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Original Article

Effects of physicochemical parameters on the reproductive pattern of sea cucumber *Holothuria scabra* in Sabah

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Abstract

Sea cucumber *Holothuria scabra* is one of the species that can produce high quality *beche-de-mer*. It has been overexploited and overfished nowadays due to high demand from the local and international markets. It is one of the most valuable species and has become a source of income for small scale fishermen in Sabah, Malaysia. This study aimed to describe the influence of physicochemical parameters on the reproductive biology of *H. scabra* at two sites, Kudat and Kunak, Sabah, Malaysia. The study was conducted for 14 months and samples were collected monthly, between July 2015 and August 2016. Gonad index and data on the physicochemical parameters (temperature, salinity, chlorophyll *a*, and organic matter) were recorded to determine the relationships that can affect the reproduction of *H. scabra*. An annual reproductive pattern was recorded in Kudat with the spawning period from July to November 2015. A continuous pattern in Kunak was recorded with a maximum gonad index in September 2015. The gonad index showed no significant (P>0.05) correlation with the selected physicochemical parameters in either Kudat or Kunak. Since maturity is influenced by environmental parameters, other environmental parameters that may regulate reproduction should be studied.

Keywords: *Holothuria scabra*, physicochemical parameter, reproductive pattern, Kudat, Kunak

1. Introduction

Holothuroids are reported to show seasonal reproductive cycles for the temperate species while the tropical species can reproduce for longer periods throughout the year (Muthiga & Kawaka, 2008). Previous studies were conducted in the tropics but at higher latitudes than the equator show a seasonal pattern of reproduction in sea cucumber (Muthiga & Kawaka, 2008). The release of gametes, sperm, and oocytes by the mature adult can be triggered by the environmental surroundings (Battaglene, Syemour, Ramofafia, & Lane, 2002; Purcell, Lovatelli, Vasconcellos, & Ye, 2010). Changes in the environmental factors perceived by the sea cucumber may be responsible for the interannual variation in various ways (Purwati & Luong-Van, 2003; Rasoloforinina *et al.,* 2005). Hence, it initiates a reaction that leads to change and modification of reproduction metabolism, possibly through gene activation or hormone synthesis (Hamel & Mercier, 2004).

Temperature, light intensity, photoperiod, salinity, tidal flux, food availability, and change in food type are the environmental factors that are believed to affect the spawning and gametogenesis in holothurians (Battaglene *et al.,* 2002; Hasan, 2005; Kumara *et al.,* 2013; Muthiga *et al.*, 2009; Muthiga & Kawaka, 2008). Kazanidis *et al.* (2014) and Mercier *et al.* (2007) mentioned that spawning in echinoderms is correlated with the phytoplankton blooms and temperature. Other than that, lunar phase can also influence not only the echinoids and crinoids but also the holothuroids (Mercier *et al.,* 2007; Ramofafia *et al.,* 2003). Studies on spawning stimulation were carried out for certain species, for example temperature, monsoon, lunar cycle, and chemicals produced by males and females were suggested to be crucial factors for spawning in *Holothuria pulla* and in snakefish *H. coluber* (Purwati *et al.,* 2003).

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Dabbagh and Sedaghat (2012) and Ramofafia *et al.* (2003) explained that the seasonal and nonseasonal reproduction in *H. scabra* was based on the distribution in different parts of the world. They stated that the reproductive pattern was potentially cued by the predictable seasonal factors such as water temperature and day length, especially at high latitudes where annual reproductive pattern was recorded. In the Indo-Pacific region, the biannual and continuous reproductive patterns with two spawning peaks were observed although the months differed during the years (Ramofafia *et al.,* 2003). This was possibly due to the influence of the local environmental conditions like temperature, salinity, and photoperiod as reported in the biannual spawning patterns in Indonesia, Philippines, New Caledonia, and India. Purwati *et al.* (2003) stated that spawning in *H. scabra* was stimulated by changes in salinity.

Gametogenesis in sea cucumber can be divided into two ways: synchronous where both males and females release mature gametes at the same time and asynchronous where the release of gametes by both male and female is not at the same time (Ramofafia *et al.,* 2003; Morgan, 2000). Different ways in gametogenesis may be influenced by changes in environmental factors (Muthiga *et al*., 2009; Ramofafia *et al.*, 2003). *H. scabra* has asynchronous gametogenesis, thus mature gametes are available year round. However, certain species, for example *H. fuscogilva* and *Actinopyga mauritiana*, have synchronous and seasonal gametogenesis (Ramofafia *et al.,* 2003). For this reason, *H. scabra* has a great advantage in aquaculture since it is able to breed year round. In Toliara, temperature seems to be involved in the synchronizing of gonad maturity in female *H. scabra* but does not appear to affect the male (Rasolofonirina *et al.,* 2005).

Many studies have been conducted on the spawning induction of holothuria including *H. scabra*. Spawning often occurs prior to the full moon and new moon, but can also be induced at other times (Agudo, 2006). Battaglene *et al.,* (2002) mentioned that *H. scabra* in the Solomon Islands and Papua New Guinea spawn around the full moon and spawning activity also increased after the full moon. It can spawn spontaneously in response to collection and transportation (Agudo, 2006) and it usually occurs during the afternoon, evening or night or both on the day the broodstock are collected (Agudo, 2006). Most studies stated that thermal stimulation is the best way to induce spawning in *H. scabra* (Battaglene *et al.,* 2002; Kumara *et al.,* 2013). Other than thermal stimulation, drying, water jetting (Hamel & Mercier, 2004), high concentration of dry algae, and UV irradiation of the water are the other induction techniques (Pitt & Duy, 2003).

In Sabah, studies on *H. scabra* species are rarely reported and information on the effects of environmental parameters on the reproduction of *H. scabra* has not been published. This information is crucial for a stock enhancement program which led to the objective of this study which was to determine the relationships of the environmental parameters with the reproduction of *H. scabra* in Sabah, Malaysia.

2. Materials and Methods

2.1 Study sites and sampling

Two sampling sites were selected based on the availability of the *H. scabra*: Limau-Limauan Kudat (N06°49’24.4”; E116°51’42.0”) at the west coast of Sabah and Telaga Tujuh Kunak (N04°39’52.05”; E118°15’49.01”) at the east coast of Sabah, Malaysia (Figure 1). Sampling was conducted monthly for each place from July 2015 to August 2016 (14 months). On each sampling occasion, approximately 10 to 15 samples were randomly collected.

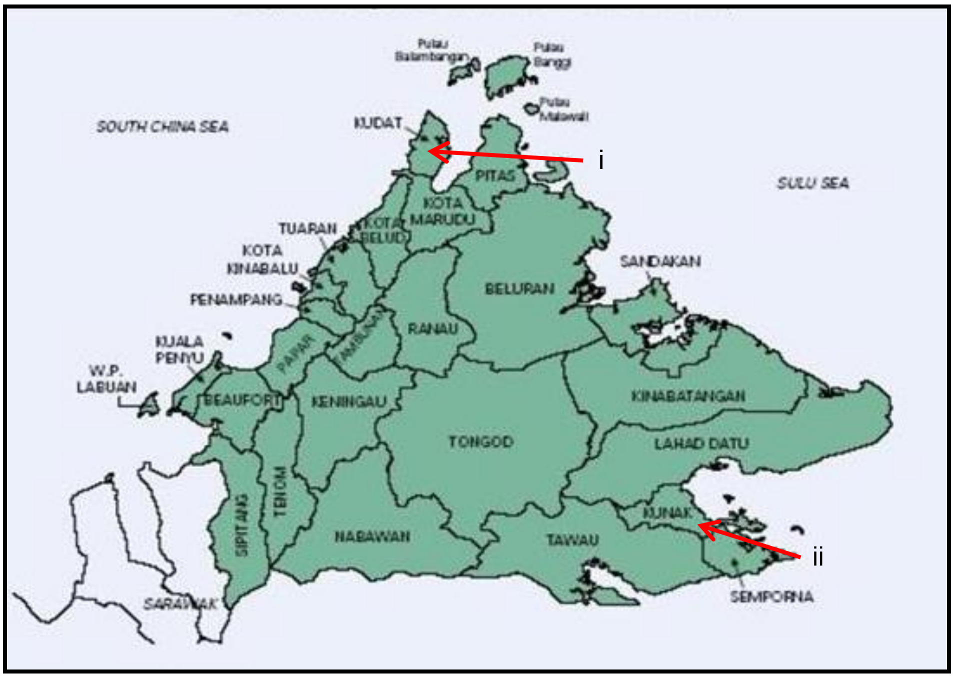

Figure 1. Location of sampling sites: i) Kudat and ii) Kunak, Sabah, Malaysia.

2.2 Gonad index (GI)

Total body weight was recorded to the nearest 0.01g using an analytical weight balance after 5 to 10 min in a dry container to expel the water from the body and total length was measured using a Vernier caliper. The gutted body weight was also recorded after dissection. Each sample was dissected at the ventral part to remove the gonad and the germinal tubule. The gonad was weighed to the nearest 0.01g. All measurements were taken on the same day of sampling at the sampling sites. The gonads underwent histological examination in the laboratory. The gonad index (GI) was calculated using this equation:

$$GI\ (\%) = GW/GBW \times 100$$

where GW is the gonad weight (g) and GBW is the gutted body weight (g).

Normal distribution of GI was checked using the Shapiro-Wilk W-test and the Kruskal-Wallis test was then used to analyze differences of gonad indices between months at each sampling site.

2.3 Environmental parameters

2.3.1 *In-situ* parameters

Water quality parameters for temperature (°C), salinity (psu), and pH were measured using a Multiprobe YSI on a monthly basis in Kudat and Kunak. The depth (m) at each sampling site was measured using a depth finder. Measurements were done in triplicate for each parameter reading that was recorded.

2.3.2 Cholorophyll *a* analysis

Chlorophyll *a* concentration was analyzed by filtering one liter of water sample through a filter paper with pore size 0.45 µm in between the top and bottom sections of the vacuum filter unit in triplicate. The water sample was collected using a van Dorn water sampler and filtered on the same day of sampling at the sampling sites. Then, the filter paper was carefully taken out using forceps and wrapped using aluminium foil for further analysis in the laboratory at Universiti Malaysia Sabah. In the laboratory, the filter paper was ground with 90% acetone using mortar and pestle in a semi-darkened condition. The ground filter paper was transferred into a centrifuge tube and left for at least 5 h before centrifugation. After 5 h, the centrifuge tube was centrifuged for 10 min at 4000 rpm. The supernatant was filled into a cuvette. The sample was measured in a spectrophotometer at 750, 664, 647, and 630 nm. The concentration of the chlorophyll *a* in the sample was calculated according to the standard method of the American Public Health Association (1998) and determined using these formulae:

$$\text{Chlorophyll } a,\ C_a = 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$$

where E is the absorbance in the respective wavelength.

$$\text{Chl } a\ (\text{mg/L}) = (C_a \times v)/(V \times L)$$

where Ca is the chlorophyll concentration in µg/mL, v is the volume of acetone (mL), V is the volume of water sample (L), and L is the cuvette length:

2.3.3 Rainfall data

Monthly rainfall data in the years 2015 and 2016 for the Kudat and Kunak areas were gathered from Department of Meteorological, Kota Kinabalu Sabah.

2.3.4 Sediment analysis

Approximately 300 g of sediment at each sampling site in each sampling occasion was taken using a grab sampler for particle size analysis and organic matter content. Particle size analysis was analyzed by oven drying 100 g of the samples in duplicate. The oven dried samples were sieved using a mechanical shaker. Six different sizes of sieve; 2000 µm, 1000 µm, 500 µm, 250 µm, 125 µm, and 63 µm were used. Then, the particle sizes retained in each sieve were weighted.

Five grams of sample from each sampling occasion was dried in an oven at 105 °C overnight in triplicate for the organic matter analysis. After the samples were dried in the oven, they were weighed (W_o) and then burned in a furnace at 400 °C for 16 h and then reweighed (W_f). The organic matter was calculated as percentage of weight loss using this equation:

$$\text{Organic matter } (\%) = [(W_o - W_f) \times 100]/Wo$$

2.4 Statistical analysis

The Tukey test was used to analyze the physicochemical parameters during the months at each site. A Pearson correlation analysis was used to test the relationship between the physicochemical parameters and precipitation with GI.

3. Results

3.1 Gonad index (GI)

Based on the 14-month sampling period, Kudat showed an annual pattern of reproductive cycle while Kunak had a continuous pattern (Figure 2). The highest mean GI in Kudat was recorded in July 2015 (1.678±1.079%) and the lowest mean GI value was in July 2016 (0.00±0.00%). In Kunak, the highest GI was recorded in September 2015 (3.491±1.699%) and the lowest GI was recorded in February 2016 (0.184±0.097%).

The GI in Kudat and Kunak were not normally distributed. A comparison of GI starting from July 2015 to August 2016 showed no significant differences during those months in Kudat and Kunak.

3.2 Physicochemical parameters in Kudat

Several physicochemical parameters such as temperature, salinity, organic matter, and chlorophyll *a* were

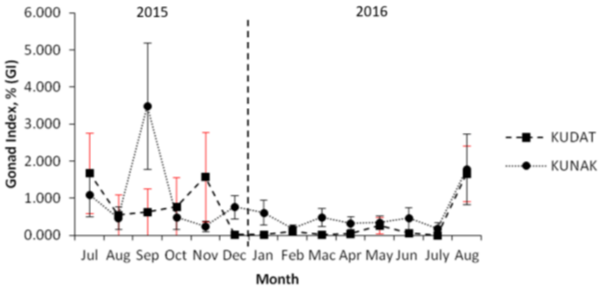

Figure 2 Monthly variations in gonad index of *H. scabra* in Kudat and Kunak (mean±SE).

recorded to document the habitat characteristics of *H. scabra* at the study sites.

The mean values of temperature, salinity, and rainfall at Kudat are shown in Figure 3. Water temperatures ranged from 28.9 °C to 31.4 °C. The highest temperature was recorded in December 2015 (31.4±0.00 °C) and it was significantly higher than in the other months (P<0.05) except in May 2016 (31.0±0.12 °C). The temperature in September 2015 (28.9±0.03 °C) was the lowest and it was significantly different compared to other months (P<0.05) except April 2016 (29.0±0.00 °C), June 2016 (29.27±0.38 °C), July 2016 (29.3±0.00 °C), and August 2016 (29.2±0.00 °C). Salinity values ranged from 31.95 psu to 35.26 psu. The highest salinity was recorded in April 2016 (35.26±0.02 psu) and it was significantly higher compared to the other months (P<0.05). The lowest salinity was recorded in July 2015 (31.95±0.00 psu) which was significantly different (P<0.05) except compared to September 2015 (32.01±0.02 psu). The rainfall ranged from 11.8 mm in March 2016 to 248.8 mm in October 2015.

The mean values of organic matter and chlorophyll *a* in Kudat are shown in Figure 4. Mean values of the organic matter ranged from 1.67% to 2.23%. The highest organic matter was recorded on October 2015 (2.23±0.02%) and the lowest was in July 2015 (1.67±0.02%); however, no significant difference in organic matter was observed during those months (P>0.05). The mean values of chlorophyll *a* ranged from 0.0163 mg/L to 0.1092 mg/L. Chlorophyll *a* was significantly higher in December 2015 (0.1594±0.006 mg/L) (P<0.05), while chlorophyll *a* in July 2016 (0.016±0.004 mg/L) was significantly lower (P<0.05).

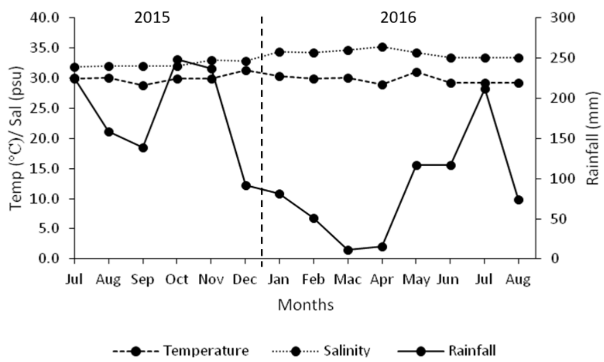

Figure 3. Mean values of temperature, salinity, and rainfall for 14 months in Kudat.

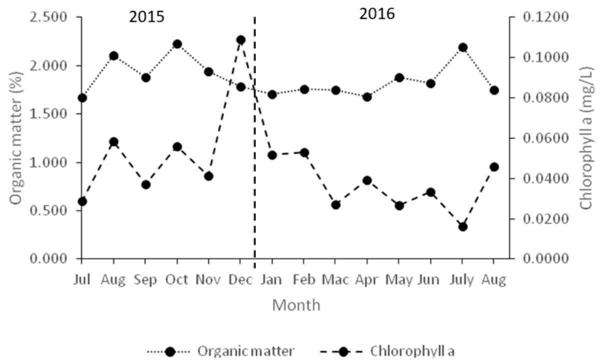

Figure 4. Mean values of organic matter and chlorophyll *a* for 14 months in Kudat.

3.3 Physicochemical parameters in Kunak

The mean values of temperature, salinity, and rainfall in Kunak are shown in Figure 5. Water temperatures ranged from 28.70 °C to 31.80 °C. The highest temperature was recorded in April 2016 (31.80±0.00 °C) and it was significantly higher (P<0.05) compared to the other months except in June 2016 (31.80±0.00 °C). February 2016 (28.73±0.07 °C) showed a significantly lower temperature (P<0.05) and it was the lowest along the sampling occasions. The mean salinity values ranged from 32.84 psu to 35.33 psu. The highest salinity was recorded in July 2016 (35.33±0.66 psu) and it was significantly different (P<0.05) compared to August 2015 (33.13±0.02 psu), September 2015 (32.84±0.09 psu), October 2015 (34.24± 0.00 psu), November 2015 (33.29±0.18 psu), December 2015 (33.85±0.04 psu), and June 2016 (34.07±0.02 psu). The lowest salinity was recorded in September 2015 (32.84±0.09 psu) which was significantly lower (P<0.05) compare to the other months except August 2015 and November 2015. Precipitation ranged from 63.6 mm in April 2016 to 374.6 mm in June 2016.

The physicochemical parameter trendline for organic matter and chlorophyll *a* are shown in Figure 6. The mean values of organic matter ranged from 1.64% to 3.89%. The highest organic matter value was recorded in April 2016 (3.89±0.14%) while the lowest value was recorded in February 2016 (1.64±0.08%). Organic matter in April 2016 was significantly higher (P<0.05) compared to the other

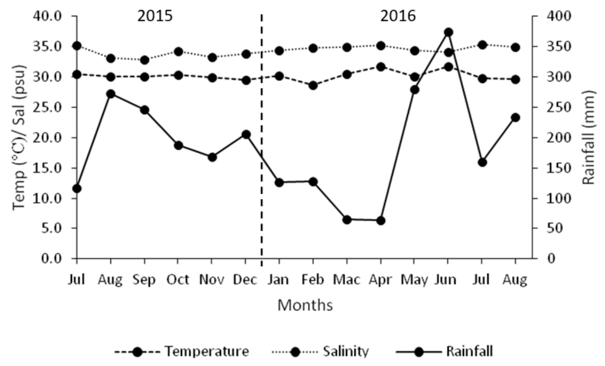

Figure 5. Mean values of temperature, salinity, and rainfall for 14 months in Kunak.

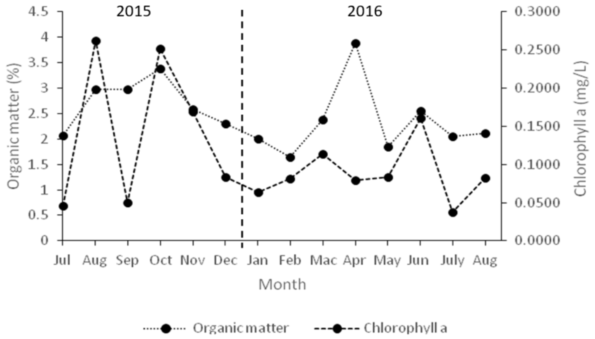

Figure 6. Mean values of organic matter and chlorophyll *a* for 14 months in Kunak.

3.4 Relationships between GI and physicochemical parameters

There were no significant correlations (P>0.05) among any of the environmental parameters (temperature, salinity, organic matter, chlorophyll *a*, and precipitation) with GI in Kudat or Kunak (Figures 7 and 8). The monthly precipitation data showed a seasonal pattern in Kudat where reproductive activity was associated with the rainy season from July 2015 to November 2015. The precipitation was minimal from December 2015 to April 2016 when the off-season of reproductive activity occurred. Meanwhile in Kunak, enhanced reproductive activity occurred in September 2015 and August 2016 during high precipitation (September 2015, 246.4 mm and August 2016, 233.8 mm).

3.5 Particle size analysis

The particle sizes of sediment were different in Kudat and Kunak (Figure 9). In Kudat, 45.3% of the sediment was categorized as sandstone since most of the sediment could be classified as coarse and medium sand (Figure 10). Compared to the sediment from Kunak, only 26.2% was categorized as coarse and the rest (73.8%) was classified as fine sand.

4. Discussion

The reproductive cycle of the sea cucumber has been studied in most of its geographical range from the Red Sea to the Philippines and to New Caledonia (Rasolofonirina *et al.,* 2005). The reproductive cycle of *H. scabra* could be annual, biannual or even continuous all year round depending on the environmental conditions (Guzman, Guevara, & Hernandez, 2003). Kudat showed an annual pattern while Kunak showed a continuous pattern in the reproductive cycle. It may vary from one geographic location to another since it can be influenced by several factors (Hamel, Himmelman, & Dufresne, 1993; Rasolofonirina *et al.,* 2005) such as temperature, light intensity, photoperiod, salinity, tidal flux, food availability, and change in food type (Leite-Castro *et al.,* 2016).

Four physicochemical parameters were recorded in this study: temperature, salinity, organic matter, and chlorophyll *a*. However, Pearson correlation analysis showed no significant correlation (P>0.05) of any environmental parameters with GI of *H. scabra* in both places.

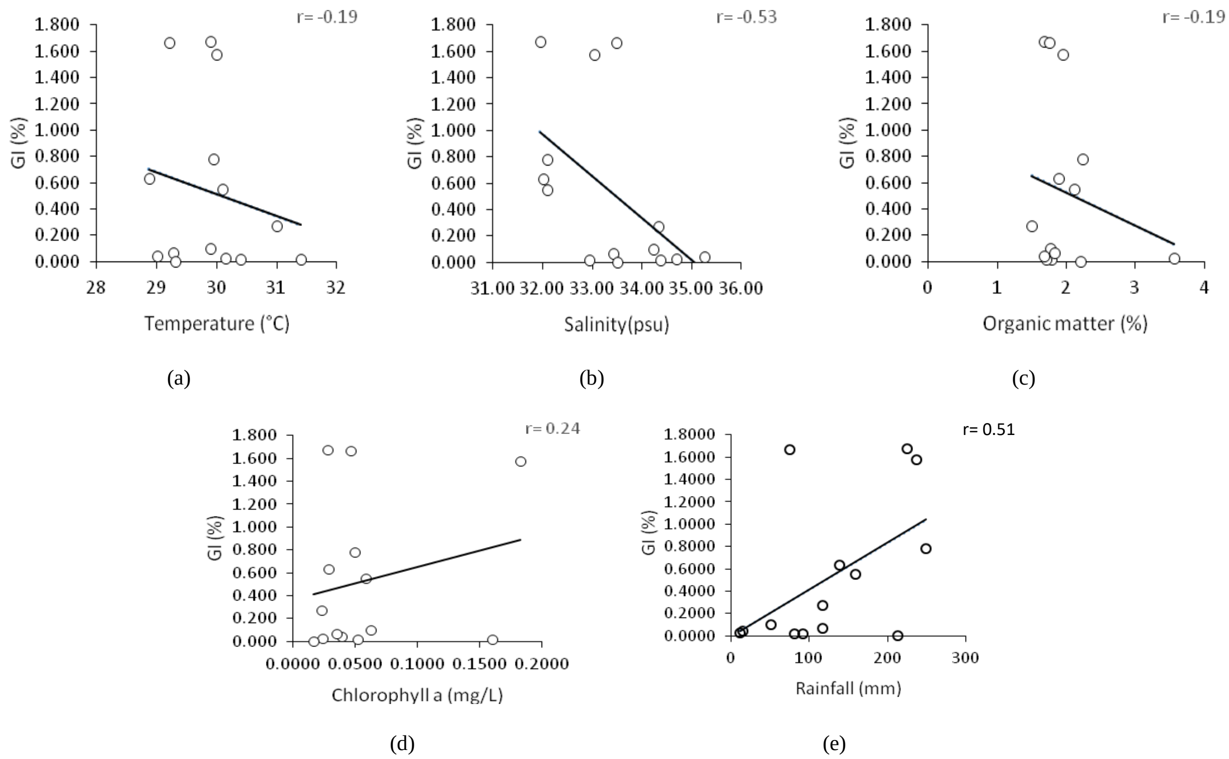

Figure 7. Pearson correlation analysis graph between mean GI and means of (a) temperature, (b) salinity, (c) organic matter, (d) chlorophyll *a*, and (e) rainfall in Kudat.

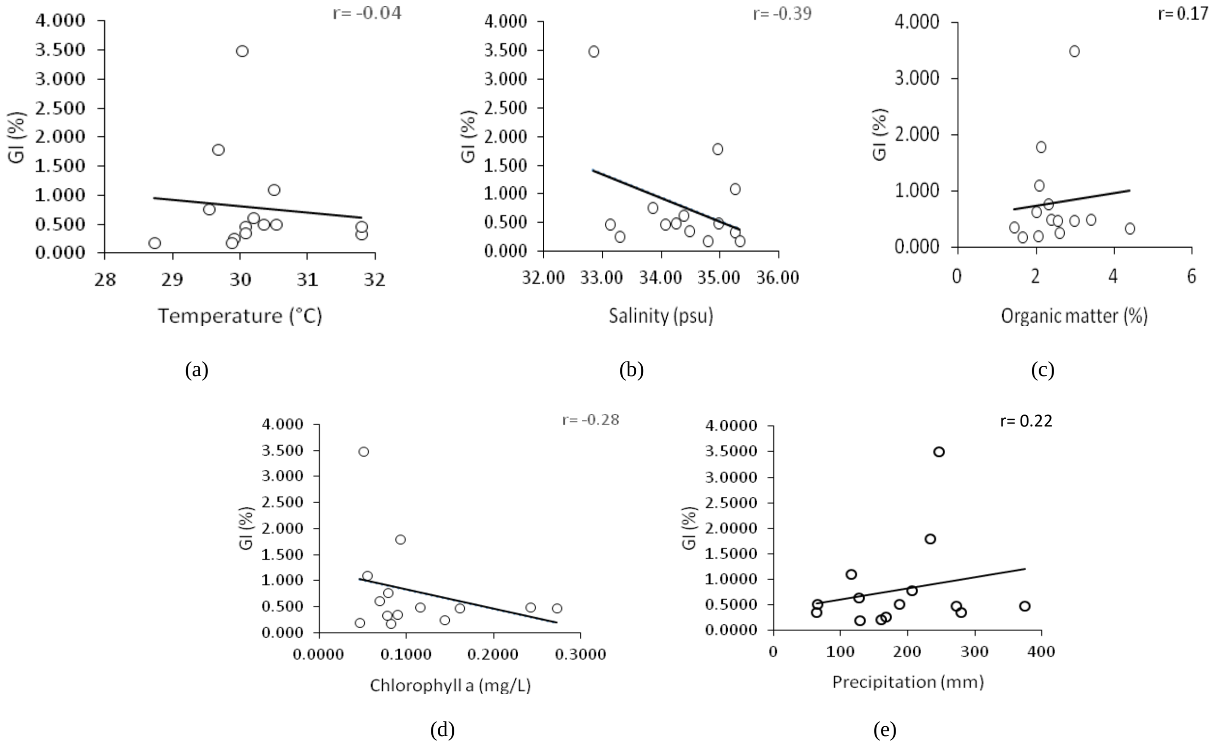

Figure 8. Pearson correlation analysis graph between mean GI and means of (a) temperature, (b) salinity, (c) organic matter, (d) chlorophyll *a*, and (e) rainfall in Kunak.

(a) (b)

Figure 9. Sediment in sampling sites: (a) Kudat (crushed shells and coral fragments) and (b) Kunak (silt and mud).

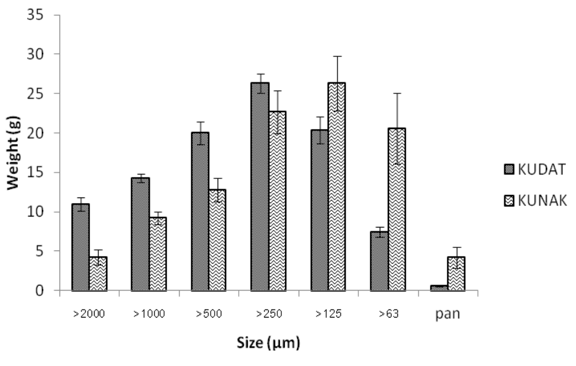

Figure 10. Percentages of sediment weight according to the particle sizes in Kudat and Kunak.

Many studies stated that temperature is the main factor to regulate gametogenesis in sea cucumber (Drum & Loneragan, 2004; Hamel *et al.*, 1993; Mezali, Soualili, Neghli, & Conand, 2014; Muthiga & Kawaka, 2009;). A study of *H. scabra* in the Solomon Islands reported that the spawning period corresponded with increased water temperature (Ramofafia *et al.*, 2003). In Toliara, a study by Rasolofonirina *et al.* (2005) showed that temperature played a role in synchronising gonad maturity of the female *H. scabra*. Besides, the white teatfish *H. fuscogilva* in Kenya also suggested that temperature may affect the reproduction of the species (Muthiga & Kawaka, 2009). Greenfish sea cucumber (*Stichopus chloronotus*) at Reunion Island showed that the spawning seasons of that species occurred during the highest seawater temperature (Hoareau & Conand, 2001). Kazanidis, Lolas, and Vafidis (2007) explained the influence of temperature on the spawning of tubular sea cucumber (*H. tubulosa*) in Pagasitikos Gulf. However, in the present study, temperature did not affect the reproduction of *H. scabra*. This finding was also reported by Hamel *et al.* (1993) on scarlet sea cucumber (*Psolus fabricii*) and Ramofafia *et al.* (2000) on *H. fuscogilva* which showed no consistent relationship between temperature and reproduction.

Jayasree and Bhavanarayana (1994) stated that reproduction of sea cucumber in the tropical areas may be induced by the reduction of salinity due to runoff and rainfall. This may affect the productivity in the coastal ecosystems leading to an adjustment in the reproductive cycle in sea cucumber (Guzman *et al.,* 2003). Asha and Muthiah (2008) proved that statement. They reported that the spawning of brown sanfish (*H. spinifera*) in Tuticorin coincided with low salinity. In spite of this, reproduction of *H. scabra* in Kudat and Kunak was not influenced by salinity since the salinity did not fluctuate extremely during the study period. In Brazil, the gray sea cucumber (*H. grisea*) also showed no significant correlation between reproduction and salinity although it was in a temperate region which supposedly has a wide variation of salinity compared to the tropical region (Leite-Castro *et al.,* 2016). The influence of salinity was stated by Purwati and Luong-Van (2003) in Ong Che's (1985) study of *H. scabra* in the Philippines and by Rasolofonirina *et al.* (2005) in Toliara.

The enhanced spawning season of *H. scabra* in Kudat (July to November 2015) and Kunak (September) overlapped with a period of rainfall. A moderate positive correlation between GI and rainfall in Kudat (r=0.52) and a weak positive correlation (r=0.21) in Kunak were recorded but it was not significantly correlated (P>0.05) at either site. Rainfall possibly caused high productivity at the coastal area which triggered the gonads of *H. scabra* to mature. Moreover, runoff generated by heavy rainfall could help increase the supply of nutrients in the water column and provide food for the plankton larvae. As a result, deposition of the plankton at the benthic creates more sources of food for the *H. scabra* (Benitez-Villalobos *et al.,* 2013).

Organic matter and chlorophyll *a* can regulate the reproduction in *H. scabra* because they can become food sources (Hamel *et al.,* 1993; Mezali *et al.,* 2014). Even though these two factors did not give any significant correlation (P>0.05) to GI in Kudat or Kunak, increased organic matter content in the sediment and a higher concentration of chlorophyll *a* could be the reasons for the onset of gametogenesis. A study in the Mediterranean Sea on the relationship of organic matter content in the sediment with the reproductive cycle of *H. sanctori* showed continuous availability of food due to the presence of organic matter throughout the year which induced the species to spawn extensively (Mezali *et al.,* 2014). In addition, organic matter content also relates to the type of sediment. Small particle sizes will provide more organic matter content due to the presence of a high number of nutritive microorganisms (Plotieau *et al.,* 2013). It was suggested that these nutritive microorganisms are important sources of food for sea cucumber to enhance the energy for reproduction (Hamel *et al.,* 1993). The presence of mature gametes in Kunak during this study was possibly due to this reason because the particle sizes were mostly fine sediments and contained high concentrations of organic matter. Reproduction of *H. grisea* in Brazil was initiated with a rise in chlorophyll *a* which enhanced the energy to complete gametogenesis (Leite-Castro *et al.,* 2016). Another study of *P. fabricii* showed increased amounts of phytoplankton may be the signal for spawning (Hamel *et al.,* 1993). Increased concentrations of chlorophyll *a* and phytoplankton might be the indicators to the sea cucumber to spawn as the condition is optimal for the larval stage to survive (Dissanayake & Stefanson, 2010).

The spawning period in each area may change depending on the yearly changes of environment (Chao, Chen, & Alexander, 1995). Researchers showed that specific environmental parameters can affect the reproduction of sea cucumber and the parameters may act independently or in combinations to determine the reproductive cycle of sea cucumber (Leite-Castro *et al.,* 2016; Mezali *et al.,* 2014). Furthermore, geographical location also plays an important role in determining the spawning period of sea cucumber since higher latitudes coincided with wider variations of environmental conditions (Keshavarz, Mohammadikia, Dabbagh, & Kamrani, 2012; Pitt & Duy, 2003; Ramofafia *et al.,* 2003). Another study reported that the reproduction of holothurians can be influenced by the cycles of the moon (Agudo, 2006). Rahanatoknam (2017) stated that *H. scabra* has a lunar spawning rhythm during the mating season as it spawns near the new moon or full moon. In this study, the *H. scabra* in Kudat had an annual reproductive pattern, whereas in Kunak, a continuous pattern was observed. A continuous pattern in the tropical regions is typical but an annual pattern in the tropics is quite unusual. Muthiga *et al.* (2009) stated that annual reproduction was usually reported at 23° N or S. This was observed at higher latitudes because synchronous gametogenesis occurred with wide variations of environmental parameters (Muthiga & Kawaka, 2008; Ramofafia *et al.,* 2003). However, there is a lack of information on seasonality in the tropical areas with less variation in environmental parameters. Some studies emphasized the seasonally of spawning in the sea cucumber in tropical areas (Kumara & Dissanayake, 2015). A previous study that reported on *H. fuscogilva* in a tropical area also had an annual reproductive cycle which was similar with the finding in the present study in Kudat (Muthiga & Kawaka, 2009). A continuous pattern was observed in *H. scabra* in Indonesia but at a different peak season with Kunak which was during September. The study in Indonesia which was conducted at Lampung, Saugi, and Ambon showed peaks in March, April, and October, respectively (Purwati, 2006). Another study of *H. scabra* in the Solomon Islands showed a continuous pattern with maximum gonad growth from September to December (Ramofafia *et al.,* 2003).

5. Conclusions

The correlations between the environmental factors did not show any influence on the reproduction of *H. scabra* at either site. Combinations of these environmental parameters might be necessary in order to see the interactions between each factor. One factor alone may not cause a change in the reproductive biology of *H. scabra*. Further research on the gut content of *H. scabra* and type of organic found in the sediment could be helpful in finding the factor that affects the reproduction of *H. scabra*. Also, using hormones to stimulate the spawning of *H. scabra* in captivity can be done to determine whether hormones have an effect on the reproductive system or not. In addition, a molecular study of *H. scabra* at both sites should be checked in order to reassure any involvement of genetic influence in promoting the reproductive cycle of *H. scabra.*

Acknowledgements

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