Asian Journal of Fisheries and Aquatic Research

2(3): 1-9, 2018; Article no.AJFAR.47061

Preliminary Trials on Hatchery and Larval Development of the Sea Cucumber, *Holothuria scabra* (Jaeger 1883), in the Sultanate of Oman

Khalfan M. Al-Rashdi[1*], Michel R. Claereboudt[2] and Igor Eeckhaut[3]

[1]Aquaculture Center, Ministry of Agriculture and Fisheries Wealth, 427 Muscat P.C 100, Oman.
[2]College of Agricultural and Marine Sciences, Sultan Qaboos University, 34 Muscat P.C. 123, Oman.
[3]Biology of Marine Organisms and Biomimetism, University of Mons-Hainaut, 7000 Mons, Belgium.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KMAR and IE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KMAR and MRC performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2018/v2i330009
Editor(s):
(1) Dr. Pınar Oguzhan Yildiz, Assistant Professor, Department of Food Engineering, The Faculty of Engineering, Ardahan University, Turkey.
Reviewers:
(1) Ali Türker Mugla, Sitki Kicman University, Turkey.
(2) Emmanuel T. D. Mensah, CSIR-Water Research Institute, Ghana.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47061

Original Research Article

Received 28 October 2018
Accepted 05 February 2019
Published 19 February 2019

ABSTRACT

Although sea cucumber *Holothuria scabra* has always been part of the traditional exploitation of the benthos of Mahout Bay in the Arabian Sea, the foreign demand for the product and its high price have put increased pressure on the resource leading rapidly to overfishing. Aquaculture of this species has developed as a response to the overfishing problem but has not been yet studied in Oman. As a first step to evaluate its potential for aquaculture in Oman it was thus necessary to conduct hatchery trials. Four hatchery trials were conducted to evaluate the quality of the local broodstock, the response and efficiency of *in-vitro* maturation and fertilization and the success of larval development and rearing. Collected animals of 200-600 g were transported by road for 5 hours with zero evisceration to the Hatchery station. *In-vitro* maturation and fertilization success of more than 90% were achieved using the maturation inducing fractions (MIF) method leading to the development of mature eggs and normal embryos larvae. Auricularia larval stages were completed within 15 days and fed normally on microalgae *Phaeodactylum tricornutum, Chaetoceros sp*. and

**Corresponding author: Email: khalfanrashdi@gmail.com;*

Nanochloropsis sp. Average settlement rate and size for pentactula juveniles were closely to 9% and 0.5 mm, respectively, nearly one month post-fertilization (PF). However, a high mortality of 70% was observed during early larval development caused by ciliated protozoans and copepods attack. The culture of *H. scabra* population in Oman is thus promising but further research is needed to ensure higher survival rates, particularly in the early larval stages where adequate water filtration and sterilization is essential.

Keywords: Sea cucumber; Holothuria scabra; hatchery; larval development, Oman.

1. INTRODUCTION

Holothurians commonly known as sea cucumbers are marine invertebrates of significant importance for marine ecosystems and for a limited number of species as a valuable fishery generally for export. From the early 1990s, the populations of various species of sea cucumbers declined due to an increasing demand of Asian markets, particularly those of China [1]. Among these species, *Holothuria scabra* is considered as one of the most valuable sea cucumber for bêche-de-mer production and have been widely fished and overfished in the tropics [2]. It is currently valued at more than 1000 US$ per kg dry weight [3], and the demand from the Asian market remains strong [4]. This increasing demand from Asian markets driving high price, an often inadequate management of the fishery and the biological characteristics of the species such as slow growth and easy to access shallow water habitat resulted globally in severe overfishing [5]. To avoid overexploitation of natural populations while maintaining a sustainable level of exports, aquaculture is considered as one of the potential solutions for *H. scabra* production [1,6]. Several countries have progressively developed a viable aquaculture production and in some cases have initiated restocking or sea ranching programs [1,7,8]. Although the sea cucumber *H. scabra* has always been a small part of the traditional exploitation of the benthos in Mahout Bay of Arabian Sea [9], the foreign demand for the product overseas and its high price have put increased pressure on this resource locally leading rapidly to overfishing [10].

The natural spawning of this species in Oman has been recently documented and showed, as holothurians in many other locations, an annual reproductive cycle [11,12,13,14,15]. Natural maturation occurred in February and March followed by spawning in April and May, which is correlated to high sea surface temperature and precipitation as both may serve as exogenous synchronizing cues for gamete maturation and spawning [11]. Alarming signs of the of the Omani population of *H. scabra* overfishing have been observed with stock densities decreasing to less than 1 individual per ha [10], a regular decrease in the size of animal processed and a progressive switch to less valuable species in only a few years of exploitation. Therefore, restocking and stock enhancement in addition to fisheries management policies are needed to ensure the sustainability of this resource. This article documents the first attempts made to develop hatchery techniques for the Omani sea cucumber *H. scabra* population as an initial step towards aquaculture development and stock enhancement programs.

2. MATERIALS AND METHODS

2.1 Culture Site and System

The Aquaculture Research Unit of Sultan Qaboos University located in Al-Hail some 40 km north of the capital of Oman, Muscat, was used to conduct *H. scabra* hatchery trials. Land based outdoors tanks were used to keep the broodstock of *H. scabra*. The tanks were continuously supplied with seawater extracted from a marine well at a salinity of 37 PSU and a temperature of 29°C. For the spawning and larval rearing experiments, the water was filtered using sand filter and 5µm particle filter and then passed through a UV sterilization unit [16].

2.2 Broodstock Collection and Transportation

In total 80 healthy animals weighing between 200-600g were collected at 4 occasions from Mahout Bay of Al-Wusta Province by local fishermen and through SCUBA diving starting between September 2009 and November 2010 (Fig. 1). Collected animals were carefully handled to avoid evisceration, cleaned by gently washing the body with seawater and packed at low density of 6-8 individuals per transparent plastic bags filled with 1-2 L of sea water and inflated with pure oxygen. The bags were placed

in cool-boxes containing dry ice to maintain a low temperature in the boxes during the 5 hours trip to Muscat. Upon arrival at the hatchery, the animals were acclimatized while still in the transport bags with ambient seawater and later transferred to clean tanks with running oxygenated sea water until experimentation.

2.3 Induced Maturation and Fertilization

For this study, 4 hatchery trials of *in-vitro* maturation and fertilization (Table 1) were conducted using an artificial maturation substance extracted from sea urchin oocytes [17]. It is referred as Maturation Inducing Fractions (MIF) and based on hatchery works in Madagascar [17,18]. Genitors were dissected through a longitudinal incision on the anterior part of the trivium to extract male and female gonads for the in-vitro fertilization.

Body fluid was drained and the gonads of males and females were gently removed using forceps and placed in separate beakers with filtered sea water. Gonads were then cut separately into small pieces using scissors to release oocytes and sperm cells from gonadal tubules. The oocytes were then sieved on 60 µm mesh with filtered sea water and placed in petri dishes at concentrations of 100 oocytes/ml. Maturation of the oocytes was induced with the MIF solution for 2 hours, then rinsed with filtered sea water to remove MIF residuals. Fertilization was achieved by adding few drops of sperm suspension to the mature oocytes. The fertilized eggs were kept in a shallow basin without aeration until hatching of gastruled embryos. Observations and photography of embryonic development stages were carried out on random samples every 30-60 minutes using a light microscope at 40X magnification.

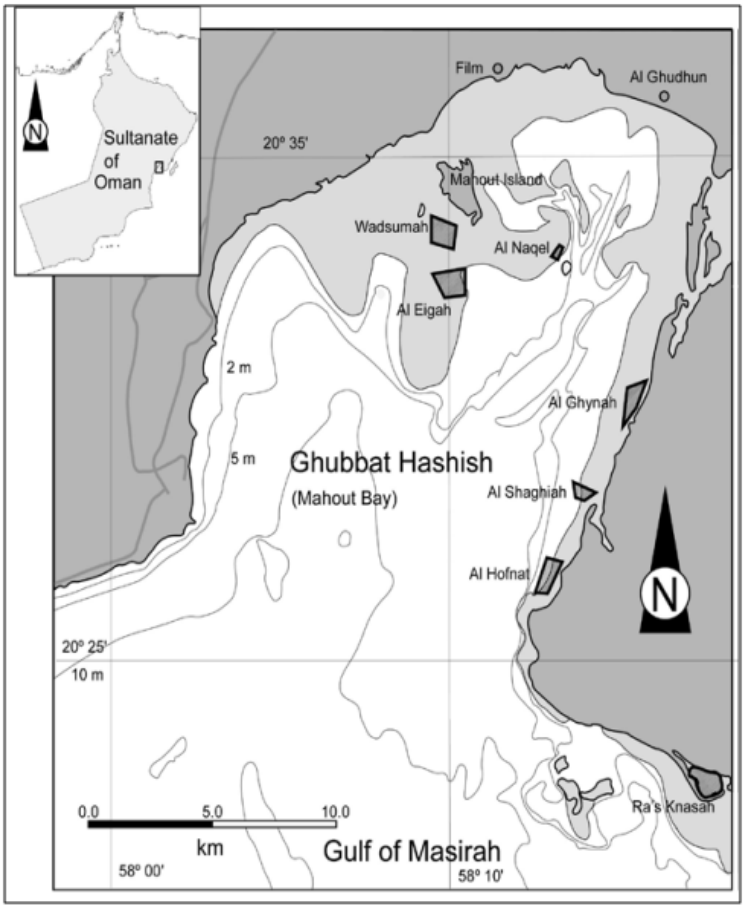

Fig. 1. Location of the *H. scabra* population used for the spawning experiments. Individuals were collected from various locations within Mahout Bay

2.4 Larval Development and Rearing

Newly-hatched gastrula larvae were transferred into 100 L tanks filled with 85 L of 2-µm filtered and UV-treated seawater and provided with aeration through air diffusers. Feeding and regular water exchange commenced with development of auricularia stage larvae from day-2 post-fertilization (PF). The water was changed by siphoning and the larvae collected onto a 60 µm wet sieve. Tanks were refilled with 2-µm filtered and UV-treated seawater. This method of water exchange was continued daily for 20 days until larval settlement. The newly settled juveniles were maintained in a flow-thru system with 2-µm filtered and UV-treated seawater.

H. scabra larvae were fed daily from the second day PF with different types of micro-algae until settlement. Three mixed algae were cultured under laboratory conditions with artificial light and aeration. A temperate species *Phaeodactylum tricornutum* was cultured at low temperature from 19 to 24°C in the laboratory. *Chaetoceros sp.* and *Nanochloropsis sp.* were cultured both under laboratory and outdoor conditions (29-30°C) and fertilized with Guillard's F/2 medium. Initially, the developing larvae were fed twice daily with *P. tricornutum* at a final concentration of approximately 2500 cells per liter. On day 8 PF, the final concentration of *P. tricornutum* was increased to 5000 cells per liter. During the Doliolaria stage (a non-feeding stage), the feeding was temporarily suspended and resumed after metamorphosis (typically on day 20 PF). At this stage both microalgae (*Chaetoceos, Nannochloropsis and Phaeodactylum*) and particulate diet (dried and powdered *Sargassum sp.*) were added to favor settlement and feed the newly settled juveniles.

2.5 Larval Settlement

As a final step of the hatchery trials, late doliolaria larvae at day 19 were introduced to new tanks with filtered and irradiated sea water under a flow-thru system for larval settlement purpose. In order to count the settlement rate, settled pentactula juveniles were detached after 10 days of introducing to the settlement tanks.

3. RESULTS

3.1 Maturation Induction and Fertilization

All collected animals survived through 5 hours road transportation and were successfully transferred to holding tanks. For the artificial maturation experiments, all female genitors positively responded to the MIF technique and produced more than 90% normal mature oocytes after 2 hours of induction. A fertilization rate of 90% was also achieved on these artificially matured oocytes. A total of more than 400,000 larvae were produced over 4 independent *in-vitro* fertilization trials and developed successfully into normal embryos and larvae (Table 1). Although 40-70% mortality was observed during larval development, 9% of the produced larvae settled successfully as pentactula Juveniles.

3.2 Embryonic and Larval Development

Larval development was normal and follows the documented stages for *H. scabra* described in the literature (Fig. 2). The mean diameter of fertilized eggs was 150 ± 3.0 µm and underwent cell division within 1.5 hours. They developed into blastula and gastrula around 12 and 16 hours post fertilization (PF), respectively. Early hatched auricularia stage was observed around 36 hours PF, and continued to develop to mid-auricularia larvae at day 6 PF. Late auricularia larvae was observed by day-13 PF and transformed to non-feeding doliolaria larvae after completing 15 days PF. There was a mass mortality reached 70% during the first 3 days of rearing. Primary microscopic observations of the larvae, revealed an abundance of ciliate protozoans and copepods in the larval tanks. However, from the 4th day of development, survival rate remained almost stable. After 13 days, the larvae reached late auricularia stage characterized by the appearance of lipid spheres at the edge of the cilia band. The first settlements were observed on day 20 PF and small juveniles began to appear on most trials on the bottom of the rearing tank 3 to 4 weeks PF. They appeared transparent and only visible using a light torch. Their size was estimated at 0.5 mm.

There was a high mortality during the first 3 days PF during all four trials. With the exception of trial 1 for which there were no microalgae to feed the larvae, from day 4 to day 15 mortality remained quite low. A second mortality was observed between day15 and day 21 in all three remaining

trials (Fig. 3). During the daily microscopic observations of the larvae, abundant ciliate protozoans and copepods were observed in the larval tanks. However, after the 4th day PF, survival was stable and reached 9% over 20 days PF.

3.3 Larval Settlement

Fig. 4 presents the results of settled larvae and their settlement rates among 3 hatchery trials after 29 days PF. Late doliolaria larvae were induced to settle by adding mixed microalgae and dried and powdered *Sargassum sp*. in a daily basis, which acted both as an inducer for the larvae to settle and serve also as a food for the newly settled pentactula. After 10 days of post-settlement, the results show high settlement rate of 9.2% in trial 1, while low rate of 8.3% was found in trial 2. Overall, the average settlement rate is almost 9% from the 3 hatchery trials. The size of settled juveniles was found at 0.5 mm± 2.0 after 30 days PF.

Table 1. Summary results for the 4 hatchery trials. The number between brackets is the number of genitors used in the trials

Hatchery trial number	Broodstock size (g)		Fertilized oocytes	Produced larvae
	Male	Female		
F1	287 (1)	287&346 (2)	160000	139200
F2	245 (1)	457&375 (2)	140000	96000
F3	319 (1)	320&425 (2)	85000	59,766
F4	200&260 (2)	600&375 (2)	120000	105,400
Total			**505000**	**400366**

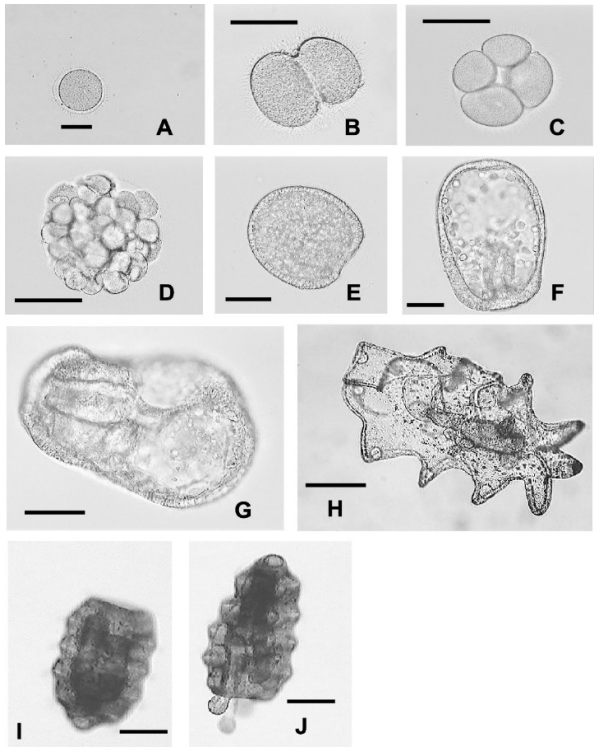

Fig. 2. Different stages of larval development in *H. scabra*. The scale bars represent 100 µm. A: Fertilized oocyte (10 min PF); B: two-cell embryo (90 min PF); C: four-cell embryo (120 min PF); D: morula stage embryo (3 hours PF); E: early stage of gastrulation (12 hours PF); F: Gastrula (15 hours PF); G: very early auricularia larva (the mouth is formed) hatched (32 hours PF); H: late auricularia larva (13 days PF); I: non-feeding doliolaria larva (15 days PF) and J: Pentactula with tentacles (20 days PF)

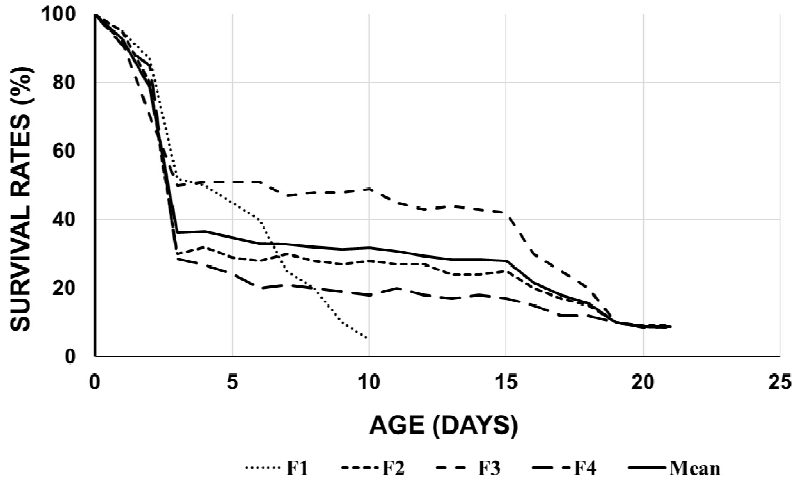

Fig. 3. Survival rates of *H. scabra* over 4 hatchery trials and the mean of the 3 trials (F2, F3, F4) during 21 days of embryonic and larval development and rearing

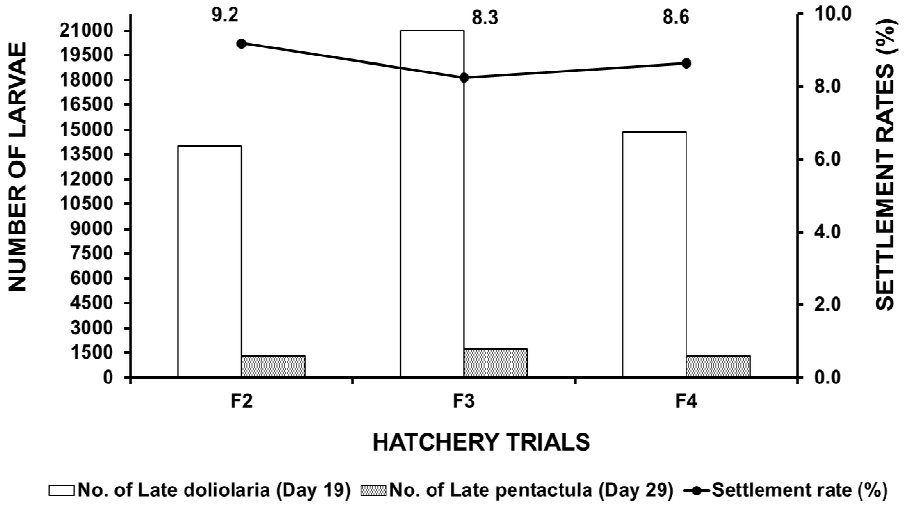

Fig. 4. Number of Doliolaria larvae introduced for settlement and number of settled pentactula juveniles after 10 days from the introduction and their Settlement rate in 3 hatchery trials (F2, F3, F4)

4. DISCUSSION

Overfishing of *H. scabra* went beyond just an ecological issue; it became a socio-economic problem as livelihoods and income of many coastal dwellers rely on its fishery.

There are several national management measures applied on this fishery, including total ban as the current case here in Oman; however, these seem to be insufficient for a sustainable use of the resources [6]. A sustainable and efficient solution so far to enhance production as well as depleted natural stock of this species is through developing aquaculture [5]. To ensure *H. scabra* aquaculture sustainability and affordability, the production of fertilizable eggs and larvae in large quantities and at any time is a key element [18]. Several methods have been described to induce *H. scabra* spawning, mainly thermal shock [19,20,21] alone or combination with water pressure and drying treatments or algae [22]. However, these methods may not be reliable to produce mature fertilizable oocytes outside the natural reproductive season of the organism [18,23]. Such critical point has been

addressed through a new methodology based on *in-vitro* maturation using artificial inducers [17] which recently have been commercialized [24]. The use of such maturation inducers allows holothurians farmers to obtain clean, fertilized eggs year-round. This method was tested in Oman *H. scabra* population and achieved more than 90% oocyte maturation and fertilization, which is consistent with Eeckhaut et al. [18] results on Madagascar *H. scabra* populations. The method requires the sacrifice of several large adult specimens which may be difficult to justify for a locally endangered species, although these few specimens will produce 1000s of juveniles [25]. Despite larval developmental timing variations among various *H. scabra* populations, the embryos underwent normal development processes up to the pentactula stage over 21 days PF which is consistent with published values for larval development in *H. scabra* [3].

In our study settlement was achieved by conditioning the tanks with algae as substrates, which is consistent also with published literatures confirming that settlement will not be successful without providing settlement cues [26,27]. The flow-thru system which was used during rearing settled pentactula was found to improve the growth and survival rates. However, the key element during this period is the stocking density [26].

The high mortality observed during the first few days PF took place before feeding and is thus unlikely the result of a contamination by the feeding solutions. Ramofafia et al. [28] also observed high mortality of 50% of the embryo to the appearing of the early auricularia stage. However, the abundant copepods and ciliates may be responsible for this temporary mortality [29]. Copepods attack larvae either directly or by repeated collisions causing bodily damage to the larvae [19]. Asha & Muthia [30] observed 65% mortality due to ciliates on the 9th day of rearing *H. spinifera* larvae. Since the water used for larval rearing was filtered and UV-treated, it is unlikely the source of the copepods and ciliates, but some of the equipment used to siphon the water, transfer the larvae from one container to another, the container themselves may have been contaminated by a concomitant fish hatchery and emphasize the need for high levels of training in sterilization and decontamination and asepsis [31]. Unfortunately, separating copepods from sea cucumber larvae is difficult as their sizes are similar [32]. To control copepod population, Pitt & Duy [19] recommended the application of trichlorofon (Dipterex), an insecticide which may be effective against some arthropod infestation. Alternative solutions using either naturally occurring substances or better management practices are needed [33]. In addition, optimization of the diet using locally available species is also necessary [34].

5. CONCLUSION

The result presented here is a first insight on the artificial production of sea cucumbers in Oman. Maturation inducing fractions (MIF) method was found to be an efficient, effective and dependable new technique to produce, in a large scale, mature and fertilized *H. scabra* oocytes leading to develop normal competent larvae. However, further studies should be made to minimize the mortality rate during early larval development and to enhance the rates of larval settlement.

The potential to develop sea cucumber aquaculture in Oman is high because (i) *H. scabra*, a high commercial value, is still present in Oman particularly in shallow waters (ii) there are some natural locations ideally suited for sea ranching such as the Bay of Mahout, (iii) the human and the basic technological potential are already in place. For example, less than 1% of the surface of the Bay of Mahout Bay could support the sea ranching of 4 M of sea cucumbers *H. scabra* if it is supplied by an efficient farm having 50,000 m^2 of ponds and an efficient hatchery with 600,000 L of adequate tanks.

ETHICAL APPROVAL

All animals used in these experiments were euthanized following to the ethical standards of the Ministry of Agriculture and Fisheries Wealth and only the minimum number necessary to obtain the number of fertilized oocytes were used.

ACKNOWLEDGEMENTS

This study was funded with thanks by the Agricultural and Fisheries Development Fund through research project; code No. 1/3/17, titled: Diversity, abundance and Aquaculture Potential of the Sea cucumber, *Holothuria scabra*, in the Sultanate of Oman. We give grateful thanks to Mohamed Al-Wahaibi, Adel Al-Bulushi, Ali Al-Ajmi and Mohamed Al-hikmani for their help in

hatchery activities. We would like to acknowledge Indian Ocean Trepang (IOT) Company for the technical support and using their patent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Purcell SW, Hair CA, Mills DJ. Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. Aquaculture. 2012;68-81.
2. Conand C. Present status of world sea cucumber resources and utilization: An international overview. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A, Editors. Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries. 2014;13-23,425,463.
3. Junus S, Kwong P, Khoo G. A review on the recent advances in the biology and aquaculture technology of *Holothuria scabra*. 2018;4(2):5-25.
4. Purcell SW, Williamson DH, Ngaluafe P. Chinese market prices of beche-de-mer: Implications for fisheries and aquaculture. Marine Policy. 2018;91:58-65.
5. Hamel JF, Conand C, Pawson DL, Mercier A. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as beche-de-mer. Advances in Marine Biology. 2001;41:129-223.
6. Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A. Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Report. Rome. 2004;463.
7. Eriksson H, de la Torre-Castro M, Purcell S, Olsson P. Lessons for resource conservation from two contrasting small-scale fisheries. AMBIO. 2014;44:204-213.
8. Watanabe S, Sumbing J, Lebata-Ramos M. Growth pattern of the tropical sea cucumber, *Holothuria scabra*, under captivity. JARQ. 2014;48(4):457-464.
9. Al-Rashdi KM, Al-Busaidi SS, Al-Rassadi IH. Status of the sea cucumber fishery in the Sultanate of Oman. SPC Beche-de-mer Information Bulletin. 2007;25:17-21.
10. Al-Rashdi KM, Claereboudt MR. Evidence of rapid overfishing of sea cucumbers in the Sultanate of Oman. SPC Beche-de-mer Information Bulletin. 2010;30:10-13.
11. Al-Rashdi KM, Claereboudt MR. Reproductive biology of the sea cucumber *Holothuria scabra* (Jaeger 1883) in Mahout Bay, Arabian Sea, Oman. International Journal of Fisheries and Aquatic Studies. 2018;6:100-108.
12. Asha PS, Muthiah P. Reproductive biology of the commercial sea cucumber *Holothuria spinifera* (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. Aquaculture. 2008;16:231−242.
13. Benitez-Villalobos F, Avila-Poveda OH, Gutiérrez-Méndez IS. Reproductive biology of *Holothuria fuscocinerea* (Echinodermata: Holothuroidea) from Oaxaca, Mexico. Sexuality and Early Development in Aquatic Organisms. 2013;1:13.
14. Muthiga NA, Kawaka J. The effects of temperature and light on the gametogenesis and spawning of four sea urchin and one sea cucumber species on Coral Reefs in Kenya. Proceedings of the 11th International Coral Reef Symposium, Florida; Session Number 11; 2008.
15. Omar HA, Abdel Razek FA, Abdel Rahman SH, El Shimy NA. Reproductive periodicity of sea cucumber *Bohadschia vitiensis* (Echinodermata: Holothuroidea) in Hurghada Area, Red Sea, Egypt. Egyptian Journal of Aquatic Research. 2013;39:115-123.
16. Al Rashdi KM. Project final report on diversity, stock and aquaculture potential of sea cucumber in Oman. Ministry of Agriculture and Fisheries Wealth, Aquaculture Centre, Muscat, Sultanate of Oman; 2012.
17. Léonet A, Rasolofonirina R, Wattiez R, Jangoux M, Eeckhaut I. A new method to induce oocyte maturation in holothuroids (Echinodermata). Invertebrate Reproduction and Development. 2018;53:13–21.
18. Eeckhaut I, Lavitra T, Léonet A, Jangoux M, Rasolofonirina R. *In-vitro* fertilisation: A simple, efficient method for obtaining sea cucumber larvae year round. In: Hair CA, Pickering TD, Mills DJ, Editors. Asia–Pacific Tropical Sea Cucumber Aquaculture. Australian Centre for International Agricultural Research Proceedings. New Caledonia. 2012;136: 40-49.
19. Pitt R, Duy NDQ. Breeding and rearing of the sea cucumber *Holothuria scabra* in

Vietnam. In: Lovatelli, A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A, Editors. Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Tech. Pap. 2004;463:333-346.

20. Duy NDQ. Seed production of sandfish *Holothuria scabra* in Vietnam. SEAFDEC. Aquaculture Extension Manual. South Asian Fisheries Development Center, Iloilo, Philippine. 2010;28.
21. Hair C, Kaure T, Southgate PC, Pickering T. Potential breakthrough in hatchery culture of Sandfish *Holothuria scabra* by using algal concentrate as food. SPC Bêche-de-mer Inf. Bull. 2011;31:60–61.
22. Agudo N. *Sandfish hatchery techniques*. The Worldfish Center, Secretariat of the Pacific Community, and Australian Center for International Agricultural Research. 2006;42.
23. Battaglene SC, Seymour JE, Ramofafia C, Lane I. Spawning induction of three tropical sea cucumbers: *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. Aquaculture. 2002;207:29–47.
24. Leonet A, Delroisse J, Schuddinck C, Ruddy W, Jangoux M, Eeckhaut I. Thioredoxins induce oocyte maturation in holothuroids (Echinodermata); 2018.
25. Purcell SW, Ngaluafe P, Foale SJ, Cocks N, Cullis BR, Lalavanua W. Multiple factors affect socioeconomics and wellbeing of artisanal sea cucumber fishers. PLoS One. 2016;11:e0165633.
26. Mercier A, Battaglene SC, Hamel JF. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology. 2000;249:89-110.
27. Li L, Li Q, Kong L. The effect of different substrates on larvae settlement in sea cucumber, *Apostichopus japonicas.* Journal of the World Aquaculture Society. 2010;41:124-130.
28. Ramofafia C, Byrne M, Battaglene SC. Development of three commercial sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*: Larval structure and growth. Journal of Marine and Freshwater Research. 2003;54:657–667.
29. James BD. Captive breeding of the sea cucumber, *Holothuria scabra*, from India. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A, Editors. Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Tech. Pap. 2004;463:385-398.
30. Asha PS, Muthia P. Spawning and larval rearing of sea cucumber *Holothuria* (*Theelothuria) spinifera* Theel. SPC Beche-de-mer Information Bulletin. 2002;16:11-15.
31. Ruth U, Remie M, Daisy A, Lance B, Neil Angelo S. Small-scale hatcheries and simple technologies for sandfish (*Holothuria scabra*) production. In: Hair CA, Pickering TD, Mills DJ, Editors. Asia–Pacific tropical sea cucumber aquaculture. Australian Centre for International Agricultural Research Proceedings. New Caledonia. 2012;136:209.
32. James BD, Rajapandian ME, Basker BK, Gopinathan CP. Successful induced spawning and rearing of the holothurians *Holothuria (Metriatyla) scabra* Jaeger at Tuticorn. Marine Fisheries Infor. Ser. 1988;87:30-33.
33. McLean E, Ibrahim FS, Al-Rashdi KM. Environmental better management practices for Omani aquaculture. Ministry of Agriculture & Fisheries Wealth, Aquaculture Centre, Muscat, Sultanate of Oman; 2011.
34. Militz AT, Leini E, Duy ND, Paul GS. Successful large-scale hatchery culture of sandfish *Holothuria scabra* using micro-algae concentrates as a larval food source. Aquaculture Reports. 2018;9:25-30.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/47061