The Effects of Physicochemical Parameters on Reproductive Pattern of Sea Cucumber, Holothuria scabra in Sabah

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Original Article

The Effects of Physicochemical Parameters on 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 produced high quality of beche-de-mer. It has been overexploited and overfishing nowadays due to the high demand from the local and international market. It is one of the most valuable species and become source of income for a small scale fisherman in Sabah, Malaysia. This study describes the influence of physicochemical parameters on the reproductive biology of H. scabra in two sites, Kudat and Kunak, Sabah, Malaysia. The study was conducted for 14 months and samples were collected monthly, between July 2015 until August 2016. Gonad index and data on physicochemical parameters (temperature, salinity, chlorophyll a and organic matter) were recorded to determine the relationship that can give effect to the reproductive of H. scabra. An annual reproductive pattern was recorded in Kudat with spawning period occurred during July to November 2015. Continuous pattern in Kunak was recorded with a maximum gonad index in September 2015. The gonad index showed no significantly (p>0.05) correlated with the studied physicochemical parameters in both Kudat and Kunak. As the maturity influenced by
the environmental parameter, other environmental parameters that may regulate the
reproduction should be further research.

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

1. Introduction

Holothuroids is 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 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 surrounding (Purcell, Lovatelli,
Vasconcellos, & Ye, 2010; Battaglene, Syemour, Ramofafia, & Lane, 2002). Changes
in environmental factors perceived by 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 believed can give effect on
the spawning and gametogenesis in holothurians (Kumara et al., 2013; Muthiga et al.,
2009; Muthiga & Kawaka, 2008; Hasan, 2005; Battaglene et al., 2002). 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 also can
give influenced not only to the echinoids, crinoids but also the holothuroids (Mercier et
al., 2007; Ramofafia et al., 2003). Study on spawning stimulation had been carried out
for certain species, for example spawning in *H. pulla* and snakefish, *H. coluber* show
temperature, monsoon, lunar cycle and chemical produced by males and females are
suggested to be crucial factors for spawning (Purwati et al., 2003).

Dabbagh and Sedaghat (2012) and Ramofafia et al. (2003) explained on the
seasonal and nonseasonal reproductive in *H. scabra* based on the distribution at
different part in the world. They stated that reproductive pattern is potentially cued by
the seasonal predictable factors such as temperature of water and day length especially
at high latitude where annual reproductive pattern was recorded. At the Indo-Pacific
region, biannual and continuous reproductive pattern with two spawning peaks has been
observed, although the months differed among years (Ramofafia et al., 2003). This may
be due to the influenced of local environment conditions like temperature, salinity and
photoperiod as reported biannual spawning pattern in Indonesia, Philippines, New
Caledonia and India. Purwati et al. (2003) stated that spawning in *H. scabra* is
stimulated by changes in salinity.

Gametogenesis in sea cucumber can be divided into two ways; synchronous
where both male and female released matured gametes at the same time and
asynchronous, the releasing of gamete 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). *Holothuria scabra* has asynchronous gametogenesis, thus mature gametes
available in the year round. However, in certain species for example *H. fuscogilva* and
Actinopyga mauritiana have synchronous and seasonal gametogenesis (Ramofafia et al., 2003). Because of this reason, H. scabra has a great advantage in aquaculture since it is able to breed year round. In Toliara, temperature seems involved in synchronizing of gonad maturity in female H. scabra but not appear any effects to 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 also can be induced at other times (Agudo, 2006). Battaglene et al., (2002) mentioned that H. scabra in Solomon Islands and Papua New Guinea spawn around the full moon and spawning activity also increased after the full moon. It can be spawned spontaneously in response to collection and transportation (Agudo, 2006) and it usually occurs during the afternoon, evening and/or night on the day broodstock are collected (Agudo, 2006). Most studies stated that thermal stimulation is the best way to induce spawning in H. scabra (Kumara et al., 2013; Battaglene et al., 2002). Other than thermal stimulation, drying, water jetting (Hamel & Mercier, 2004), high concentration of dry algae and UV irradiation of water are the other induction technique (Pitt & Duy, 2003).

In Sabah, study on H. scabra species is rarely reported and information on the effect of environmental parameters on the reproductive of H. scabra has not published yet. The information will be crucial for the stock enhancement programme and this leads to the objective of this study which is to determine the relationship of the environmental parameters with the reproductive of H. scabra in Sabah, Malaysia.
2. Materials and Methods

Study sites and sampling

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

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

Gonad Index (GI)

Total body weight was recorded to the nearest 0.01g by using analytical weight balance after left in a dry container for five to ten minutes to expel the water from the body and total length was measured by using Vernier caliper. Then, gutted body weight; weight of sample after dissected was taken. Each sample was dissected at the ventral part to remove the gonad and the germinal tubule. Then, the gonad was weighted to the nearest 0.01g. All measurements were taken on the same day of sampling at the sampling sites. Gonads collection were proceed with histological examination in laboratory. The GI was calculated by using the following equation:

$$\text{GI} \,(\%) = \frac{\text{GW}}{\text{GBW}} \times 100$$

Where:

- \( \text{GW} \) = Gonad weight (g)
GBW = Gutted body weight (g)

Normal distribution of GI was checked by using Shapiro-Wilk W test; Kruskal-
Wallis test was then used to analyze differences of gonad indices between months in
each sampling sites, Kudat and Kunak.

Environmental parameters

In-situ parameters

Water quality parameters for temperature (°C), salinity (psu) and pH were measured by
using multiprobe YSI on monthly basis in Kudat and Kunak. Depth (m) at each
sampling site was measured by using depth finder. Triplicate for each parameter reading
were recorded.

Chlorophyll a analysis

Chlorophyll a concentration was analysed 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 by using Van-dorn
water sampler and filtered on the same day of sampling at the sampling sites, Kudat and
Kunak. Then, the filter paper was carefully taken out using a forcep and wrapped by
using aluminium foil for further analysed in the laboratory of Universiti Malaysia
Sabah. In the laboratory, the filter paper was grinded with 90% acetone using mortar
and pestle in semi-darkened condition. Then, grinded filter paper transferred into
centrifuge tube. It was left for at least five hours before centrifuged. After five hours,
the centrifuge tube was centrifuged for 10 minutes at 4000 rpm. The supernatant was
produced after the centrifuged was extracted out and filled into a cuvette. Then, the
sample was measured in spectrophotometer by using following wavelengths: 750, 664,
647 and 630 nm. The concentration of the chlorophyll $a$ in the sample was calculated as in the standard method APHA (1998) and determined using the formula as described below:

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

Where $E = \text{The absorbance in the respective wavelength.}$

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

Where:

- $C_a = \text{Chlorophyll concentration in } \mu g/mL$
- $v = \text{Volume of acetone (mL)}$
- $V = \text{Volume of water sample (L)}$
- $L = \text{Cuvette length}$

Rainfall data

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

Sediment analysis

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

For organic matter, five g of sample in each sampling occasions was dried in an oven at 105°C overnight in triplicate. After dried in the oven, the samples were weighted ($W_o$) then sample was burned in a furnace at 400°C for 16 hours and then...
The organic matter was calculated as a percentage weight loss by
using the equation as follows:

\[
\text{Organic matter (\%) = \left(\frac{W_o - W_f}{W_o}\right) \times 100}
\]

162 **Statistical analysis**

Tukey test was used to analyse the physicochemical parameters among months in each sites. Pearson Correlation was used to test the relationship between the physicochemical parameters and precipitation with GI.

3. Results

**Gonad Index (GI)**

Based on 14 months sampling period, Kudat shows annual pattern of reproductive cycle while Kunak has 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 in July 2016 (0.00 ± 0.00%). In Kunak, the highest GI was recorded in September 2015 (3.491 ± 1.699%). The lowest GI was recorded in February 2016 (0.184 ± 0.097%).

GI in Kudat and Kunak were not normally distributed. Comparison of GI starting from July 2015 until August 2016 showed no significant difference among those months in Kudat and Kunak.

Figure 2 - Monthly variations in gonad index of *H. scabra* in Kudat and Kunak (Mean ± SE)
Physicochemical Parameters in Kudat

Several physicochemical parameters such as temperature, salinity, organic matter and chlorophyll $a$ were recorded to document the habitat characteristics of $H. scabra$ at study sites.

The mean values of temperature, salinity and rainfall at Kudat were shown in Figure 3. Water temperatures values ranged from 28.9°C to 31.4°C. The highest temperature value was recorded in December 2015 (31.4 ± 0.00°C) and it is significantly higher ($p< 0.05$) than the other months except in May 2016 (31.0 ± 0.12°C). Temperature on September 2015 (28.9 ± 0.03 °C) was the lowest and showed significantly different ($p<0.05$) to other months 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, where the highest salinity was recorded on April 2016 (35.26 ± 0.02 psu) and showed significantly higher ($p< 0.05$) compare to the other months. The lowest was recorded in July 2015 (31.95 ± 0.00 psu) which showed significant difference ($p<0.05$) except 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 were shown in Figure 4. Organic matter mean values ranged from 1.67 to 2.23%. The highest organic matter was recorded on October 2015 (2.23 ± 0.02%) and the lowest in July 2015 (1.67 ± 0.02%). However, no significant difference ($p>0.05$) of organic matter showed among those months. The chlorophyll $a$ mean values ranged from 0.0163 mg/L to 0.1092 mg/L. Chlorophyll $a$ was significantly higher ($p<0.05$) in December 2015 (0.1594 ±
While July 2016 (0.016 ± 0.004 mg/L) showed significantly lower (p<0.05) than other months.

Figure 3 - The mean values of temperature, salinity and rainfall for 14 months in Kudat

Figure 4 - The mean values of organic matter and chlorophyll a for 14 months in Kudat

Physicochemical Parameters in Kunak

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

Physicochemical parameters trendline for organic matter and chlorophyll a were 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 (p<0.05) higher compare to the other months. For chlorophyll a,
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the mean values ranged from 0.0374 mg/L to 0.2624 mg/L. The highest chlorophyll $a$ value was recorded in August 2015 ($0.2624 \pm 0.0114$ mg/L) and the lowest in July 2016 ($0.0374 \pm 0.0138$ mg/L). August 2015 showed significantly higher ($p<0.05$) to other months except to chlorophyll $a$ in October 2015 ($0.2520 \pm 0.0153$ mg/L).

Figure 5- The mean values of temperature, salinity and rainfall for 14 months in Kunak

Figure 6- The mean values of organic matter and chlorophyll $a$ for 14 months in Kunak

Relationship between GI and Physicochemical Parameters

There was no significant correlation ($p>0.05$) of all environmental parameters (temperature, salinity, organic matter, chlorophyll $a$ and precipitation) with GI in Kudat and Kunak (Figure 7 and 8). For monthly precipitation data, it showed a seasonal pattern in Kudat where season of reproductive activity associated with rainy season in July 2015 to November 2015. The precipitation was minima from December 2015 to April 2016 where the off season of reproductive activity occurred. Meanwhile in Kunak, enhanced reproductive activity during September 2015 and August 2016 were occurred during high precipitation recorded (September 2015: 246.4 mm; August 2016: 233.8 mm).

Figure 7: Pearson correlation analysis graph between mean GI and mean 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 mean of a) temperature, b) salinity, c) organic matter, d) chlorophyll $a$ and e) rainfall in Kunak

Particle size analysis
Particle size of sediment was different in Kudat and Kunak (Figure 9). In Kudat, 45.3% sediment was categorized as sandstone since most of the sediment can be classified as coarse and medium sand (Figure 10). Compare to sediment from Kunak, only 26.2% was categorized as coarse and the rest, 73.8% was classified as fine sand.

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

Figure 10: Percentage of sediment weight according to the particle size in Kudat and Kunak

4. Discussion

The reproductive cycle of the sea cucumber has been studied at most of its geographical range, from 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 how the environment conditions are (Guzman, Guevara, & Hernandez, 2003). Kudat shows annual pattern while Kunak shows continuous pattern of 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 have been recorded in this study which are temperature, salinity, organic matter and chlorophyll *a*. However, Pearson correlation analysis shows no significant correlation (p>0.05) of any environmental parameters with GI of *H. scabra* in both places.
Many studies stated that temperature is the main of any other factor that can regulate gametogenesis in sea cucumber (Mezali, Soualili, Neghli, & Conand, 2014; Muthiga & Kawaka, 2009; Drum & Loneragan, 2004; Hamel et al., 1993). Study on *H. scabra* in Solomon Islands, spawning period corresponded with increasing water temperature (Ramofafia et al., 2003). In Toliara, study by Rasolofonirina et al. (2005) showed that temperature plays a role in synchronising gonad maturity of female *H. scabra*. Besides, white teatfish, *H. fuscogilva* in Kenya also suggest that temperature may give effect to the reproduction of the species (Muthiga & Kawaka, 2009). Greenfish sea cucumber, *Stichopus chloronotus* at Reunion Island showed spawning seasons of that species occurred during the seawater temperature at the highest (Hoareau & Conand, 2001). Kazanidis, Lolas and Vafidis (2007) explained on the influence of temperature to the spawning of tubular sea cucumber, *H. tubulosa* in Pagasitikof Gulf. However, in the present study, temperature did not give any effect on the reproduction of *H. scabra*. This finding also reported by Hamel et al. (1993) on scarlet sea cucumber *Psolus fabricii* and Ramofafia et al. (2000) on *H. fuscogilva* that showed no consistent relationship between the temperature and the reproduction.

Jayasree and Bhavanarayana (1994) stated that reproduction of sea cucumber in the tropical area may be induced by the reduction of salinity due to runoff and rainfall. This may affect the productivity in the coastal ecosystems, hence adjust the reproductive cycle in sea cucumber (Guzman et al., 2003). Asha and Muthiah (2008) proved that statement, as spawning of brown sanfish, *H. spinifera* in Tuticorin was coincided with low salinity. In spite of this, reproductive of *H. scabra* in Kudat and Kunak was not influenced by the salinity, as the salinity was not fluctuate extremely when the study was conducted. In Brazil, gray sea cucumber, *H. grisea* also showed no
significant correlation between reproductive and salinity, although it is in temperate region which supposedly has 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 Philippines and by Rasolofonirina et al. (2005) in Toliara.

Enhanced spawning season of *H. scabra* in Kudat (July to November 2015) and Kunak (September) overlapped with a period of rainfall. Moderate positive correlation between GI and rainfall in Kudat (*r* = 0.52) and weak positive correlation (*r* = 0.21) in Kunak were recorded, but not significant correlated (*p* > 0.05) in both sites. Rainfall may cause high productivity at the coastal and triggered the gonad of *H. scabra* to be matured. Moreover, runoff generated by heavy rainfall could help to increase the nutrients supply in the water column and provide food for the plankton larvae. As a result, deposition of the plankton at the benthic create more source of food for the *H. scabra* (Benitez-Villalobos et al., 2013).

Organic matter and chlorophyll *a* can regulate the reproduction in *H. scabra* as they can become one of the food source (Mezali et al., 2014; Hamel et al., 1993). Eventhough these two factors did not give any significant correlation (*p* > 0.05) to GI in Kudat and Kunak, increasing of organic matter content in the sediment and arising of chlorophyll *a* concentration can be the reasons for the onset of gametogenesis. A study in Mediterranean Sea on the relationship of organic matter content in the sediment with reproductive cycle of *H. sanctori* showed continuous available of food due to the presence of organic matter throughout the year induce the species to spawn extensively (Mezali et al., 2014). In addition, organic matter content also relates to the types of the sediment. Small size of the particle will provide more organic matter content as high
number of nutritive micro-organisms present (Plotieu et al., 2013). And these nutritive micro-organisms are suggested to be important source of food for sea cucumber to enhance the energy for the reproduction (Hamel et al., 1993). Presence of mature gametes in Kunak during this study could be due to this reason as the particle size are mostly categorized as fine sediments and contained high organic matter. Reproduction of H. grisea in Brazil initiated as the chlorophyll a rising, therefore enhance the energy to complete gametogenesis (Leite-Castro et al., 2016). Other study on P. fabricii showed increasing of phytoplankton may be the signal for its spawning (Hamel et al., 1993). Increasing of chlorophyll a and phytoplankton concentration might give indicator to the sea cucumber to spawn as the condition is optimal for the larval stage to survive (Dissanayake & Stefanson, 2010).

Spawning period in each area may change directly to the yearly changes of environment (Chao, Chen, & Alexander, 1995). Researchers have showed specific environmental parameters that can give effect to 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 as increasing latitude coincided with wide variations of environment condition (Keshavarz, Mohamnadikia, Dabbagh, & Kamrani, 2012; Pitt & Duy, 2003; Ramofafia et al., 2003). There is also study reported that holothurians reproductive can be influenced by the cycles of the moon (Agudo, 2006). Rahanatoknam (2017) stated that H. scabra has lunar spawning rhythm during mating season where it spawns nearly to new moon or full moon. In this study, H. scabra in Kudat has annual pattern of reproductive cycle whereas in Kunak, continuous pattern was observed. Continuous
pattern in the tropical regions is typical but annual pattern in tropical is quite unusual. Muthiga et al. (2009) stated that annual reproduction usually reported at 23° N or S. This is because, at higher latitude, synchronous gametogenesis occurred with wide variations of environmental parameters (Muthiga & Kawaka, 2008; Ramofafia et al., 2003). However, lack of information on seasonality experienced in the tropical area with less variation in environmental parameters reported. Some studies have emphasized on seasonally spawn of sea cucumber in tropical area (Kumara & Dissanayake, 2015). Previous study reported on H. fuscogilva at the tropical area also has an annual pattern reproductive cycle which is similar with finding in the present study in Kudat (Muthiga & Kawaka, 2009). Continuous pattern observed in H. scabra at Indonesia but different peak season with Kunak which is during September whereas, study in Indonesia conducted at three different sites; Lampung, Saugi and Ambon showed different peaks; March, April and October, respectively (Purwati, 2006). Other study on H. scabra in Solomon Islands shows continuous pattern with maximum gonad growth from September to December (Ramofafia et al., 2003).

Conclusion

The correlation between the environmental factors studied did not show any influence on the reproductive of H. scabra in both sites. Combinations of these environment parameters might be necessary in order to see the interactions between each factors. Only one factor alone may not cause change in reproductive biology of H. scabra. Further research on the gut content of H. scabra and type of organic found in the sediment could be help in finding the factor that may give effect on the reproduction of H. scabra. Besides, usage of hormones to stimulate the spawning of H. scabra in
captivity can be done to determine either hormones have effect on the reproductive system or not. In addition, molecular study of *H. scabra* in both sites should be check in order to reassure any involvement of genetic influence in promoting the reproductive cycle of *H. scabra*.

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

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

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