to Figure 2).

The optimum dietary protein levels for the prawn (*M. japonicus*) have been well documented. Studies by Deshimaru & Kuroki (1975) and Deshimaru & Yone (1978) reported that optimum dietary protein level of juvenile *M. japonicus* was 50% and 52–57%, respectively, when casein-albumin (9:1) was used as a protein source. We also showed that larval *M. japonicus* required 45–55% protein in a casein-based diet (Teshima

Table 8. Relationship between dietary protein level and NPU value in the prawn

Diet No.	CP(%) in diet	Av. BW (g)	Endogenous N loss (mg prawn^{-1} d^{-1})[a]	Carcass N increment [b] (mgN prawn^{-1} d^{-1}) Apparent	Carcass N increment [b] (mgN prawn^{-1} d^{-1}) True	True intake [c] (mg d^{-1}) Diet	True intake [c] (mg d^{-1}) N	NPU (%)
1	8.1	0.72	0.13	0.017	0.14	14.3	0.18	79
2	12.9	0.85	0.15	0.159	0.31	16.9	0.34	90
3	18.6	0.91	0.16	0.230	0.39	18.2	0.54	72
4	24.0	0.99	0.17	0.315	0.49	19.8	0.76	64
5	29.0	1.11	0.19	0.450	0.64	22.2	1.03	63
6[d]	50.0	2.41	0.42	0.812	1.23	48.1	3.85	32

[a]Body nitrogen loss · body weight; body nitrogen loss was obtained from body protein loss in the prawn fed a protein-free diet (see Table 9).
[b]Initial average body protein (or N) of prawn in feeding trial-2 was 95.5 mg protein (or 15.5 mg N).
[c]Provisional value; true feed intake was calculated on the assumption that feeding rate is 2% of body weight.
[d]Value obtained in feeding trial-1.

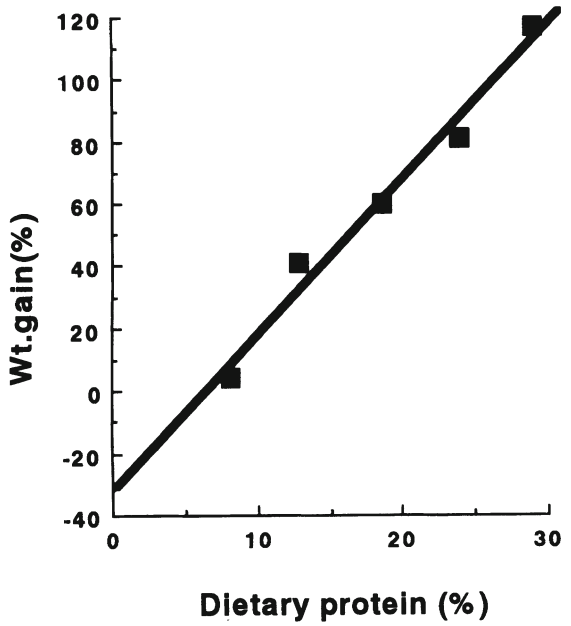

Figure 2. Relationship between dietary protein levels (%; X) and body weight gain (%; Y) in the prawn *M. japonicus*. $Y = -0.319 + 5X$ ($r^2 = 0.978$).

& Kanazawa, 1984). These informations indicated the necessity of high levels of dietary proteins for good growth and survival of this species, suggesting a low utilization of dietary protein for growth. The present study confirmed that a high dietary level of protein similar to those reported in the earlier studies (Deshimaru & Kuroki, 1975; Deshimaru & Yone, 1978; Teshima & Kanazawa, 1984) was needed for good growth of the juvenile prawn. In the prawn, however, optimum dietary protein levels (%) which meet the protein requirement (g^{-1} BW d^{-1}) should be evaluated in relation to the water stability and feeding rate of diets along with sources or qualities of proteins. Optimum dietary protein levels for prawns will decrease with increasing feeding levels and also fluctuate with the spilling rate of diets into water during mastication. Avoiding the fluctuation of optimum dietary level due to the above mentioned factors such as water stability and feeding level, the method adopted in the present study is conceived to be useful in the estimation of protein requirement and to deduce optimum dietary protein level for juvenile *M. japonicus*, because it is generally difficult to measure the aquantity of diets ingested actually in prawn species. In general, however, true or absolute protein requirement in aquatic animals varies during development and with water temperature (Guillaume, 1997). In the case of poikilothermal animals like prawns, feed intake is often variable according to environmental conditions such as water temperature, etc. The protein requirements of *M. japonicus* and other crustaceans are desirable to be investigated more in detail in relation to water temperatures and prawn age.

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The effect of fixatives in the quantification of morphological lipofuscin as an age index in crustaceans

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Key words: lipofuscin, age pigments, fixatives, *Nephrops norvegicus*

Abstract

The role of fixation in studies using histological techniques to measure lipofuscin as an age indicator was evaluated. Four fixatives were used: Bouin, 10% buffered saline formaldehyde, Davidson and San Felice. The species used was the Norway lobster (*Nephrops norvegicus*). Similar sized males, with a maximum carapace length difference of less than 10 mm were used and attributed randomly to each treatment. Results showed that San Felice and Davidson were not appropriate for lipofuscin studies. Bouin and 10% formaldehyde produced the best results in terms of tissue quality and lipofuscin granule brightness. This work suggests that Bouin can be a substitute of formaldehyde in lipofuscin studies, in particular in situations when other structures, such as gonads, are needed for parallel or complementary studies.

Introduction

The absence of permanent calcified structures in crustaceans has led many researchers to look for different alternatives to estimate age. Recent studies have indicated that the neural accumulation of granular lipofuscin, an autofluorescent pigment resulting from the peroxidation of unsaturated lipids by oxygen free radicals (Katz & Robison, 1986), can be used as an index of crustacean age (Sheehy, 1990). The species of decapods in which lipofuscin was used for age estimation include the crayfish species *Cherax quadricarinatus*, *C. cuspidatus*, *Pacifastacus leniusculus* (Sheehy, 1989; Sheehy, 1990; Sheehy, 1992; Belchier et al., 1998), the clawed lobsters *Homarus americanus*, *H. gammarus* and *Nephrops norvegicus* (Tully, 1993; Kerros et al., 1995; O'Donovan and Tully, 1996; Sheehy et al., 1996; Wahle et al, 1996), the rock lobster *Panulirus cygnus* (Sheehy et al., 1998) and the shrimp species *Penaeus monodon* and *Penaeus japonicus* (Sheehy et al., 1995; Vila et al,. 2000). In the nervous tissues used (brain and eye stalk) the lipofuscin forms granules which density and size are used as age indices. These granules are observed under the microscope, using fluorescent light in a process that is highly dependent on histological and image analysis techniques to produce images with distinct lipofuscin granules and a good contrast between the granules and the background.

During a recent study concerning lipofuscin analysis in *Nephrops norvegicus*, some problems related to the quality of the tissue were identified as being the result of deficient fixation when too many individuals were put in the same container, resulting in different exposures to the fixation liquid. The identified problems included poor intensity of the auto-fluorescent lipofuscin granules, tissue shrinkage and high levels of background fluorescence that affected lipofuscin quantification. Thus, a study was undertaken to assess the effect of fixation on tissue quality and autofluorescence intensity. A secondary objective was the assessment of alternative fixatives that are better for histological analysis of other internal structures. As an example, the 10% formaldehyde, generally used for a period of at least 2 days for lipofuscin studies, makes other structures such as gonads too hard and in poor condition for histological observation. The use of alternative fixatives could allow different observations to be made on the same individuals. The fixatives studied were 10% formaldehyde (buffered, and prepared with seawater), Bouin (Hopwood, 1990), San Felice (Gabe, 1968) and Davidson's.

The species used was *Nephrops norvegicus* and the lipofuscin was observed in the brain olfactory lobe cell masses.

Methodology

Experimental setup

The experiment was conducted in two phases. The first was a preliminary phase with small samples (15 per treatment) using Bouin, Davidson's, 10% formaldehyde and San Felice for determining the interest in using the chosen fixatives. After verifying that San Felice did not produce samples where lipofuscin could be quantified, a second trial was conducted using the remaining three fixatives and samples of 30 individuals per treatment. The composition of the different fixatives used is presented in Table 1.

These two phases were conducted in different periods and with individuals from different fishing grounds. Therefore, the data from both trials were analysed separately.

The specimens of *Nephrops norvegicus* were obtained from commercial trawlers operating in the South coast of Portugal. The individuals were decapitated at arrival on deck. Only males with carapace lengths ranging from 30.0 to 39.9 mm were used. Groups of 15 specimens were selected randomly and placed in covered buckets containing 4 l of the designated fixative. After a minimum of 48 h, the specimens were rinsed in running water and preserved in 70% alcohol until they were processed. All samples were processed after 3 days.

Histological processing

The brain was extracted under a stereomicroscope, leaving the circum-oesophageal commissures longer than the optic nerve for posterior orientation. The brains were then prepared for paraffin embedding using the following protocol on an automatic tissue processor: alcohol 50% – 1 h; alcohol 70% – 1 h; alcohol 90% – 1 h; alcohol 95% – 1 h; 1st alcohol 100% – 1 h; 2nd alcohol 100% – 1 h; 1st xylene – 1.5 h; 2nd xylene – 1.5 h; 1st wax – 2 h; 2nd wax – over night. Orientation of the brains was chosen as to ensure that the circum-oesophageal commissures would be perpendicular to the cutting surface of the block as described by Sheehy (1989).

Serial 6-μm sections were cut throughout the tissue block. Selected unstained wax sections were slide mounted in DPX after dewaxing in xylene (2×3 min baths).

The sections were observed under a microscope with objective magnification of 40× and fitted with a halogen light with a 450–490 nm excitation filter.

Image analysis

Although lipofuscin granules could be observed throughout the olfactory lobe cell mass (OCLM), the highest concentrations were found in the region where the fibre tracts (connections of the OCLM to other areas of the brain) were visible (Tully, 1993). Five equidistant sections showing fibre tracts were chosen and from each one 4 images were taken, resulting in 20 images for each individual.

The images were captured using a high resolution B&W Sony camera and the image analysis was done using Optimas®, version 6, for Windows 95 (Optimas Corporation, 1996).

The methodology for image treatment consisted in the identification of the region of interest in the OCLM and the application of a filter (Laplacian – HDC & Wallis Filter) to enhance the brightness of the lipofuscin granules in relation to the background fluorescence. A threshold level was then chosen for isolation of lipofuscin granules, adjusting the brighter points to coincide with the lipofuscin granules. The parameters used, number of granules in the image, total area with lipofuscin granules and total area observed were then automatically estimated using a computer program. The data were afterwards stored in an Excel spreadsheet.

Data analysis

In the first trial, only a qualitative analysis of tissue quality was carried out. Samples were classified as either good, when lipofuscin granules could be analysed, or bad when this analysis was difficult or impossible. In the second trial, both qualitative and quantitative data analysis was done. Qualitative analysis focused not only on tissue quality but also on lipofuscin granule brightness. At this time four different categories were defined – excellent, good, medium and bad, depending on the condition, resolution and uniformity of the cells of the OLCM as well as brightness intensity of the granules.

For the qualitative analysis the basic variables total area observed, total area with lipofuscin and total number of granules present were added for each in-

Table 1. Composition of the four different fixatives used in the trials. Table values correspond to ml necessary to produce 1 l of fixative

Compound	Fixative			
	Bouin	Davidson	Formaldehyde 10%	San Felice
Absolute alcohol	–	300	–	–
Chromic acid solution (1%)	–	–	–	640
Formaldehyde 37%	200	200	250	32
Acetic acid	50	100	–	40
Picric acid solution (1.2)	750	–	–	–
Glycerol	–	100	–	–
Seawater	–	300	750	288

dividual and transformed into the following indices of lipofuscin abundance (according to Tully, 1993):

1. Percentage area covered with lipofuscin (equal to total area with lipofuscin divided by observed area and multiplied by 100);
2. Density of lipofuscin granules per 100 μm^2 (equal to total number of granules divided by total area observed and multiplied by 100);
3. Mean size of lipofuscin granules (total area with lipofuscin divided by total number of granules).

Individuals classified as 'bad' were excluded for the purpose of the quantitative analysis. Statistical analyses included comparison of proportions (χ^2 test as in Fleiss, 1973), *t*-tests and one-way ANOVAs for comparison of means.

Results

Results from the first trial revealed that *Nephrops* fixed with Bouin and 10% formaldehyde had a higher percentage of samples with tissue quality suitable for lipofuscin analysis, 87 and 80%, respectively, while only 64% of the samples in Davidson's were in good condition. None of the samples fixed with San Felice were viable for lipofuscin quantification. The results of the first phase of the experiment are presented in Table 2.

In the second phase of the experiment, 10% formaldehyde gave the best results (0% rejection and 60% with excellent tissue quality), Bouin and Davidson produced similar results with respect to tissue condition (40 and 50% excellent or good) but Bouin had better results than Davidson when granule brightness was evaluated. The results of the qualitative data analysis from phase two of the experiments are presented in Table 3. Regarding the brightness of lipofuscin granules, results showed that when using Davison as a fixative, the yellow fluorescing irregularly shaped granules showed less contrast with the blue-green background fluorescence. A test comparing the proportions of excellent samples in relation to granule brightness with 10% formaldehyde and Bouin showed no significant differences (p-value = 0.38).

Table 2. Results of tissue quality obtained in the first phase of the experiment. The classification 'good' corresponds to situations where lipofuscin can be identified clearly, 'bad' was used when tissue shrinkage or degradation made the quantification of lipofuscin difficult

Fixative	*n*	%Good	%Bad
Boulin	15	86.7	13.3
Davidson	14	64.3	35.7
Formaldehyde	15	80.0	20.0
San Felice	15	0.0	100.0

An ANOVA on each one of the variables, testing the null hypothesis that fixation does not affect lipofuscin measurement, led to the rejection of H_0 in all cases (p-values <0.001). The mean values and standard deviations for each variable and treatment level are presented in Table 4. Again, Davidson clearly shows the worst performance, leading to underestimation of the area covered and granule density. A *t*-test was used to compare the two treatments showing better results (Bouin and 10% formaldehyde). Significant differences were found for percentage of lipofuscin cover and average granule size ($p<0.001$) but not for granule density (p=0.224).

Table 3. Qualitative results of tissue quality and granule brightness in the experimental trial. In the groups 'excellent', 'good' and 'medium' lipofuscin could be identified clearly with varying grades of tissue condition or contrast between the lipofuscin granules and the background. The 'bad' classification was used when the tissue was deteriorated or when the contrast between lipofuscin granules and the background was not clear resulting in the impossibility of defining the granule edge

	Fixative	*n*	%Excellent	%Good	%Medium
Tissue quality	Boulin	30	16.7	23.3	30.0
	Davidson	30	6.7	43.3	20.0
	Formaldehyde	30	60.0	20.0	20.0
Granule brightness	Douin	30	73.3	20.0	6.7
	Davidson	30	10.0	63.3	16.7
	Formaldehyde	30	93.3	6.7	0.0

Table 4. Results of lipofuscin indices (mean and standard deviation) using different fixatives

Fixative	Total Area (μm^2)	% Covered Area	Av. G-Density	Av. G-Size (μm^2)
Bouin	27562.7	0.909±0.22 a	0.229±0.05 a	3.952±0.53 a
Davidson	31587.9	0.499±0.36 b	0.105±0.04 b	4.367±1.17 b
Formaldehyde	32340.7	1.14±0.48 b	0.210±0.06 b	5.246±1.16 c

Discussion

From the results of the preliminary trial and with regards to sample quality and rejection rate, we could say that besides formaldehyde (the fixative used in lipofuscin studies), other fixatives such as Bouin could also be used, while San Felice was not appropriate and Davidson should be avoided. Excluding San Felice, all the other fixatives allowed the identification of the yellow fluorescent granules occurring in the OLCM of *Nephrops* and these looked similar in appearance to those reported by Tully (1993) and Sheehy & Wickins (1994). According to the results of this study, Bouin is an alternative to formaldehyde.

Concerning the use of lipofuscin as an age index in crustaceans, Davidson's fixative proved to be less appropriate. Results revealed an underestimation of lipofuscin values, compared to formaldehyde and Bouin. Lipofuscin contents expressed as percent area covered and average granule density in the OCLM were half the values found in samples fixed with formaldehyde and Bouin. This seems to be related to the exclusion of smaller granules during quantification by image analysis; the glycerol or alcohol could be the agent responsible for this occurrence since they are not present in the other two fixatives.

The importance of lipofuscin granule brightness is observed in the result from samples fixed with Bouin. In fact, although tissue quality was not the most appropriate (30% medium and only 17% excellent), the sharp contrast between granules and background permitted an easy quantification of lipofuscin in the OCLM, and even smaller granules could be quantified. One or both of the acids used in the composition of Bouin could be responsible for the enhancement of the granules fluorescence, picric acid being the most likely agent to producing it since it has a strong staining effect (giving a yellowish colour to the samples, Hopwood, 1990).

Overall, 10% formaldehyde proved to be the most appropriate fixative for use in lipofuscin studies, and is in fact widely used for this purpose. It should be noted that reported concentrations of formaldehyde varied from 4% (Sheehy & Wicking, 1994; Sheehy et al., 1998) to 10% (Sheehy, 1990; Tully, 1993; O'Donovan and Tully, 1996). Soaking time was also variable from 24 to 48 h (Tully, 1993; O'Donovan and Tully, 1996; Sheehy et al., 1998) to 2 weeks (Sheehy & Wicking, 1994). In our case, some early attempts to lower the concentration of formaldehyde from 10% to 4% for working safety reasons failed

because samples preserved this way (48 h soaking) did not show quantifiable lipofuscin. Perhaps lower concentration of formaldehyde would have to be associated with longer soaking times, but Sheehy et al. (1998), working with the rock lobster *Panulirus cygnus*, showed good results using 4% formaldehide and 48 h soaking.

In conclusion, formaldehyde is overall the best fixative for lipofuscin studies but Bouin can be considered a viable alternative. The use of Bouin can be important if internal organs other than the brain have to be extracted for other purposes, such as maturation studies using gonads.

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