


Article

A High Abundance of *Holothuria (Halodeima) atra* (Holothuroidea Aspidochirotida) in a *Halimeda* Dominated Habitat

Vanyarat Kongsap¹, Ekkalak Rattanachot^{2,*}, Anchana Prathep^{2,3}, Witthaya Buaphol⁴ and Jaruwan Mayakun^{1,3,*} 

¹ Molecular Evolution and Computational Research Unit, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkhla, Thailand

² Excellence Center for Biodiversity of Peninsular Thailand, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkhla, Thailand

³ Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Songkhla, Thailand

⁴ Mu Ko Phetra National Park, Department of National Parks, Wildlife and Plant Conservation, Langu 91110, Satun, Thailand

* Correspondence: ekkalak.r@psu.ac.th (E.R.); jaruwan.may@psu.ac.th (J.M.)

Abstract: High-value sea cucumber species are overexploited, and the focus of fishing has shifted to low-value species, e.g., *Holothuria (Halodeima) atra*. In this study, the population of *H. atra* was investigated in three different habitats: a seagrass habitat, a seaweed habitat, and bare sand, at an intertidal zone of Lidee Island, Mu Ko Phetra National Park, Satun Province, Thailand. The habitat type was the predictor which had a significant impact on the density and frequency distribution of the body length of *H. atra* individuals. *H. atra* was only found in the seaweed and seagrass habitats. The highest density of this species, 91.1 ± 9.6 inds 100 m^{-2} , was found in the seaweed habitat. The frequency distribution of the body length of *H. atra* individuals in the seagrass habitat showed no significant change throughout the study period, but the mode of the length frequency distribution in the seaweed habitat gradually rose from 9 to 22 cm from January–September 2019. Asexual reproduction was the major source of recruitment. The occurrence of recently fissioned individuals was slightly higher in the seaweed habitat than in the seagrass habitat ($6.0 \pm 1.1\%$ and $2.2 \pm 0.6\%$, respectively). This study showed that *H. atra* was most abundant in the seaweed habitat, which is likely because of the greater availability in this habitat of food sources, microhabitats, and protection against sun irradiance, desiccation, and stress during low tides.

Keywords: habitat; *Halimeda macroloba*; *Halophila ovalis*; *Holothuria atra*; population structure; length-frequency distribution



Citation: Kongsap, V.; Rattanachot, E.; Prathep, A.; Buaphol, W.; Mayakun, J. A High Abundance of *Holothuria (Halodeima) atra* (Holothuroidea Aspidochirotida) in a *Halimeda* Dominated Habitat. *J. Mar. Sci. Eng.* **2023**, *11*, 451. <https://doi.org/10.3390/jmse11020451>

Academic Editors: Michael Karydis and Maurizio Azzaro

Received: 17 January 2023

Revised: 14 February 2023

Accepted: 15 February 2023

Published: 18 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Sea cucumber (Class Holothuroidea, Phylum Echinodermata) are important and common marine invertebrates with 1775 species reported worldwide [1]. They are widely distributed throughout the world and are most diverse in tropical shallow water zones, ranging from the intertidal zone to the abyssal depths [2]. They play many important ecological roles, such as bioturbation, sediment cleaning and mixing, and nutrient recycling in benthic communities [3–6].

Besides their ecological importance, sea cucumbers are also economically important as medical and luxury foods [7,8]. Holothuroids are commercially exploited worldwide and around 20,000 to 40,000 tons of sea cucumbers are sold each year, mostly to Asian markets [2]. In Hong Kong stores, the prices for high-value sea cucumber species can reach up to USD 3583 kg^{-1} [7,9]. Overfishing of sea cucumbers around the world has caused the average harvested body size and density to decrease [2,10–12]. This overexploitation

has led to a severe decline in the wild stocks of high-economic-value species and fishing targets have shifted from high-value to low-value species [11,12]. The overexploitation of high-commercial-value species of sea cucumbers, e.g., *Holothuria (Metriatyula) scabra* Jaeger, 1833 is a common occurrence. Furthermore, fisheries seem to have shifted towards the low-value species [2,12]. In tropical areas, especially in Thailand, *H. scabra* is the most important and economically valuable species that is overexploited and illegally harvested without regulation. The low-value species, *H. atra*, then tends to be harvested after the stocks of *H. scabra* begin to deplete.

H. atra is widespread in the tropical Indo-Pacific region [13]. It is a heavily exploited species in many countries in Asia [14] even though it has low commercial value. It is a high-yielding species in the Chinese market [15] because it commonly occupies shallow intertidal areas where it can be easily harvested, with the potential for future commercialization [13,16–20]. However, in Thailand, *H. atra* is overexploited and harvested without management or regulations [21]. Smaller enterprises, such as culture ponds and sea ranching systems, are ongoing in Thailand; however, the essential baseline knowledge of the biology, reproduction, population, ecology, and behaviour of *H. atra* is still insufficient for successful aquaculture and fishery management.

The population of *H. atra* might be affected by various factors, such as environmental stresses, the availability of food, human disturbances, and habitat preference [17,18,22]. Previous studies have reported that the density, body size, biomass, fission rate, and abundance of *H. atra* varies between habitats, such as between shallow- and deep-tide pools, back reef and front reef, and protected and unprotected areas [17,18,22]. Recently, coastal development, pollution, and nutrient inputs have resulted in the degradation of nearshore habitats [23–25]. Habitat degradation can result in population loss and affect the structure of *H. atra* populations [21,26].

However, in Thailand, the population structures, dynamics, and habitat preferences of sea cucumbers in different habitats still remain unclear and more information is needed. A thorough investigation of *H. atra* populations will provide a better understanding of the needs of a sustainable sea cucumber population. Therefore, the main objective of this study is to investigate the population of *H. atra* in three nearby different habitat types: seagrass beds, seaweed beds, and bare sand. It is hypothesized that the abundance of *H. atra* in seaweed and seagrass habitats is higher than in a bare sand habitat which is likely because the availability of food sources and microhabitats.

2. Materials and Methods

2.1. Study Site

The study site was located in an intertidal zone of the Andaman Sea between Lidee Lek Island and Lidee Yai Island which are located in Mu Ko Phetra National Park ($6^{\circ}47'09.7''$ N $99^{\circ}45'56.3''$ E), Satun Province, in south-western Thailand (Figure 1). There are two main seasons, both monsoon-dominated. The rainy season is dominated by the Southwest monsoon from May to October and the dry season is dominated by the Northeast monsoon from November to April. Salinity in the study area varies from 28–35 psu. In this area, *Halimeda macroloba* Decaisne is the dominant calcified green algal species, with a density of 55.6 ± 7.5 thalli m^{-2} . The dominant seagrass species, with a cover of $47.6 \pm 29\%$, is *Halophila ovalis* (R. Br. Hook. J. H. (*Halodeima*) *atra* is common and abundant, with a density of 72.0 ± 4.5 inds $100 m^{-2}$.

The study site was surveyed and divided into three habitat types: a seaweed habitat with dominant *H. macroloba*, a seagrass habitat with dominant *H. ovalis*, and a bare sand habitat. The estimated area of each habitat was around 1 km^2 .

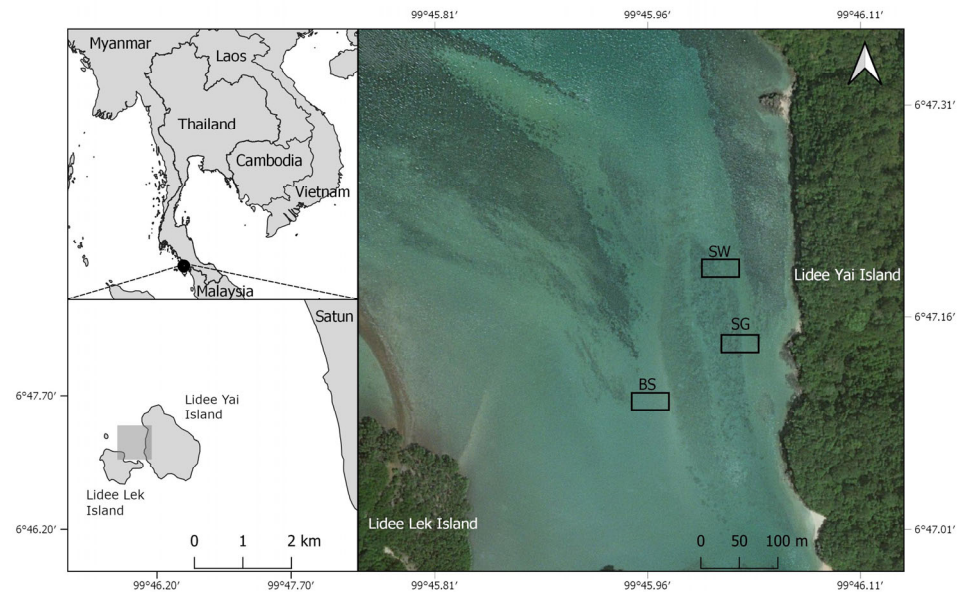


Figure 1. Study site at Lidee Islands, Mu Ko Phetra National Park, in the Andaman Sea, Satun Province. SW: seaweed habitat; SG: seagrass habitat; BS: bare sand habitat.

2.2. Field Sampling Periods

The field studies were carried out monthly from November 2018 to March 2020 except during March, May, August 2019, and February 2020 due to unfavourable weather. The recruitment of *H. atra* was carried out on a monthly basis from September 2019 to March 2020.

2.3. Density, Size, and Recruitment from Asexual Reproduction of *H. atra*

In each habitat type, three permanent belt transects, each 50 m long and 10 m apart, were laid perpendicular to the shoreline of Lidee Yai Island. The number of *H. atra* were counted along a 1 m strip on each side of the transect line and the total length (from mouth to anus) of counted individuals was measured. Asexual reproduction by fission was observed as per Conand [18] to categorize *H. atra* into 3 groups: normal, fissioning, and recently fissioned. Then, the occurrence of the recently fissioned individuals was calculated.

2.4. Recruitment from Sexual Reproduction of *H. atra*

In this survey area, the aboveground part of seaweed and seagrass were examined as substrates for newly settled *H. atra*. The aboveground part of macrophytes were collected using a quadrat which was placed on its natural percent cover or density. For *H. macroloba*, the aboveground parts in the 20 × 20 cm quadrat were cut and kept in bottles. The aboveground part of *H. ovalis* was collected by cutting the sediment in a 10 × 10 cm quadrat to cut the rhizome. Then, the content was scooped and gently washed to remove the sediment and retain only the seagrass. The petioles and leaves of *H. ovalis* were collected. There were 10 replicates for each substrate type. After a few minutes, 99% ethanol was added to the bottle and then gently shaken every 15 min. for 1 h. The contents of the bottle were poured through a 63 μm sieve. The aboveground parts were washed thoroughly with fresh water to detach all remaining larvae from the leaves. The material that remained on a sieve was stored in 99% ethanol before observing for juvenile (adapted from Mercier [27]). The contents of a bottle were observed under a light microscope using a Sedgewick rafter counting chambers. The juveniles were measured (contracted length) under the microscope using an eyepiece micrometre. The remaining content was observed under stereomicroscope to find the small juveniles.

2.5. Abundance and Aboveground of Macrophytes

Quadrats (50 × 50 cm) were placed at 5 m intervals along each line transect to estimate the percentage cover of *H. ovalis* and density of *H. macroloba*. Seagrass and seaweed specimens were identified as per Coppejans et al. [28]. For aboveground biomass of macrophytes, a quadrat, 20 × 20 cm, was placed randomly at 0 m, 25 m, and 50 m of each line transect in each seagrass and seaweed habitat; then, all macrophytes in the quadrat were collected. The above ground part of macrophytes was cleaned, dried in the oven at 60 °C for 48 h, and weighted, respectively.

2.6. Properties of Sediment

Sediment samples (2.5 cm depth) were randomly collected at 0 m, 25 m, and 50 m along each line transect using a 7 cm diameter core drill. Samples were kept in an ice box during the transportation to the laboratory at Faculty of Science, Prince of Songkla University [29]. In the laboratory, sediment samples were dried in an oven at 60 °C for 48 h. To analyse organic matter and grain size, 10 g of dry sediment was sieved through a 1 mm mesh sieve, then ground until homogeneous and weighed to 3 g. The sample was then burned at 450 °C for 5 h, and reweighed. Organic matter was calculated by loss of weight on ignition (LOI; adapted from Storer [30]). For grain-size analysis, the rest of the dried sediments were sieved through 2 mm, 1 mm, 500 µm, 250 µm, 125 µm, and 63 µm mesh sieves and then sediments in each sieve were weighed and mean grain size calculated by using GRADISTAT v.9.1 [31,32].

2.7. Environmental Factors

Water temperature and light intensity were recorded using the Onset Hobo LI light logger (Onset Computer Corporation, Bourne, MA, USA) that was set up at 10 cm from aboveground at each habitat site (from 7 am to 4 pm). Light intensity in Lux was transformed into µmole photon m⁻¹ s⁻¹ [33]. Salinity was measured using a refractometer. Gypsum balls were used to indirectly measure water velocity [34]. Gypsum balls were dried at 60 °C until a constant weight was achieved and weighed before exposure. In the field, the gypsum balls were set at 10 cm above the ground in each transect line for one tide (6–8 h) when the water level was at least 1 m height; then, they were collected and brought back to the laboratory. Used gypsum balls were weighed and the dissolution rate calculated (g h⁻¹).

2.8. Statistical Analyses

A two-way analysis of variance (ANOVA) was used to test the difference in density of *H. atra*, the occurrence of recently fissioned individuals of *H. atra*, and mean grain size among the habitat types and months. A one-way analysis of variance (ANOVA) was used to test the difference of percentage cover of *H. ovalis*, the biomass of *H. ovalis*, the density of *H. macroloba*, the biomass of *H. macroloba*, light intensity, and temperature between months. A Scheirer-Ray-Hare test was used to test the difference in length of *H. atra* and organic matter among habitat types and months. A Kruskal-Wallis test was used to test the distribution of sediment size among habitat types and dissolution rate of gypsum balls between months. Post hoc comparisons were made using Tukey's post hoc test and Dunn's Kruskal–Wallis multiple comparisons. Simple linear regression was used to test the relationship between the density of *H. atra* and the abundance of seaweed and seagrass. Multiple regression was used to test the relationship between density and 2 habitat types (seaweed and seagrass), sediment properties, and physical factors. The R statistical software was used for statistical analysis [35].

3. Results

3.1. Abundance and Aboveground Biomass of Macrophyte Vegetation

In the seagrass habitat, there was a significant difference in the percentage cover of *H. ovalis* between months, with a mean of $46.8 \pm 2.7\%$ (one-way ANOVA, $F_{12,26} = 4.863$, $p < 0.001$, Figure 2A). The lowest percentage cover of *H. ovalis* was found in December 2018 and January 2020, with $17.7 \pm 2.8\%$ and $30.0 \pm 0.9\%$, respectively. The highest was found in November 2019, with $67.8 \pm 8.5\%$. For the aboveground biomass of *H. ovalis*, there was a significant difference between months, with mean 8.4 ± 0.6 gdw m^{-2} (one-way ANOVA, $F_{10,88} = 14.55$, $p < 0.001$; Figure 2B). The lowest aboveground biomass of *H. ovalis* was in December 2019 and January 2020: 3.2 ± 1.1 and 3.8 ± 0.5 gdw m^{-2} , respectively. The highest was found in June 2019: 19.5 ± 1.8 gdw m^{-2} .

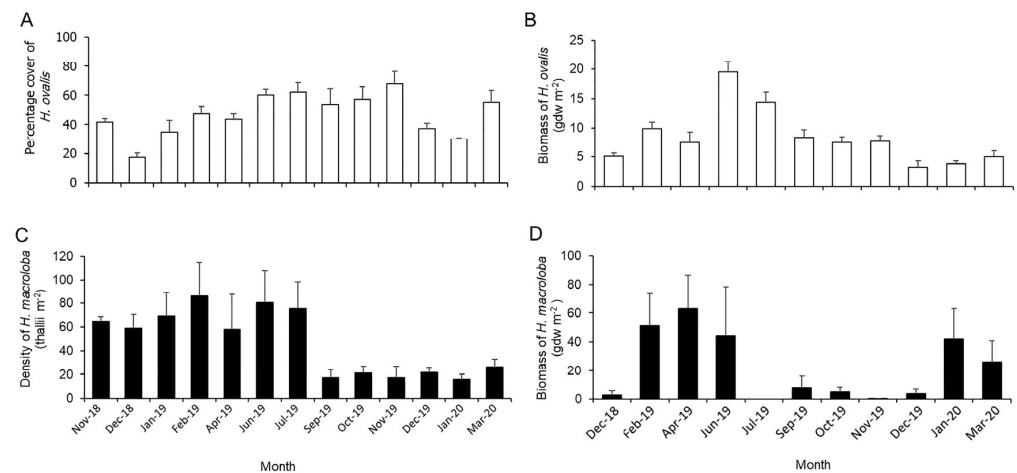


Figure 2. Abundance of macrophyte (mean \pm SE) from November 2018 to March 2020 except March, May, and August 2019, and February 2020, and biomass of macrophytes (mean \pm SE) from December 2018 to March 2020 except January, March, May, and August 2019, and February 2020: (A) percentage cover of *H. ovalis* in seagrass habitat; (B) aboveground biomass of *H. ovalis* in seagrass habitat; (C) density of *H. macroloba* in seaweed habitat and (D) aboveground biomass of *H. macroloba* in seaweed habitat.

In the seaweed habitat, the highest densities of *H. macroloba* were found in November 2018 until July 2019: 57.8 ± 4.1 to 85.7 ± 2.0 thalli m^{-2} . Interestingly, the density of *H. macroloba* dramatically decreased in September 2019 from 75.3 ± 22.9 thalli m^{-2} in July 2019 to 17.2 ± 7.1 thalli m^{-2} in September 2019 (one-way ANOVA, $F_{12,26} = 2.497$, $p < 0.05$; Figure 2C). For the aboveground biomass of *H. macroloba*, there was a significant difference between the months, with a mean of 22.4 ± 5.2 gdw m^{-2} (one-way ANOVA, $F_{10,88} = 2.059$, $p < 0.05$; Figure 2D). The lowest aboveground biomass of *H. macroloba* was found in December 2019 (3.2 ± 1.1 gdw m^{-2}) and the highest was found in June 2019 (19.5 ± 1.8 gdw m^{-2}). Sexual reproduction of *H. macroloba* was observed in September 2019.

3.2. Properties of Sediment

The percentage of sedimentary organic matter was significantly lower in bare sand than in the seagrass and seaweed habitats ($5.4 \pm 0.1\%$, $6.9 \pm 0.2\%$ and $6.8 \pm 0.1\%$, respectively; Table 1). In all three habitats, the highest proportion of sedimentary organic matter was found in April 2019 ($8.5 \pm 0.5\%$) and the lowest proportion was found in June 2019 (5.1 ± 0.14 ; Figure 3A; Table 1). There was no significant difference in the percentage of sedimentary organic matter between seagrass and seaweed habitats ($6.9 \pm 0.2\%$ and $6.8 \pm 0.1\%$, respectively; Table 1).

Table 1. Results of Scheirer-Ray-Hare testing of data of the effects of habitat type and month on total length of *H. atra* and sedimentary organic matter.

Source of Variation	Total Length of <i>H. atra</i>			Organic Matter		
	df	SS	H	Df	SS	H
Habitat type	1	4,006,900	153.930 ***	2	851,280	84.595 ***
Month	12	1,921,400	73.815 ***	12	1,046,093	103.954 ***
Habitat type × month	12	1,304,300	50.106 ***	24	427,825	42.515 *
Residuals	5573	13,849,000		308		

df, degrees of freedom; SS, sum of squares; ^{ns}, no significant differences; ^{*}, $p < 0.05$; ^{***}, $p < 0.001$.

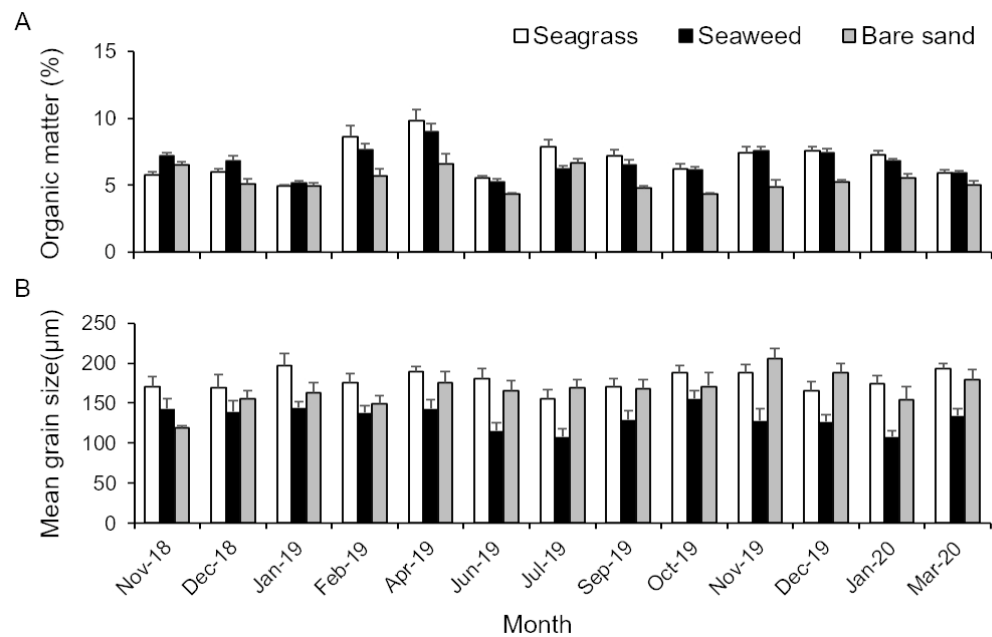


Figure 3. Properties of sediment (mean ± SE): (A) organic matter and (B) grain size in seagrass, seaweed, and bare sand habitats from November 2018 to March 2020.

The mean grain size was significantly different in all three habitats. The smallest mean grain size was found in the seaweed habitat ($131.6 \pm 3.1 \mu\text{m}$) compared with the seagrass habitat and bare sand ($178.3 \pm 3.1 \mu\text{m}$ and $166.7 \pm 3.6 \mu\text{m}$, respectively). The smallest grain size ($144.1 \pm 7.7 \mu\text{m}$) was found in July 2019 in the seaweed habitat and the largest grain size was found in November 2019 in the bare sand habitat ($174.0 \pm 9.6 \mu\text{m}$; Figure 3B; Table 2).

Table 2. Results of two-way ANOVA testing of data of the effects of habitat type and month on density, occurrence of recently fissioned individuals of *H. atra*, and mean grain size.

Source of Variation	Density			Occurrence of Recently Fissioned Individual			Mean Grain Size		
	df	MS	F	df	MS	F	df	MS	F
Habitat type	1	28,425	45.187 ***	1	10.81	0.575 ^{ns}	2	68,820	61.713 ***
Month	12	2159	3.433 ***	5	40.89	2.176 ^{ns}	12	3039	2.725 **
Habitat type × month	12	659	1.048 ^{ns}	5	6.27	0.334 ^{ns}	24	1967	1.764 *
Residuals	52	629		24	18.79		308	1115	

df, degrees of freedom; MS, mean squares; ^{ns}, no significant differences; ^{**}, $p < 0.05$; ^{***}, $p < 0.01$; ^{****}, $p < 0.001$.

The fractions of sediment samples were significantly different among habitats (Kruskal-Wallis, $p < 0.01$). The highest amount of gravel (>2 mm) composed of shell and coral rubble

and fine sand (125 μm) were found in the seaweed habitat. The highest amount of medium sand (250 μm) was found in the seagrass habitat. Meanwhile, the highest amounts of very coarse sand (1 mm), coarse sand (500 μm), very fine sand (63 μm), and mud (<63 μm) were found in the bare sand habitat (Figure 4).

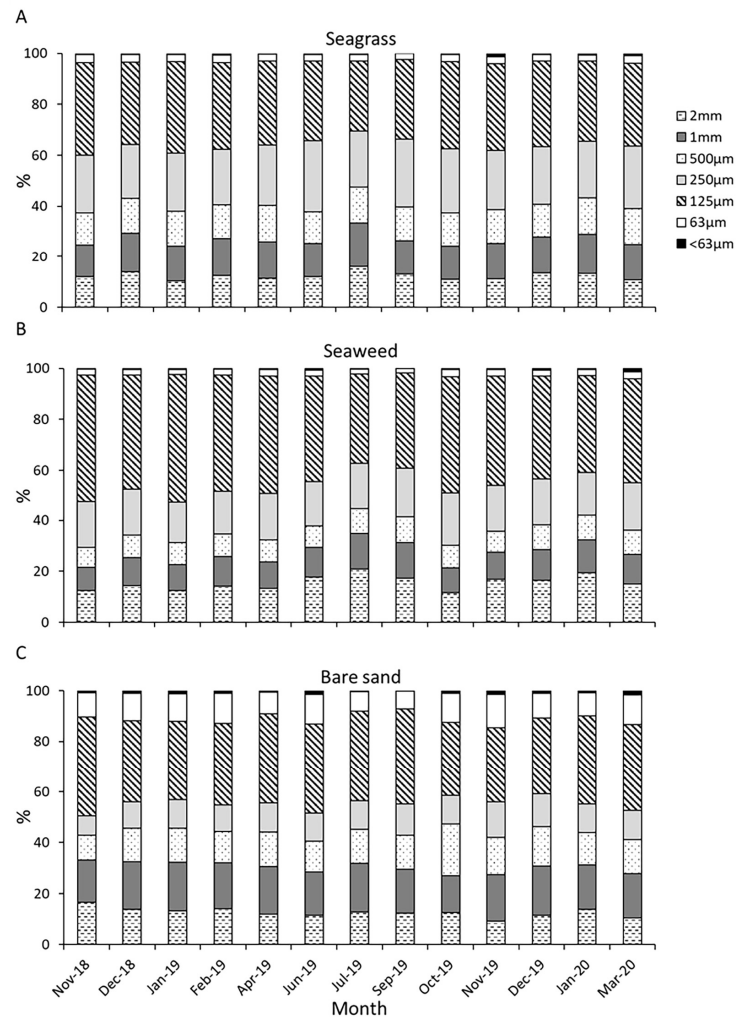


Figure 4. Distribution of sediment size (mean \pm SE) in (A) seagrass; (B) seaweed; and (C) bare sand habitats from November 2018 to March 2020.

3.3. Environmental Factors

Light intensity was highest in February 2019 ($1044.1 \pm 75.3 \mu\text{mole photon m}^{-2} \text{s}^{-1}$), while the lowest light intensity was recorded in November 2019 ($535.5 \pm 75.7 \mu\text{mole photon m}^{-2} \text{s}^{-1}$; one-way ANOVA, $F_{11,23} = 7.767$, $p < 0.001$). The temperature ranged from a low of $28.4 \text{ }^\circ\text{C}$ in January 2020 to a high of $32 \text{ }^\circ\text{C}$ in June 2019 (one-way ANOVA, $F_{11,23} = 15.52$, $p < 0.001$). The water velocity varied between habitat types and months (two-way ANOVA; habitat type, $F_{2,62} = 5.376$, $p < 0.01$; month, $F_{10,62} = 70.924$, $p < 0.001$; interaction, $F_{20,62} = 4.257$, $p < 0.001$). The dissolution rate of gypsum balls was significantly highest in November 2019 ($0.6 \pm 0.04 \text{ g h}^{-1}$) and lowest in October 2019 ($0.3 \pm 0.1 \text{ g h}^{-1}$; Kruskal-Wallis, $H_{10} = 77.969$, $p < 0.001$).

The density of *H. atra* was not significantly correlated with the percentage cover of *H. ovalis*, the density of *H. macroloba*, and the aboveground biomass of *H. macroloba* ($p > 0.05$; Figure 5A,C,D). However, the density of *H. atra* in the seagrass habitat was significantly negatively correlated with the biomass of *H. ovalis* (linear regression, $Y = -1.28X + 59.74$, $R^2 = 0.18$, $F_{1,31} = 6.59$, $p < 0.05$; Figure 5B).

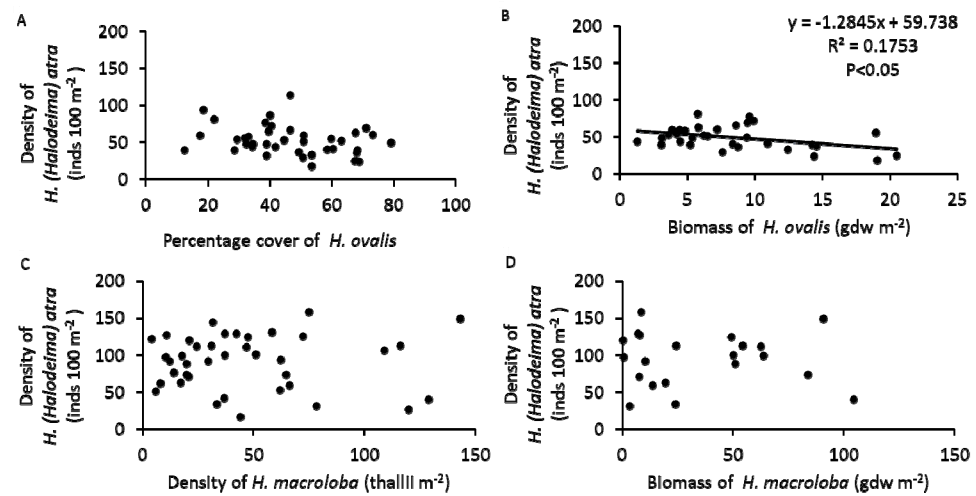


Figure 5. Relationship between density of *H. atra* and abundance of macrophytes: (A) percentage cover of *H. ovalis*; (B) aboveground biomass of *H. ovalis*; (C) density of *H. macroloba*; and (D) aboveground biomass of *H. macroloba*.

For multiple regression analysis between the density of *H. atra* with habitat type, organic matter, grain size, and the physical factors of light, temperature, and the dissolution rate of gypsum balls, the multiple regression analysis revealed that the habitat type was the predictor which had the significantly strongest impact on the density of *H. atra* followed by mean grain size and temperature. The density of *H. atra* was significantly positively correlated with the seaweed habitat and the mean grain size, but negatively correlated with temperature (Table 3).

Table 3. Results of multiple regression analysis testing the influence of habitat type, organic matter, mean grain size, light, temperature, and dissolution rate of gypsum ball on the density of *H. atra*.

Variable	Coefficient	SE	t-Value	VIF
Intercept	351.171	194.561	1.805 ^{ns}	
Habitat type	67.647	11.631	5.816 ^{***}	2.84
Organic matter	4.834	3.458	1.398 ^{ns}	1.54
Mean grain size	0.571	0.207	2.754 ^{**}	2.71
Light	0.024	0.025	0.962 ^{ns}	1.25
Temperature	−14.749	5.837	−2.527 [*]	1.41
Dissolution rate of gypsum ball	−7.348	43.969	−0.167 ^{ns}	1.65

^{ns}, no significant differences; ^{*}, $p < 0.05$; ^{**}, $p < 0.01$; ^{***}, $p < 0.001$.

3.4. Population of *H. atra*

The average density of *H. atra* in the seagrass habitat, 52.8 ± 3.2 inds 100 m^{-2} , was significantly lower than in the seaweed habitat, 91.0 ± 5.9 inds 100 m^{-2} (Table 1). From June to July 2019, the average density of *H. atra* in the seagrass habitat was slightly lower than in other months and increased afterwards. The average density of *H. atra* in the seaweed habitat was considerably low during June–July 2019 but had recovered by September 2019 (Figure 6, Table 2). However, no individuals were found in the bare sand habitat during this study.

The average length of *H. atra* in the seaweed habitat, 13.9 ± 0.1 cm, was significantly longer than *H. atra* in the seagrass habitat, 12.1 ± 0.1 cm (Table 1). The longest average length of *H. atra* was found in September 2019, with 14.3 ± 0.3 cm, while the shortest average length was found in April 2019, with 12.5 ± 0.2 (Table 1). The monthly length-frequency distribution of *H. atra* showed a unimodal distribution in both the seagrass and seaweed habitats. In the seagrass habitat, the mode of length-frequency distribution did not significantly change throughout the study period, ranging from 8 to 12 cm. In the seaweed

habitat, the mode of length-frequency distribution rose from 9 to 22 cm in January 2019 to September 2019, then decreased to 11 cm in October 2019 (Figure 7).

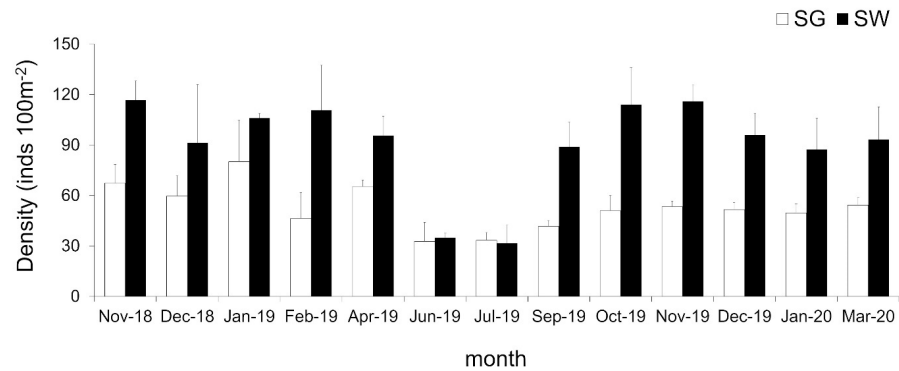


Figure 6. *H. atra* densities (mean ± SE) in the seagrass (SG) and seaweed (SW) habitats from November 2018 to March 2020.

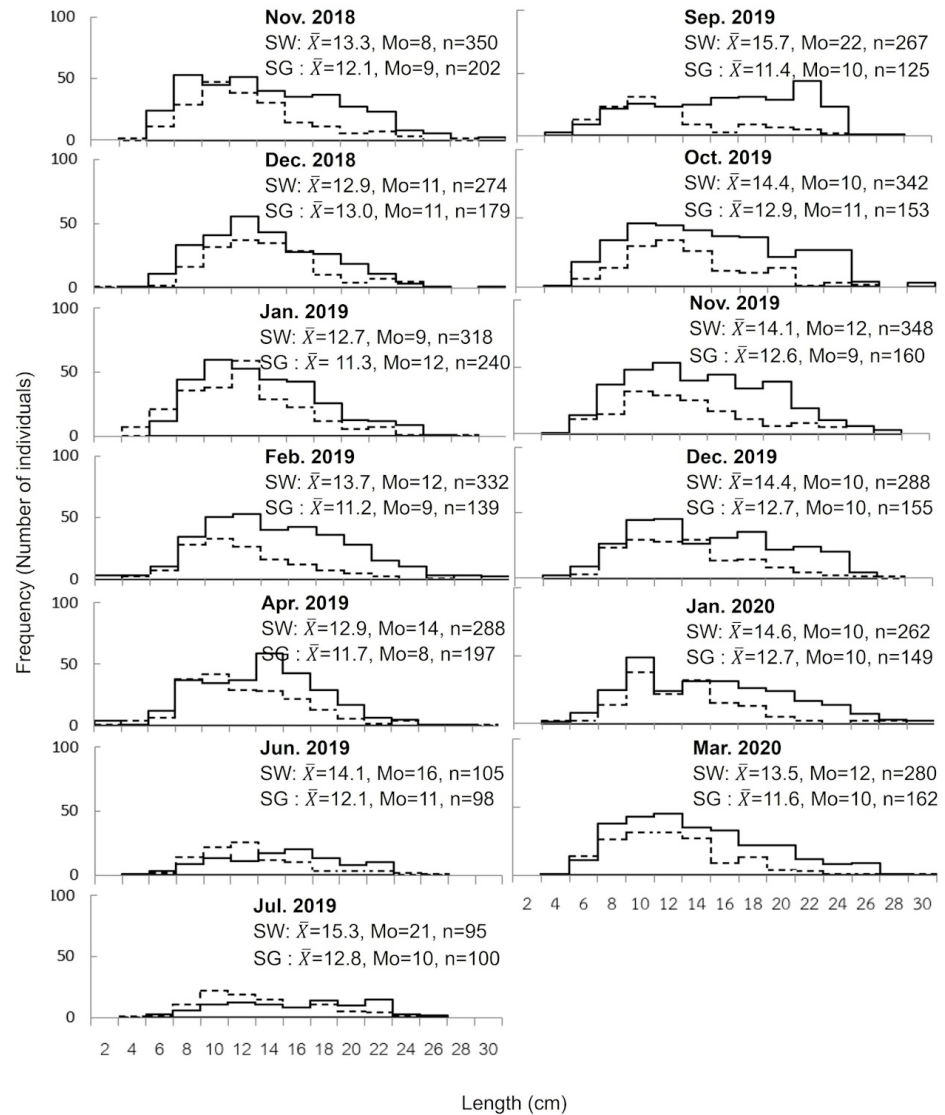


Figure 7. *H. atra* length-frequency distribution, sampled monthly from November 2018 to March 2020 in seagrass (dash line) and seaweed habitats (solid line).

3.5. Recruitment of *H. atra*

For recruitment from asexual reproduction during September to December 2019, the occurrence of recently fissioned individuals of *H. atra* was slightly higher in the seaweed habitat than in the seagrass habitat ($6.0 \pm 1.1\%$ and $2.2 \pm 0.6\%$, respectively), but the difference was not statistically significant (Figure 8, Table 2).

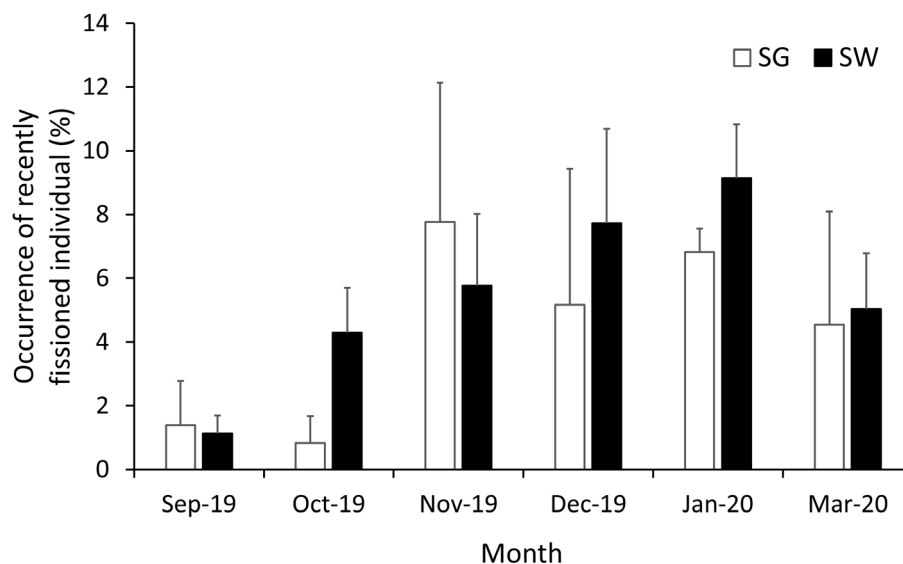


Figure 8. Occurrence of recently fissioned individuals (mean \pm SE) in the seagrass habitat (SG) and seaweed habitat (SW), from September 2019 to March 2020.

4. Discussion

In the present study, the abundance of *H. atra* was higher in the seaweed habitat than in the seagrass habitat and absent in the bare sand habitat. This might be because of habitat structure. The structure of *H. macroloba* is more complex than the structure of *H. ovalis* based on leaf morphology, dimensions of shoots, canopy level, and leaf length [36]. *H. macroloba* has a large, erect thallus that protrudes approximately 5–15 cm above the substrates, and its branching is highly dimensional. *H. ovalis* is very short, protruding approximately 1–2 cm above the substrate, and has thin leaves compared with *H. macroloba*. The different structures of these habitat types might provide differences in protection and food availability for sea cucumbers.

In this study site, the sea cucumber was exposed to the air for around 3 h twice a day during the spring tide period. Therefore, the complex structure of *H. macroloba* can provide the sea cucumber with better protection and shelter from low-tide stress, i.e., high temperature, solar irradiance, and desiccation, than the seagrass habitat and bare sand [22,37–44]. In addition, multiple regression revealed that *H. atra* density was positively related to seaweed habitat, but negatively related to temperature. Furthermore, we often found *H. atra* tightly wreathed around *H. macroloba* by its tube feet (Figure 9A–D). This behaviour might help to stabilize its body firmly against tidal currents that could dislodge it. Thus, *H. macroloba* presents a more suitable habitat for *H. atra* and it acts like a pillar for sea cucumbers to attach to (personal observation). Therefore, *H. atra* might prefer to live in the habitats that provide better protection and shelter from low-tide stress and tidal currents.



Figure 9. *H. atra* attaches and wreathes its body around *H. macroloba* (A–D).

Regarding food availability, we verified that the bare sand habitat had the lowest amount of organic matter due to less ability to trap and retain organic matter [36,45]. Moreover, the high content of organic matter in the macrophyte-dominated habitat was composed of dead matter from the macrophytes [46,47]. *H. ovalis* and *H. macroloba* both have high growth rates and fast turnover rates. *H. ovalis* can produce new leaf pairs in 4 days [48] and has a turnover rate of 10 leaves per shoot⁻¹ year⁻¹ [49] while *H. macroloba* produces one-two new segments per thallus⁻¹ day⁻¹ and a thallus turnover rate of 14.7–29.4 days [50]. Previous studies reported that *H. atra* shows selective feeding on the sediments rich in its food, i.e., organic matter and microalgae [51–53]. Therefore, *H. atra* might prefer to live in high-organic matter habitats, including the seaweed habitat and seagrass habitat.

In addition, this study reported that there was variation in the abundance and biomass of macrophytes between months. The abundance and biomass of *H. ovalis* were slightly lower in the dry season, similar to a previous study in the southern Andaman Sea [47,48]. This might be because a longer exposure period or higher irradiance in the dry season can damage the tissue and create associated stress symptoms [54]. In the seaweed habitat, a high abundance and biomass of *H. macroloba* was found in December 2018 until July 2019, and dramatically decreased in September 2019. This might be because of the die-off after the sexual reproduction period [50]. After that, the biomass of *H. macroloba* recovered in January 2020. Interestingly, the analyses of linear regression indicated that there was no relationship between the density of *H. atra* and the abundance of *H. macroloba*. This might be because during low-density periods of *H. macroloba*, the seaweed habitats still provide a better availability of food, shelter, and protection than the seagrass and bare sand habitats. In addition, the abundance of the seaweed will recover in a few months [50]. The regression analysis demonstrated that the density of *H. atra* was negatively related to the biomass of *H. ovalis*. However, this relationship was unclear. A possible reason might be because the belowground network of *H. ovalis* is compact, and their leaves are dense (two leaves per node), occupying both aboveground and belowground spaces and obstructing the sea cucumber from obtaining food from the sand. From field observation, *H. atra* found in the seagrass habitat always live in the sparse spots of seagrass where they can easily obtain food on the surface of substrates.

From the multiple regression analysis, it was determined that the main factors influencing *H. atra* density were the habitat types and temperature, as the environmental stressors that were discussed in the previous section. Moreover, grain size was positively related to the density, which might be due to the fact that sand particles were dominant in

these areas, while mud ($<63 \mu\text{m}$) was less than 5%. In addition, *H. atra* prefers to feed on the sand more than fine sediment, which relates to a previous report showing that around 90% of sediment in *H. atra* intestine was composed of sand and gravel, while 10% was silt [55].

In this study, the length-frequency distribution of *H. atra* was different between habitats. In the seagrass habitat, the length-frequency distribution of *H. atra* was dominated by small individuals and the mode did not change throughout the year. This was similar to previous studies carried out in shallow waters near Ananij Island, Wanlitung, and Reunion Island [17,56–58]. It might be that fission produces a small individual but compensates for overall mortality and emigration [17,59–62]. In the seaweed habitat, the mode gradually rose from 8 to 22 cm from January until September 2019. After that, it rapidly decreased to 11 cm by October 2019. The findings were quite similar to a previous study by Ongkers et al. [63] which showed that the average size of *H. atra* in the coastal waters of Morella gradually increased from April to September 2018. Uthicke [59] also reported temporal variation in the mode of the length-frequency distribution of *H. atra* at Fantome Island and Brook Island. However, the reason for this phenomenon in the seaweed habitat was unclear. Therefore, for a better understanding of population dynamics, such as migration and recruitment from both sexual and asexual reproduction, and seasonal variation, long-term studies are needed.

For asexual recruitment, we hypothesized that there was a higher occurrence of recently fissioned individuals of *H. atra* in the seagrass habitat than in the seaweed habitat, since a triggering factor for the asexual reproduction of *H. atra* is a stressful environment [17,56,64,65]. Thus, the more complex structure of the seaweed habitat might reduce the trigger for fission. However, slightly higher fission in the seaweed habitat than in the seagrass habitat might be because *H. atra* utilized the strong-flat thallus of *H. macroloba* as a substrate to hold while twisting its body during fission ([17], personal observation, Figure 10A,B).

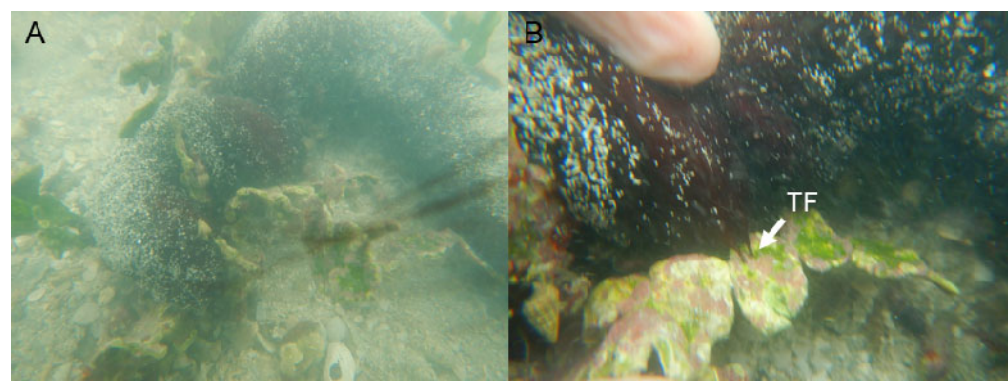


Figure 10. Using its tube feet (TF), *H. atra* holds *H. macroloba* to help twist its body (A,B).

Regarding sexual recruitment, newly settled and small juveniles were not found during this study. This might be because of a failure of fertilization and an unsuitable environment [66,67]. A failure of fertilization occurs due to the low density of mature individuals [66]. Dissanayake and Stefansson [68] reported that the size of the mature individuals of *H. atra* were 16 cm long, which showed low abundance in this study site. Moreover, Chao et al. [66] showed that small individuals ($<190 \text{ g wet wt}$) in the fission population showed no signs of spawning even if they showed mature gonads. Second, previous studies have reported the survivorship of sea cucumbers in these stages to depend on proper environmental conditions [69–71]. Stressful environments, i.e., high temperature, desiccation, and varied salinity might cause the mortality of larvae, newly settled, and small juveniles of *H. atra* [38,66,67]. Thus, the absence and unpredictability of the newly settling and small juveniles of *H. atra* can indicate that asexual reproduction might be the major source of the recruitment to maintain this population [56,66].

5. Conclusions

In this study, we studied the population structures of *H. atra* in three different habitats: a seagrass habitat, a seaweed habitat, and bare sand. Our studies found *H. atra* in the seaweed and seagrass habitats, but no *H. atra* individuals were found in the bare sand habitat. Different habitat types are correlated to the density and length-frequency distribution of this sea cucumber. *H. atra* is more abundant in vegetated areas where shelter, food, and protection are available. These findings regarding the habitat of *H. atra* have implications for aquaculture in terms of site selection. In addition, these results suggest that different habitats could influence the population structure and dynamics of *H. atra*. Therefore, habitat preservation is an important management strategy to maintain sustainable populations of *H. atra* and possibly other sea cucumber populations.

Author Contributions: Conceptualization, J.M., E.R. and A.P.; methodology, V.K., E.R., J.M. and W.B.; formal analysis, V.K.; investigation, V.K.; writing—original draft preparation, V.K.; writing—review and editing, J.M. and E.R.; visualization, E.R. and J.M.; supervision, J.M., E.R. and A.P.; project administration, J.M. and E.R.; funding acquisition, E.R., J.M. and V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Faculty of Science Research Fund, Prince of Songkla University (PSU) (J.M., Research Grant, 2020, contract no. 163003) and the Faculty of Science Research Fund, Prince of Songkla University (PSU) (V.K., contract no. 1-2562-02-007). Financial support to E.R. from The Thailand Research Fund and Office of the Higher Education Commission (contract no. MRG6180174) is acknowledged.

Institutional Review Board Statement: The license to use animals for scientific purposes, ID U1-07886-2561, was provided by the Institute of Animals for Scientific Purpose Development (IAD), National Research Council of Thailand (NRCT).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the Seaweed and Seagrass Research Unit TEAM, Prince of Songkla University, Watana Chaithongkeaw, and the staff of Mu Ko Phetra National Park, Langu, Satun, Thailand for field assistance. Thanks to Thomas Duncan Coyne for assistance with the English.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. World Register of Marine Species. Available online: [https://www.marinespecies.org/aphia.php?p=browser&id\[\]=148744&id\[\]=1393249#focus](https://www.marinespecies.org/aphia.php?p=browser&id[]=148744&id[]=1393249#focus) (accessed on 31 October 2022).
2. Toral-Granda, V.; Lovatelli, A.; Vasconcellos, M. *Sea Cucumbers: A Global Review of Fisheries and Trade*; Food and Agriculture Organization: Rome, Italy, 2008; pp. 1–304.
3. Mercier, A.; Battaglene, S.C.; Hamel, J.F. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *J. Exp. Mar. Biol. Ecol.* **1999**, *239*, 125–156. [[CrossRef](#)]
4. Schneider, K.; Silverman, J.; Woolsey, E.; Eriksson, H.; Byrne, M.; Caldeira, K. Potential influence of sea cucumbers on coral reef CaCO₃ budget: A case study at One Tree Reef. *J. Geophys. Res.* **2011**, *116*, G04032.
5. MacTavish, T.; Stenton-Dozey, J.; Vopel, K.; Savage, C. Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically Enriched Coastal Sediments. *PLoS ONE* **2012**, *7*, e50031. [[CrossRef](#)] [[PubMed](#)]
6. Domínguez-Godino, J.A.; González-Wangüemert, M. Habitat associations and seasonal abundance patterns of the sea cucumber *Holothuria arguinensis* at Ria Formosa coastal lagoon (South Portugal). *Aquat. Ecol.* **2020**, *54*, 337–354. [[CrossRef](#)]
7. Purcell, S.W. Value, Market Preferences and Trade of Bêche-de-mer from Pacific Island Sea Cucumbers. *PLoS ONE* **2014**, *9*, e95075. [[CrossRef](#)]
8. Xia, S.D.; Wang, X.Y. Nutritional and medicinal value. In *The Sea Cucumber *Apostichopus japonicus*: History, Biology and Aquaculture*; Yang, H.S., Mercler, A., Hamel, J.F., Eds.; Academic Press: New York, NY, USA, 2015; Volume 39, pp. 353–366.
9. Purcell, S.W.; Williamson, D.H.; Ngaluafé, P. Chinese market prices of Bêche-de-mer: Implications for fisheries and aquaculture. *Mar. Policy* **2018**, *91*, 58–65. [[CrossRef](#)]

10. Lovatelli, A.; Conand, C.; Purcell, S.; Uthicke, S.; Hamel, J.F.; Mercier, A. *Advances in Sea Cucumber Aquaculture and Management*; Food and Agriculture Organization: Rome, Italy, 2004; pp. 1–418.
11. Hasan, M.H. Destruction of sea cucumber populations due to overfishing at Abu Ghosoun area, Red Sea. *J. Basic. Appl. Zool.* **2019**, *80*, 5. [[CrossRef](#)]
12. Anderson, S.C.; Flemming, J.M.; Watson, R.; Lotze, H.K. Serial exploitation of global sea cucumber fisheries. *Fish.* **2010**, *12*, 317–339. [[CrossRef](#)]
13. Purcell, S.W.; Samyn, Y.; Conand, C. *A Commercially Important Sea Cucumber of the World*; Food and Agriculture Organization: Rome, Italy, 2012; pp. 80–81.
14. Choo, P.S. Population status, fisheries, and trade of sea cucumbers in Asia. In *Sea Cucumbers: A Global Review of Fisheries and Trade*; Toral-Granda, V., Lovatelli, A., Vasconcellos, M., Eds.; Food and Agriculture Organization: Rome, Italy, 2008; Volume 516, pp. 81–118.
15. Li, X. Fishery and resource management of tropical sea cucumbers in the islands of the South China Sea. In *Advances in Sea Cucumber Aquaculture and Management*; Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.H., Mercier, A., Eds.; Food and Agriculture Organization: Rome, Italy, 2004; Volume 463, pp. 261–265.
16. Bonham, K.; Held, E. Ecological Observations on the Sea Cucumbers *Holothuria atra* and *H. eucospilota* at Rongelap Atoll, Marshall Islands. *Pac. Sci.* **1963**, *17*, 305–314.
17. Chao, S.M.; Chen, C.P.; Alexander, P.S. Fission and its effect on population structure of *Holothuria atra* (Echinodermata: Holothuroidea) in Taiwan. *Mar. Biol.* **1993**, *116*, 109–115. [[CrossRef](#)]
18. Conand, C. Asexual reproduction by fission in *Holothuria atra*: Variability Asexual Reproduction Regeneration Holothurian Strategy Indo-Pacific of some parameters in populations from the tropical Indo Pacific. *Oceanol. Acta* **1996**, *19*, 209–216.
19. Conand, C. Holothurians (sea cucumbers, Class Holothuroidea). In *The Living Marine Resources of the Western Central Pacific*; Carpenter, K.E., Niem, V.H., Eds.; Food and Agriculture Organization: Rome, Italy, 1998; Volume 2, pp. 1158–1164.
20. Thorne, B.V.; Eriksson, H.; Byrne, M. Long term trends in population dynamics and reproduction in *Holothuria atra* (Aspidochirotida) in the southern Great Barrier Reef; the importance of asexual and sexual reproduction. *J. Mar. Biol. Asso. UK* **2013**, *93*, 1067–1072. [[CrossRef](#)]
21. Bussarawit, S.; Thongtham, N. Sea Cucumber Fisheries and Trade in Thailand. In *Proceedings of the Conservation of Sea Cucumber in Malaysia, Their Taxonomy, Ecology and Trade*, Kuala Lumpur, Malaysia, 25 February 1999; pp. 26–36.
22. Asha, P.S.; Diwakar, K.; Santhanavalli, G.; Manissery, M.K. Comparative distribution and habitat preference of the sea cucumber *Holothuria atra* Jaeger at protected and unprotected sites in Thoothukudi region of Gulf of Mannar, south-east coast of India. *Indian J. Fish.* **2015**, *62*, 52–57.
23. Short, F.T.; Wyllie-Echeverria, S. Natural and human-induced disturbance of seagrasses. *Environ. Conserv.* **1996**, *23*, 17–27. [[CrossRef](#)]
24. Edmunds, P.J.; Carpenter, R.C. Recovery of *Diadema antillarum* reduces macroalgal cover and increases abundance of juvenile corals on a Caribbean reef. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5067–5071. [[CrossRef](#)]
25. Cheevaporn, V.; Menasveta, P. Water pollution and habitat degradation in the Gulf of Thailand. *Mar. Pollut. Bull.* **2003**, *47*, 43–51. [[CrossRef](#)]
26. Choo, P.S. The Philippines: A hotspot of sea cucumber fisheries in Asia. In *Sea Cucumbers: A Global Review of Fisheries and Trade*; Toral-Granda, V., Lovatelli, A., Vasconcellos, M., Eds.; Food and Agriculture Organization: Rome, Italy, 2008; Volume 516, pp. 119–140.
27. Mercier, A.; Battaglione, S.C.; Hamel, J.F. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia* **2000**, *440*, 81–100. [[CrossRef](#)]
28. Coppejans, E.; Prathep, A.; Lewmanomont, K.; Hayashizaki, K.; De Clerck, O.; Leliaert, F.; Terada, R. *Seaweeds and Seagrasses of the Southern Andaman Sea Coast of Thailand*; Kagoshima University Museum: Kagoshima, Japan, 2017; pp. 13–245.
29. Sloan, N.A.; von Bodungen, B. Distribution and Feeding of the Sea Cucumber *Isostichopus badionotus* in Relation to Shelter and Sediment Criteria of the Bermuda Platform. *Mar. Ecol. Prog. Ser.* **1980**, *2*, 257–264. [[CrossRef](#)]
30. Storer, D.A. A simple high sample volume ashing procedure for determining soil organic matter. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 759–772. [[CrossRef](#)]
31. Wentworth, C.K. A Scale of Grade and Class Terms for Clastic Sediments. *J. Geol.* **1922**, *30*, 377–392. [[CrossRef](#)]
32. Blott, S.J.; Pye, K. GRADISTAT: A grain size distribution and statistics package for the analysis unconsolidated sediments. *Earth Surf. Process. Landf.* **2001**, *26*, 1237–1248. [[CrossRef](#)]
33. Stankovic, M.; Kaewsrihkhaw, R.; Rattanachot, E.; Prathep, A. Modeling of suitable habitat for small-scale seagrass restoration in tropical ecosystems. *Estuar. Coast. Shelf Sci.* **2019**, *231*, 106465. [[CrossRef](#)]
34. Komatsu, T.; Umezawa, Y.; Nakaoka, M.; Supanwanid, C.; Kanamoto, Z. Water flow and sediment in *Enhalus acoroides* and other seagrass beds in the Andaman Sea, off Khao Bae Na, Thailand. *Coast. Mar. Sci.* **2004**, *29*, 63–68.
35. R Core Team. The R Project for Statistica. Available online: <https://www.R-project.org/> (accessed on 10 November 2021).
36. Panyawai, J.; Tuntiprapas, P.; Prathep, A. High macrophyte canopy complexity enhances sediment retention and carbon storage in coastal vegetative meadows at Tangkhen Bay, Phuket, Southern Thailand. *Ecol. Res.* **2019**, *34*, 201–212. [[CrossRef](#)]
37. Adams, A.J.; Locascio, J.V.; Robbins, B.D. Microhabitat use by a post-settlement stage estuarine fish: Evidence from relative abundance and predation among habitats. *J. Exp. Mar. Biol. Ecol.* **2004**, *299*, 17–33. [[CrossRef](#)]

38. Seeruttun, R. A Study on the Factors Influencing the Growth and Survival of Juvenile Sea Cucumber, *Holothuria atra*, under Laboratory Conditions. *Univ. Maurit. R. J.* **2008**, *14*, 1–15.
39. Seabra, R.; Wethey, D.S.; Santos, A.M.; Lima, F.P. Side matters: Microhabitat influence on intertidal heat stress over a large geographical scale. *J. Exp. Mar. Biol. Ecol.* **2011**, *400*, 200–208. [[CrossRef](#)]
40. Dissanayake, D.C.T.; Stefansson, G. Habitat preference of sea cucumbers: *Holothuria atra* and *Holothuria edulis* in the coastal waters of Sri Lanka. *J. Mar. Biol. Assoc. UK* **2012**, *92*, 581–590. [[CrossRef](#)]
41. Lampe, K. Holothurian density, distribution and diversity comparing sites with different degrees of exploitation in the shallow lagoons of Mauritius. *SPC Bêche-de-mer Inf. Bull.* **2013**, *33*, 23–29.
42. Cabansag, J.B.P.; Margarita, T.; Gajelan-Samson, M.B.P.; Diaz, F.A. Abundance and density of *Holothuria atra* and *Bohadschia marmorata* in Marine Protected Areas (MPAs) of Guiuan Eastern Samar and Palompon Leyte, Philippines. *Int. J. Agric. Technol.* **2014**, *10*, 119–131.
43. Setyastuti, A. Echinodermata, *Holothuria atra*, in an intertidal seagrass bed off The Bama beach, Baluran National Park, East Java, Indonesia. *J. Ilmu Teknol. Kelaut. Trop.* **2014**, *6*, 31–39.
44. Darnell, M.Z.; Backwell, P.R.Y.; Stockbridge, J.; Dyson, M.L.; Munguia, P. Thermal and desiccation constraints drive territory preference in fiddler crabs. *J. Exp. Mar. Biol. Ecol.* **2019**, *518*, 151173. [[CrossRef](#)]
45. Hendriks, I.E.; Bouma, T.J.; Morris, E.P.; Duarte, C.M. Effects of seagrasses and algae of the Caulerpa family on hydrodynamics and particle-trapping rates. *Mar. Biol.* **2010**, *157*, 473–481. [[CrossRef](#)]
46. Honkoop, P.J.C.; Berghuis, E.M.; Holthuijsen, S.; Lavaleye, M.S.S.; Piersma, T. Molluscan assemblages of seagrass-covered and bare intertidal flats on the Banc d'Arguin, Mauritania, in relation to characteristics of sediment and organic matter. *J. Sea Res.* **2008**, *60*, 255–263. [[CrossRef](#)]
47. Tuntiprapas, P.; Hayashizaki, K.; Ogawa, H.; Panyawai, J.; Tamada, S.; Stankovic, M.; Prathep, A. The contributions of allochthonous and autochthonous materials to organic carbon in coastal sediment: A case study from Tangkhen Bay, Phuket, Thailand. *Ecol. Res.* **2019**, *34*, 718–729. [[CrossRef](#)]
48. Kaewsrikhaw, R.; Ritchie, R.J.; Prathep, A. Variations of tidal exposures and seasons on growth, morphology, anatomy and physiology of the seagrass *Halophila ovalis* (R.Br.). Hook. f. in a seagrass bed in Trang Province, Southern Thailand. *Aquat. Bot.* **2016**, *130*, 11–20. [[CrossRef](#)]
49. Vermaat, J.E.; Agawin, N.S.R.; Duarte, C.M.; Fortes, M.D.; Marbà, N.; Uri, J.S. Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Mar. Eco. Prog. Ser.* **1995**, *124*, 215–225. [[CrossRef](#)]
50. Mayakun, J.; Prathep, A. Calcium carbonate productivity by *Halimeda macroloba* in the tropical intertidal ecosystem: The significant contributor to global carbonate budgets. *Phycol. Res.* **2019**, *67*, 94–101. [[CrossRef](#)]
51. Moriarty, D.J.W. Feeding of *Holothuria atra* and *Stichopus chloronotus* on Bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. *Aust. J. Mar. Fresh. Res.* **1982**, *33*, 255–263. [[CrossRef](#)]
52. Uthicke, S.; Karez, R. Sediment patch selectivity in tropical sea cucumbers (Holothurioidea: Aspidochirotida) analyzed with multiple choice experiments. *J. Exp. Mar. Bio. Ecol.* **1999**, *236*, 69–87. [[CrossRef](#)]
53. Viyakarn, V.; Chavanich, S.; Heery, E.; Raksasab, C. Distribution of sea cucumbers, *Holothuria atra*, on reefs in the upper Gulf of Thailand and the effect of their population densities on sediment microalgal productivity. *Estuar. Coast. Shelf Sci.* **2020**, *235*, 106514. [[CrossRef](#)]
54. Ralph, P.J.; Burchett, M.D. Photosynthetic responses of the seagrass *Halophila ovalis* (R. Br.) Hook. f. to high irradiance stress, using chlorophyll a fluorescence. *Aquat. Bot.* **1995**, *51*, 55–66. [[CrossRef](#)]
55. Hartati, R.; Zainuri, M.; Ambariyanto, A.; Widianingsih, W. Feeding selectivity of *Holothuria atra* in different microhabitat in Panjang Island, Jepara (Java, Indonesia). *Biodiversitas* **2020**, *21*, 2233–2239. [[CrossRef](#)]
56. Ebert, T.A. Growth and Size of the Tropical Sea Cucumber *Holothuria (Halodeima) atra* Jager at Enewetak Atoll, Marshall Islands. *Pac. Sci.* **1978**, *32*, 183–191.
57. Jaquemet, S.; Rousset, V.; Conand, C. Asexual reproduction parameters and the influence of fission on a *Holothuria atra* sea cucumber population from a fringing reef on Reunion Island (Indian Ocean). *SPC Bêche-de-mer Inf. Bull.* **1999**, *11*, 12–18.
58. Conand, C. Monitoring a fissiparous population of *Holothuria atra* on a fringing reef on Reunion Island (Indian Ocean). *SPC Bêche-de-mer Inf. Bull.* **2004**, *20*, 22–25.
59. Uthicke, S. Influence of asexual reproduction on the structure and dynamics of *Holothuria (Halodeima) atra* and *Stichopus chloronotus* populations of the Great Barrier Reef. *Mar. Fresh. Res.* **2001**, *52*, 205–215. [[CrossRef](#)]
60. Lane, D. Sea cucumber diversity and resources in Brunei, Borneo Island. In Proceedings of the 11th International Echinoderm Conference, Munich, Germany, 6–10 October 2004; pp. 231–237.
61. Lee, J.; Byrne, M.; Uthicke, S. The influence of population density on fission and growth of *Holothuria atra* in natural mesocosms. *J. Exp. Mar. Biol. Ecol.* **2008**, *365*, 126–135. [[CrossRef](#)]
62. Ebert, T.A. Dynamics of *Holothuria atra* at Enewetak Atoll, Republic of the Marshall Islands, based on tetracycline tagging. In *Echinoderms: Durham: Proceedings of the 12th International Echinoderm Conference, Durham, UK, 7–11 August 2006*; Taylor & Francis: Washington DC, USA, 2009; pp. 609–614.
63. Ongkers, O.T.S.; Pattinasarany, M.; Mamesah, J.A.B.; Uneputti, P.R.A.; Pattikawa, J.A. Size distribution and growth pattern of *Holothuria atra* and *Holothuria scabra* in the coastal waters of Morella, Central Maluku Indonesia. *Int. J. Fish. Aquat. Stud.* **2018**, *6*, 301–305.

64. Harriott, V.J. Reproductive biology of three congeneric sea cucumber species, *Holothuria atra*, *H. Impatiens* and *H. edulis*, at Heron Reef, Great Barrier Reef. *Mar. Freshw. Res.* **1985**, *36*, 51–57. [[CrossRef](#)]
65. Uthicke, S. Seasonality of asexual reproduction in *Holothuria (Halodeima) atra*, *H. (H.) edulis* and *Stichopus chloronotus* (Holothuroidea: Aspidochirotida) on the Great Barrier Reef. *Mar. Biol.* **1997**, *129*, 435–441. [[CrossRef](#)]
66. Chao, S.M.; Chen, C.P.; Alexander, P.S. Reproduction and growth of *Holothuria atra* (Echinodermata: Holothuroidea) at two contrasting sites in southern Taiwan. *Mar. Biol.* **1994**, *119*, 565–570. [[CrossRef](#)]
67. Indriana, L.F.; Firdaus, M.; Soffa, F.B. Fissiparous sea cucumber (*Holothuria atra*) population in north lombok, Indonesia. *Mar. Res. Indones.* **2018**, *43*, 1–8. [[CrossRef](#)]
68. Dissanayake, D.C.T.; Stefansson, G. Reproductive biology of the commercial sea cucumber *Holothuria atra* (Holothuroidea: Aspidochirotida) in the northwestern coastal waters of Sri Lanka. *Invertebr. Reprod. Dev.* **2010**, *54*, 65–76. [[CrossRef](#)]
69. Hamel, J.; Mercier, A. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *J. Fish. Aquat. Sci.* **1996**, *53*, 253–271. [[CrossRef](#)]
70. Balch, T.; Scheibling, R.E. Larval supply, settlement, and recruitment in echinoderms. In *Echinoderm Studies*; Jangoux, M., Lawrence, J.M., Eds.; A. A Balkema: Rotterdam; The Netherland; Brookfield, VT, USA, 2001; Volume 6, pp. 1–83.
71. Asha, P.S.; Muthiah, P. Effects of temperature, salinity and pH on larval growth, survival and development of the sea cucumber *Holothuria spinifera* Theel. *Aquaculture* **2005**, *250*, 823–829. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.