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Egg and Larval Development of Induced Spawned Sandfish (*Holothuria scabra*) in Hatchery

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Abstract: *Holothuria scabra*, commonly known as sandfish has a high market demand, especially in South East Asian countries such as Malaysia, Philippines and Vietnam. This species that is very popular among the Chinese is served as luxorius delicacy because it has high protein and medicinal properties. The increasing demand for sandfish has led to the over-exploitation worldwide. Hatchery production enables continuous seed production of *H. scabra* throughout the year by artificial spawning. Fifty healthy broodstocks collected from Kampung Baru-Baru, Tuaran Sabah (6° 18' 18.1656" N, 116° 17'43.1052" E) were acclimatized in Universiti Malaysia Sabah (UMS) hatchery for 2 weeks prior to spawning induction. The broodstocks were fed twice a day with ground *Sargassum* sp. and *Enhalus* sp. The combination of 3 spawning induction methods: thermal shock, desiccation and algal bath were applied. Total fertilized eggs obtained were approximately 700,000, with a 9 % hatching rate. The larval rearing was carried out in high density polyethylene (HDPE) tank filled with filtered and UV treated seawater (29-30°C). The larvae were fed twice daily with *Nannochloropsis* sp. at a specific feeding rate. The egg and larval development were recorded daily. The duration for each stages of larval development was as followed: Gastrula (24 hours after fertilization), Early auricularia (Day 2), Mid auricularia (Day 4), Late auricularia (Day 6), Early doliolaria (Day 21), Late doliolaria (Day 24), Pentactula (Day 26).

Key Word: *Holothuria scabra*, sandfish, larval development, spawning induction

Introduction

Overexploitation of sea cucumbers is not a current issue. Sea cucumbers especially Holothurians have been facing this issue for over two centuries. According to Ram *et al*. (2014), at least 58 species are harvested and traded around the world. *Holothuria scabra* (sandfish) was the main targeted species for global trading and listed among the most highly valued tropical species in Asian markets. In Malaysia, more than 80 species of sea cucumber were recorded present in this country (Kamarudin *et al.*, 2010) and Sabah is the only state in Malaysia that has significant sea cucumber fishery. Annual Fisheries Statistics reported that 139 tonnes of sea cucumbers were landed in Sabah in year 2005 (Choo, 2012). The Fisheries Department of Sabah also reported that this species could be found mainly in district of Pitas, Kunak, Kudat and Semporna. Sandfish contains bioactive substances that exhibit the antibacterial, antifungal and anticancer properties (Caulier *et al*., 2013). Due to this, the price can reach USD 150-200/kg for premium grade and as a result, increased the demand and leads to the overexploitation of this species. The continuous overexploitation for export has led to the depletion of the broodstocks and become vulnerable to extinction (Pakoa *et al.*, 2012). Hatchery production by artificial spawning will enables continuous seed supply for aquaculture. Reproductive cycle, egg and larval development of *H. scabra* is species specific and different between geographically separated populations. This study provides a preliminary understanding on the egg and larval development of *H. scabra*, particularly in Sabah.

Materials and Methods

Broodstock management

Fifty healthy broodstocks (200-300 g) with no skin lesion were collected from Kampung Baru- Baru, Tuaran, Sabah (6° 18' 18.1656" N, 116° 17'43.1052" E). The broodstocks were packaged in the oxygen-filled plastic bags and transported in the insulated polystyrene container. The broodstocks were acclimatized in UMS hatchery for 2 weeks prior to spawning

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induction. A 2 tonne tank containing 5 cm depth of treated sand was used as the broodstock's tank. Wet paste and powdered *Sargassum* sp. and *Enhalus* sp. were given as the feed, twice daily (9 a.m. and 4 p.m.). *In situ* water quality parameters of the broodstock's tank was monitored using YSI Professi-onal Plus handheld multiparameter instrument. The water quality parameter was recorded twice daily (9 a.m. and 4 p.m.).

Spawning induction

Ten broodstocks were dissected before the spawning induction to check the presence of gonad**.** Forty broodstocks were transferred into 1 tonne high density polyethylene (HDPE) tank in the absence of sand for overnight defecation. This is important to prevent contamination of foreign substances such as the faeces during the spawning induction. The combination of 3 spawning induction methods: desiccation, thermal shock and algal bath were used to induce the sandfish. Desiccation which is also known as drying method is a process in which the broodstocks were placed in an empty container without seawater for a period of time (Agudo,2006). For thermal shock, the temperature of the seawater in the spawning tank was raised by 3-5°C above the ambient temperature (Agudo, 2006; Battaglene *et al*., 2002; Mazlan and Hashim, 2015). Boiled seawater was added into the spawning tank until the desired temperature was obtained. Commercial Spirulina powder was used at 60 g/L as feed stimulant (Agudo, 2006). The spirulina bath was stirred to evenly mix the spirulina powder. The spawning induction was carried out in a flat- bottomed fiberglass tank with filtered and UV treated seawater.

Larval rearing

Fertilized eggs were siphoned from the spawning tank and collected using the 30µm hand net. The eggs were rinsed with filtered and UV-sterilized seawater to remove excess sperms as excess sperms might cause polyspermy. The egg density was determined before it was transferred into the 2-tonne larval rearing tank. Aquarium heaters were used to maintain the temperature in the larval rearing tank at 29- 30°C. The larvae were fed twice a day (9 a.m. and 4 p.m.) with *Nannochloropsis* sp. The feeding rate was increased gradually from 20,000 cells ml^{-1} to 40,000 cells ml^{-1}. Polyethylene plastic sheets coated with Spirulina were installed into the larval rearing tank once the first doliolaria was observed. The settlement plates were important to induce the metamorphosis of the doliolaria into pentactula (Mercier *et al.*, 2000). Spirulina powder was added twice a day (9 a.m. and 4 p.m.) at a concentration of 0.25 g m^{-3} to feed the pentactula. Partial water changes (20%) were carried out every 2 days (Agudo, 2006).

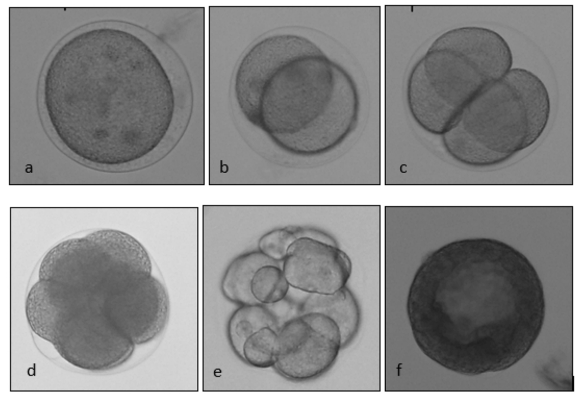

Fig. 1: Egg development of *H. scabra*. a) Fertilized egg, b) 2 cells stage, c) 4 cells stage, d) 8 cells stage, e) Multi cell stage, f) Blastula

Results

Egg and larval development

The number of fertilized eggs obtained from the spawning induction was 700,000 eggs.The fertilized eggs of *H. scabra* are round, white in colour and can be seen by naked eyes floating in the water column. The diameter of the eggs was 80-200µm (Fig. 1). The fertilized eggs were hatched as gastrula (Fig. 2a) and it was observed 24 hours after fertilization. The total number of fertilized eggs hatched was 60,000 eggs and the hatching rate was 9%. At 48 hours after fertilization, auricularia was started to appear. Auricularia has transparent slipper- shaped and swim at the water column by using the ciliary band. At this stage the larvae began to feed on microalgae. In the early auricularia stage, the ciliary band was visible and can be clearly observed (Fig. 2b). The eosophagus, stomach and buccal cavity started to develop in mid auricularia (Fig. 2c). Five hyaline spheres on each side of the larvae were observed in the late auricularia (Fig. 2d). The late auricularia started to develop into early doliolaria on day 21 (Fig. 2e). At this stage , the larvae shrank down almost 50% from its initial size because the buccal cavity disappeared and the hyaline spheres were compressed. Doliolaria was a non- feeding larvae stage, but the feed was still supplied at a lower concentration (10,000 cells/ml). Larval development

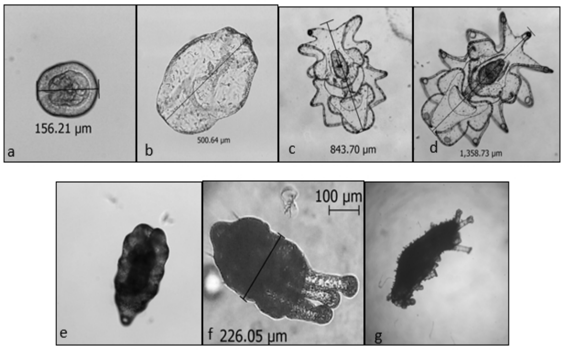

Fig. 2: Larval development of *H. scabra*. a) Gastrula, b) Early auricularia, c) Mid auricularia, d) Late auricularia, e) Early doliolaria, f) Late doliolaria, g) Pentactula

might be different. Some larvae in the larval rearing tank had slow development and still in the auricularia stage. On day 26, the dolioalaria metamorphosized into pentactula and started to settle onto the settlement plate. The size of the pentactula was within 2-5 mm and the pentactula fed on the Spirulina powder. The duration for each stages of larval development was summarized in Table 1.

Tab. 1: Larval development of *H. scabra*

Larval stage	Day after fertilization (Day)
Gastrula	1
Early auricularia	2
Mid auricularia	4
Late auricularia	6
Doliolaria	21
Pentactula	26-40

Water quality parameter of larval rearing tank

The range of water quality parameters recorded throughout this study was shown in Table 2. The water quality was maintained within the range recommended by Agudo (2006).

Tab. 2: *In situ* water quality parameters.

Water parameter	Range
Temperature	29- 30.8°C
Dissolved oxygen	4.11- 6.14 ppm
Salinity	33.77-35.18 ppt
pH	7.08- 8.39

Discussions

Spawning induction can be defined as introducing short environmental stress to the broodstocks (Battaglene *et al.,* 2002). There are various methods used to induce the spawning of *H. scabra*; thermal stimulation, drying, feed stimulant, water pressure and gonad extraction. Thermal stimulation is the most common method of spawning induction in many invertebrates. It is also the most cited stimulants for sandfish induction (Battaglene *et al*., 2002).

Table 3 shows the comparison of larval development of *H. scabra* in Sabah, Peninsular Malaysia, Philippine, Iran, India and Australia. The larval development period recorded from gastrula up to late auricularia are almost similar in those places. However, there is a large difference in the development of larvae at the doliolaria and pentactula stage. In this study, the doliolaria was started to be observed at Day 21. The fastest development of auricularia into doliolaria stage was recorded at Day 10 in Philippine, India and Australia. In Peninsular Malaysia and Iran, the doliolaria was started to appear on Day 11 and Day 14, respectively. This study also recorded that the doliolaria started to metamorphosis into pentactula stage at Day 26. In Peninsular Malaysia, the pentactula appeared at Day 18 (Mazlan and Hashim, 2015). In Philippine, Iran, India and Australia the pentactula were observed at Day 15, Day 20, Day 13 and Day 12, respectively (Nievales, 2014; Dabbagh and Sedaghat, 2012; James,2004; Agudo, 2004). The larval development recorded in this study was slower compared to previous studies. There are several factors that affect the larval development such as water quality (temperature, dissolved oxygen, pH and salinity), diet types and genetic. The water quality was maintained within the range recommended by Agudo (2006). According to previous studies, it is recommended to feed the *H. scabra* larvae with the *Chaetoceros* sp., *Isochrysis* sp. and *Rhodomonas salina* (Agudo, 2006; Asha *et al*., 2007; Asha *et al*. 2011). However, in this study the larvae were fed with *Nannochloropsis* sp. due to the insufficient supply of *Chaetoceros* sp. Different nutrition composition in the microalgae given might affect the larval development of *H. scabra*. According to Banerjee *et al.* (2011), *Chaetoceros calcitrans* has high protein (41.6%) and lipid (26.8%) content compared to *Nannochloropsis oculata* (protein= 32.8%, lipid=13.0%). High protein and lipid content in the *Chaetoceros* sp. might enhance the larval development of *H. scabra*. The quality of the broodstock as well gave impact on the quality of the eggs, hatching rate and larval development.

Table 3: Comparison of larval development with previous studies

Larval stage	Day after fertilization (days)					
	Sabah, Malaysia	Peninsular Malaysia	Philippine	Iran	India	Australia
Gastrula	1	-	-	-	-	1
Early auricularia	2	2	2	2	3	2
Mid auricularia	4	-	4	4	-	4
Late auricularia	6	-	7	8	-	5
Doliolaria	21	11	10	14	10	10
Pentactula	26-40	18	15	20	13	12
References	This study	Mazlan and Hashim, 2015	Nievales, 2014	Dabbagh and Sedaghat, 2012	James, 2004	Agudo, 2006

Conclusion

Hatchery seed production is seen as a way to restore the depleted wildstock of sandfish through the sea ranching programme. It is important for the sustainability of this species. Continuous seed production of sandfish in hatchery can be achieved by artificial spawning. However, larval rearing is also one of the most crucial stage in aquaculture. Intensive studies on the larval rearing especially on the diet types, stocking density, salinity and temperature must be done to improve the hatching rate, growth and survival of the sandfish from the early stage. It can ensure the success of the sea ranching programme.

References

- ✓ Agudo, N. (2006) Sandfish Hatchery Techniques. Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and the World Fish Centre, Noumea, pp. 44.
- ✓ Asha P.S., Rajagopalan M. and Diwakar K. (2007) Stock enhancement of sea cucumbers- a solution for the depletion of natural stocks of *Holothuria scabra* along Gulf of Mannar. Marine Fisheries Information Service, 193: 7-10.
- ✓ Asha P.S., Rajagopalan M. and Diwakar K. (2011) Influence of Salinity on Hatching Rate, Larval and Early Juvenile Rearing of Sea Cucumber *Holothuria scabra* Jaeger. Journal of the Marine Biological Association of India, 53 (2): 218-224.
- ✓ Banerjee S., Hew W.E., Khatoon H., Shariff M. and Yusoff F.M. (2011) Growth and Proximate Composition of Tropical Marine *Chaetoceros calcitrans* and *Nannochloropsis oculata* Cultured Outdoors and Under Laboratory Conditions. African Journal of Biotechnology, 10(8): 1375-1383.
- ✓ Battaglene S.C. (1999) Culture of Tropical Sea Cucumbers for Stock Restoration and Enhancement. Naga, The ICLARM Quarterly, 22 (4): 4-11.
- ✓ Battaglene S.C., Seymour J.E., Ramofafia C. and Lane I. (2002) Spawning Induction of Three Tropical Sea Cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. Aquaculture, 207: 29-47.
- ✓ Caulier G., Flammang P., Rakotorisoa P., Gerbaux P., Demeyer M. and Eeckhaut I. (2013) Preservation of The Bioactive Saponins of *Holothuria scabra* through The Processing of Trepang. Cahiers de Biologie Marine, 54: 685-690.
- ✓ Choo P.S. (2012). The Sea Cucumber Fishery in Semporna, Sabah, Malaysia. SPC Bche-de-mer Information Bulletin, 32: 43-48.
- ✓ Dabbagh A.R. and Sedaghat M.R. (2012) Breeding and rearing of The Sea Cucumber *Holothuria scabra* in Iran. SPC Beche-de- mer Information Bulletin, 32: 49-52.
- ✓ James B.D. (2004) Captive Breeding of The Sea Cucumber, *Holothuria scabra*, from India. Aquaculture Advances, Session III: 385-395.
- ✓ Kamarudin, K.R., Rehan, A.M., Hashim, R. and Usup, G. (2010) An Update on Diversity of Sea Cucumbers (Echinodermata: Holothuroidea) in Malaysia. Malayan Nature Journal, 62: 315-334.
- ✓ Mazlan, N. and Hashim, R. (2015) Spawning Induction and Larval Rearing of The Sea Cucumber *Holothuria scabra* in Malaysia. SPC Beche-de-mer Information Bulletin, 35: 32-36.
- ✓ Mercier, A., Battaglene, S.C., Hamel, J.F. (2000). Settlement Preferences and Early Migration of The Tropical Sea Cucumber Holothuria scabra. Journal of Experimental Marine Biology and Ecology, 249: 89-110.
- ✓ Nievales, M. F. (2014) Larval Rearing Techniques of Sandfish. Southeast Asian Fisheries Department Centre Sandfish Training.
- ✓ Ram R. and Southgate P.C. (2014) The Influence of Processing Techniques on The Quality and Nutritional Composition of Tropical Sea Cucumbers. SPC Fisheries Newsletter, 143: 17-18.