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Influence of salinity on hatching rate, larval and early juvenile rearing of sea cucumber *Holothuria scabra* Jaeger

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Abstract

The fertilized eggs, auricularia larvae and one month old juveniles of *Holothuria scabra,* obtained from induced spawning were used for various experiments to assess the effect of salinity on hatching rate and larval and early juvenile growth. The experiments were conducted for two days on hatching rate of fertilized eggs, for ten days on larval survival, growth and development and for 30 days on juvenile's growth rate. The maximum hatching of 39% at 35 ppt, followed by 32% at 33 ppt indicated the suitability of an ambient salinity of 33 to 35 ppt for effective hatching of fertilized eggs. High survival, growth rate and fastest development of auricularia were obtained at salinity between 33 and 35 ppt. The maximum growth rate in length and weight, was at 30 ppt, which may be the optimum for juvenile rearing. The one way ANOVA on differences in the hatching rate, larval growth and survival rate and juvenile growth rate at different salinities indicated high level of significance (p<0.001).

Keywords: *Holothuria scabra,* hatching rate, larval rearing, juvenile rearing

Introduction

The dried product of sea cucumbers of the families *Stichopodidae* and *Holothuridae* called *beche-de-mer* is a source of income to the fishermen from Gulf of Mannar and Palk Bay located in the southeast coast of India. The *beche-de-mer* is a product mainly from *Holothuria scabra* and *H. spinifera* (Asha and Muthiah, 2002; Chellaram *et al*., 2003). Like in many countries, sea cucumbers were overexploited in India (Asha *et al*., 2006). Subsequently the Ministry of Environment and Forest, Government of India, has banned the fishery and export of sea cucumbers since June 2001. The ban has affected the livelihood of poor coastal communities.

The ranching of hatchery produced juveniles of sea cucumbers to the natural habitat, a process called restoration, restocking or reseeding is gaining momentum worldwide, as the means to replenish the depleted stock of holothurians (Battaglene, 1999).

H. scabra, commonly called sand fish, is one of the most commercially valued tropical species of sea cucumbers with a wide distribution in the Indo-Pacific areas. The A-grade *beche-de-mer* processed from sand fish commands one of the highest price in the international market. Since mass production of juveniles of *H. scabra* through hatchery has been proved successful (James *et al*., 1988; Battaglene,1999; Agudo, 2006), the species is considered as an ideal candidate for farming and restocking. Stock enhancement of this species is currently being practiced in many countries (Hamel *et al*.,2001; Pitt 2001; Agudo, 2006).

Agudo (2006) reported that *H. scabra* can tolerate reduced salinities up to 20 ppt for a short duration but successful larval rearing in laboratory is carried out at salinities 26-30 ppt. Chen and Chian (1990) raised the larvae of *Actinopyga echinites* at 35 ppt. Asha and Muthiah (2002, 2005) indicated successful larval rearing of *H. spinifera* at salinities ranging from 34.8 to 36.0 ppt and confirmed 35 ppt as the optimum for the larvae. Pitt and Duy (2004) reported successful metamorphosis and settlement of *H. scabra* larvae in salinities from 20 to 40 ppt with maximum settlement at 35 ppt. Giraspy and Ivy (2008) reared the larvae of

H. scabra var. *versicolor* at salinity between 34 and 35.5 ppt. Thierry *et al.* (2009) indicated that drastic salinity drop during low tide was a major problem while rearing the juveniles of *H. scabra*.

In natural conditions, the larvae of holothurians are exposed to surface salinities between 29.6 and 36.2 ppt in the inshore waters off Tuticorin (Asha and Diwakar, 2007). Considerable reduction in salinity, up to 14 ppt has been observed due to freshwater influx during monsoon season in Tuticorin waters (Asha *et al*., 2009). Hence salinity is a key factor in determining the success of holothurian larval and juvenile rearing at Tuticorin. The present study was conducted to assess the effect of salinity on hatching rate and larval and juvenile rearing of *H. scabra,* so as to improve the rearing conditions suitable for mass production of juveniles of *H.scabra* in hatcheries.

Material and Methods

Broodstock of *H. scabra* (20 numbers) collected by skin divers were maintained in 1000 litres FRP tanks with 150 mm thick coral sand at the bottom. Exchange of water was carried out daily and that of sand in every fortnight. The brooders were fed with *Sargassum* sp. powder at the rate of 5% of body weight. The brooders were induced to spawn by addition of feed constituted by rice bran, soya powder and *Sargassum* sp. powder (4:1:2) at a ratio of 50 g 500 l^{-1} as suggested by Asha and Muthiah (2007, 2008). Fertilized eggs were washed through a 40 µm sieve and used for the experiment on hatching rate up to the stage dipleurula.

Auricularia larvae (48 hours old; mean size: 390.1 ± 9.2 µm) and 30 days old juveniles of 13-20 mm length and 0.17-0.33 g weight from the same brood, were used for the experiment on the effect of salinity on larval and juvenile rearing.

The salinity selected for the experiments were 15, 20, 25, 30, 33, 35, 38 and 40 ppt. The experiments were conducted in plastic circular aquarium bowls (capacity: 3 l; diameter: 20 cm; height: 16 cm) filled with sand filtered sea water. Three replicate bowls were maintained for each treatment. The larvae and juveniles were transferred to the experimental salinities by slowly changing the conditions over an acclimatization period of 1 hour.

The hatching rate was calculated after 48 hours and the percentage of dipleurula stage was estimated in each treatment by counting the numbers in one ml sub samples. In the larval rearing experiment, 100% water was changed on alternate days and 50% on other days. During 100% water change, the larvae retained on a 40 µm sieve were transferred to 3 l glass beakers. Enumeration of larvae was carried out as suggested by Asha and Muthiah (2006, 2007). The larvae were fed with the microalage *Chaetoceros* sp. at concentrations as specified by Asha (2004) and the experiment was conducted for ten days.

For juvenile rearing experiment, the water was changed daily and the growth rate in length and weight were estimated on 30th day at the end of the experiment. The juveniles were fed with *Sargassum* sp. powder and fine sand at a proportion of 1:2 at 1% of body weight sieved through 80 µm sieve.

The mean differences in the number of fertilized eggs hatched on 2nd day, mean difference in the size and number of 8 day old larvae and 30 day old juveniles from the initial value for each treatment were considered in one way analysis of variance (ANOVA). The differences among treatment means were tested for significance by a post – hoc multiple comparisons (Fisher's LSD) test.

Results and Discussion

The dipleurula larvae did not hatch from the fertilized eggs at salinities 15, 20, 25 and 40 ppt, but hatching was observed at salinities 30, 33, 35 and 38 ppt. The highest percentage of hatching (39.0%) was observed at 35 ppt, followed by 34.0%, 31.7% and 18.6% at salinities 33, 30 and 38 ppt respectively, indicating 35 ppt as the optimum salinity for effective hatching of fertilized eggs (Fig. 1).

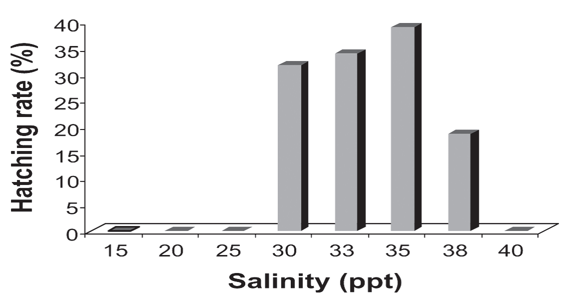

Fig. 1. Hatching rate of fertilized eggs of *H. scabra* at different salinities

Agudo (2006) suggested that large difference in temperature and salinity of water affects the survival of holothurian's eggs. Morgan (2000) indicated that hatch rate and number of spawned eggs are important indicators of egg viability of broodstock maintained in captivity. In the present work, hatching was not observed below 30 ppt and above 38 ppt. At lower salinity, during the process of development, the blastula became less dense in the iso-osmotic environment because of their enlarged size and could not overcome the density gradient (Kashenko, 2002).

The one way ANOVA on the difference in mean hatching rate of the larvae at different salinities indicated high level of significance (p<0.001) (Table 1). Among the paired comparison of different salinity treatments, except between 15 and 20 ppt, the comparisons between 15 and 25 ppt, 20 and 25 ppt, 30 and 33 ppt, 30 and 35 ppt, 15 and 40 ppt were significantly different (Table 1).

Total mortality of larvae was noticed on 2nd day at 15 ppt. At 20 ppt, the survival rate of larvae drastically decreased from 52.4% on day 4 to 9% on day 8 and total mortality was observed on day 10. At 40 ppt, none of the larvae survived after day 6. At salinities between 25 and 38 ppt, maximum percentage of survival (17.7%) was observed at 35 ppt, followed by 16.7% at 33 and 38 ppt (Table 2). Similarly the growth rate of larvae was nil on day 6 at 40 ppt and on day 8 at 20 and 25 ppt. Maximum growth rate of 74.8µm day $^{-1}$ was observed at 35 ppt followed by 74.03 µm day $^{-1}$ at 33 ppt and 56.4µm day $^{-1}$ at 30 ppt respectively (Table 3).

A high degree of significance (p<0.001) was observed both in the mean survival percentage and mean growth rate of the larvae held at different salinities (Tables 4 and 5). Except between salinities 20 and 25, 20 and 30, 20 and 33, 20 and 35, 20 and 38, no significant difference was noticed among pair-wise comparisons of survival percentage at different salinity treatments. Where as except between 20 and 25, 20 and 40, 25 and 40, 38 and 40, statistically significant difference was observed among all other

Table 1. ANOVA on number of *Holothuria scabra* dipleurulae hatched on second day at different salinities with results of Least Significant Difference (LSD) post-hoc multiple comparisons test

Treatments		Sum of squares	*df*	Mean Square	*F* ratio		*p* value
Between groups		6432.76	7	918.97	22.561		<0.001
Within groups		651.72	16	40.733			
Total		7084.48	23				
LSD							
	20	25	30	33	35	38	40
15	>0.05	>0.05	<0.001	<0.001	<0.001	<0.01	>0.005
20		>0.05	<0.001	<0.001	<0.001	<0.01	>0.05
25			<0.001	<0.001	<0.001	<0.01	>0.05
30				>0.05	>0.05	<0.05	<0.001
33					>0.05	<0.01	<0.001
35						<0.01	<0.001
38							<0.001

Table 2. Mean (±S.E., n = 30) survival percentage of the auricularia of *Holothuria scabra* at various salinities

Days	Salinity (ppt)						
	20	25	30	33	35	38	40
4	52.4 ± 11.8	48.3 ± 5.1	45.7 ± 1	77.4 ± 14.8	94.07 ± 3.4	77.4 ± 14.9	16.7 ± 3.5
6	26.07 ± 7.3	28.2 ± 9.8	33.6 ± 13.8	27.7 ± 5.9	67.7 ± 13.6	27.7 ± 5.9	8.75 ± 2.6
8	9 ± 0.7	17.03 ± 4.6	18.01 ± 1	21.7 ± 4.7	27.8 ± 7.5	21.7 ± 4.7	_
10	_	12.4 ± 2	13.03 ± 0.3	16.7 ± 4.05	17.7 ± 3.4	16.7 ± 4.05	_

Table 3. Mean (±S.E., n = 30) growth rate of the auricularia of *Holothuria scabra* at various salinities

Days	Salinity (ppt)						
	20	25	30	33	35	38	40
4	28.6 ± 18.7	97.3 ± 10.8	77.93 ± 2.9	112.1 ± 5.9	110 ± 3.8	105.8 ± 8.7	67.83 ± 9.3
6	38.05 ± 2.9	63.4 ± 10.8	55.13 ± 3.14	76.6 ± 1.3	77.6 ± 0.6	80.9 ± 0.725	-
8	32.11 ± 2.6	37.4 ± 43	51.8 ± 2.4	73.4 ± 1.7	76.8 ± 2.3	71.02 ± 1.14	-
10	-	-	56.4 ± 3.3	74.03 ± 0.8	74.8 ± 1.4	74.2 ± 0.94	-

pair-wise comparison in the mean growth rate of the larvae among different treatments (Tables 4 and 5).

On day 10, about 60% of the larvae attained late auricularia stage at 35 ppt followed by 45% at 33 ppt and 30% at 38 ppt. None of the auricularia larvae attained the later stage at 30 ppt. The percentage of metamorphosed doliolaria larvae was 10 at 35 ppt followed by 2 at 33 ppt (Fig. 2).

Pitt and Duy (2004) observed mortality of larvae of *H. scabra* at 15 ppt within 3 days but metamorphosis and settlement were achieved at salinities between 20 and 40ppt, with maximum survival rates of recently hatched larvae to juveniles at 35 ppt. Giraspy and Ivy (2005) opined that salinity ranging from 33 to 37 ppt is good for successful larval and juvenile rearing of *H. scabra*. Kashenko (2002) pointed out that the auricularia of *Apostichopus japonicus* migrated away from diluted surface layer at reduced salinity, to a depth of normal salinity and the adaptive plasticity and behavioral mechanisms allow larvae in some stages to survive and avoid the

Table 4. ANOVA on number of *Holothuria scabra* larvae survived on day 8 at different salinities with results of Least Significant Difference (LSD) post-hoc multiple comparisons test

Treatments		Sum of squares	*df*	Mean Square		F ratio
Between groups		1070.22		6	178.37	12.835
Within groups		194.67		14	13.89	
Total		1264.78		20		
LSD	25	30	33	35	38	40
20	<0.01	<0.01	<0.001	<0.001	<0.001	>0.05
25		>0.05	>0.05	>0.05	>0.05	>0.05
30			>0.05	>0.05	>0.05	>0.001
33				>0.05	>0.05	>0.001
35					>0.05	>0.001
38						>0.001

Table 5. ANOVA on growth rate of *Holothuria scabra* larvae on day 8 at different salinities with results of Least Significant Difference (LSD) post-hoc multiple comparisons test

Treatments		Sum of squares	*df*	Mean Square	F ratio	*p* value
Between groups		24713.61	8	4118.94	1001.88	<0.001
Within groups		57.557	14	4.111		
Total		24771.17	20			
LSD	25	30	33	35	38	40
20	>0.05	<0.001	<0.001	<0.001	>0.05	>0.05
25		<0.001	<0.001	<0.001	>0.05	>0.05
30			<0.001	<0.001	<0.001	<0.001
33				>0.05	<0.001	<0.001
35					<0.001	<0.001
38						>0.05

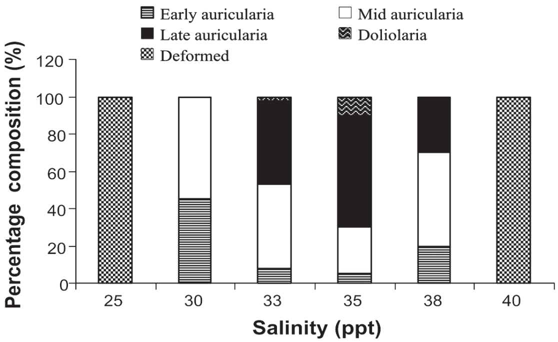

Fig. 2. Proportion of auricularia larvae of *H. scabra* on day 10 at different salinities

effects of reduced salinity. Harder (1968) also indicated that stratification of water column in estuaries and bay leads to accumulation of marine invertebrate larvae in waters of reduced salinity, causing osmotic shock and loss of locomotor activity.

In the juvenile rearing experiment, the juveniles at 15 ppt showed bulging and extension of body within 24 hours and absolute mortality occurred within 48 hours. The juveniles survived at all other salinities during the experimental period. The juveniles did not grow at 40 ppt. The growth rate was only 0.09 mm day^{-1} for juveniles reared at salinities 20, 25 and 38 ppt, followed by 0.14 and 0.17 mm day^{-1} for those at salinities 35 and 33 ppt respectively (Fig. 3a). The growth rate was only 0.0032 g day^{-1} for juveniles reared at 20 ppt, followed by 0.006 g.day^{-1}, 0.0062 g.day^{-1}, 0.0069 g.day^{-1} and 0.0071 g.day^{-1} for juveniles at 38, 35, 25 and 33 ppt respectively (Fig. 3b).

The highest growth rate in length (0.24 mm day^{-1}) and weight (0.008 g.day^{-1}) were observed for juveniles grown at 30 ppt. Hence 30 ppt is suggested

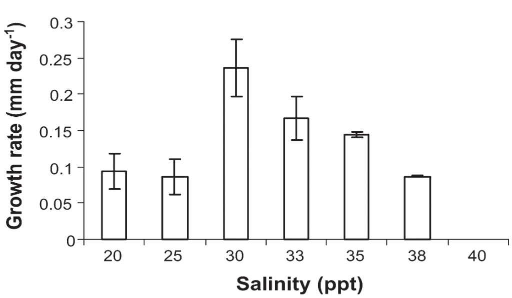

Fig. 3a. Growth rate in length of *H. scabra* juveniles reared at different salinities

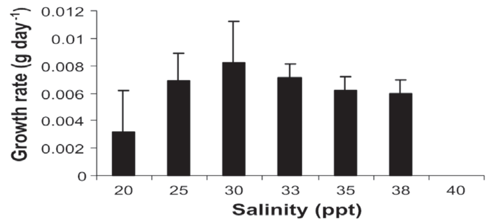

Fig. 3b. Growth rate in weight of *H. scabra* juveniles reared at different salinities

as the optimum for rearing the one month old juveniles of *H. scabra*. Wang and Chen (2004) indicated that sea cucumber juveniles could tolerate wide range of salinity from 26 to 33 ppt and recommended 31.6 ppt as the optimum for the juvenile. Jiaxin (2003) pointed out that the salinity for juvenile *Apostichopus japonuicus* ranged from 27 to 35ppt. Thierry *et al.* (2009) reported mortality of *H. scabra* juveniles reared at 20 ppt salinity after 22 days. Pitt *et al*. (2004) and Pitt and Duy (2004) pointed out that the holothurians could live in water with salinity of around 20 ppt for several weeks and would probably survive for shorter periods at lower salinities. Mercier *et al.* (1999) observed decrease in salinity from 35 to 30 ppt, 25 and 20 ppt induced the burrowing of all juveniles within minutes and acclimatization occurred most rapidly at 30 ppt salinity and was slowest at 20 ppt. Liu *et al*. (2004) indicated that salinity tolerance varied with respect to the size of juvenile holothurians.

A high degree of significance (p<0.05) was observed in the mean growth rate of juveniles held at different salinities (Table 6). In the pair- wise comparison except between salinities 15 and 30, 20 and 30, 25 and 30, 15 and 33, 15 and 35, 30 and 38, 30 and 40, 33 and 40, 35 and 40 and 38 and 40, no significant difference was observed in growth rate in length among different treatments. Similarly, except between 20 and 30, 25 and 40, 30 and 40, 33 and 40, 38 and 40 ppt, no significant difference was noticed in the growth rate of juveniles in weight among treatments (Table 6).

In the present study, the highest hatching rate of fertilized eggs, highest larval survival, growth rate and fastest development of auricularia of *H. scabra* were observed at salinity 35 ppt, whereas the highest growth rate in length and weight of the

Table 6. ANOVA on growth rate in length of *Holothuria scabra* juveniles at different salinities with results of Least Significant Difference (LSD) post-hoc multiple comparisons test

Treatments			Sum of squares	*df*	Mean Square	*F* ratio	
Between groups			0.136	7	194	6.88	
Within groups			0.4970	16	3110		
Total			0.186	23			
LSD	20	25	30	33	35	38	40
15	>0.05	>0.05	<0.001	<0.01	<0.01	>0.05	>0.05
20		>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
25			<0.01	>0.05	>0.05	>0.05	>0.05
30				>0.05	>0.05	<0.01	<0.001
33					>0.05	>0.05	<0.01
35						>0.05	<0.05
38							<0.05

juveniles was obtained at 30 ppt. It is inferred that one month old juveniles of *H. scabra* could tolerate lower salinity much effectively than fertilized eggs and larvae.

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References

Agudo, G. 2006. *Sandfish Hatchery Techniques*. Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and the WorldFish Center, Noumea, 44 pp.

Asha, P. S. 2004. Effect of feed concentration on larval growth, survival and development of *Holothuria* (*Theelothuria*) *spinifera* Theel. *J. Mar. Biol. Ass. India*, 46: 80-86.

Asha, P. S. and P. Muthiah. 2002. Spawning and larval rearing of sea cucumber *Holothuria* (*Theelothuria*) *spinifera. SPC Beche-de-mer Inform. Bull.*, 16: 11-15.

Asha, P. S. and P. Muthiah. 2005. Effect of temperature, salinity and pH on the larval growth, survival and development of the sea cucumber *Holothuria spinifera* Theel. *Aquaculture*, 250: 823-829.

Asha, P. S. and P. Muthiah. 2006. Effect of single and combined microalgae on the larval growth, survival and development of the commercial sea cucumber *Holothuria spinifera* Theel. *Aquacult. Res.*, 37: 113-118.

Asha, P. S. and P. Muthiah. 2007. Growth of the hatchery produced juveniles of the sea cucumber *Holothuria spinifera* Theel. *Aquacult. Res.*, 38: 1082-1088.

Asha, P. S., M. Rajagopalan and K. Diwakar. 2006. Effect of sea weed, sea grass and powdered algae in rearing the hatchery produced juveniles of *Holothuria* (Metriatyla) *scabra* jaeger. *Proceedings on National Symposium on Recent Trends in Fisheries Education and Research,* FCRI, Tuticorin, p. 79-85.

Asha, P. S. and K. Diwakar. 2007. Recent trends in the hydrobiology of coastal and inshore waters off Tuticorin in the Gulf of Mannar, India. *J. Mar. Biol. Ass. India*, 49: 7-11.

Asha, P. S. and P. Muthiah. 2008. Reproductive biology of the commercial sea cucumber *Holothuria spinifera*. Theel. *Aquacult. Int.*, 16: 231-242.

Asha, P. S., K. K. Joshi and K. Diwakar. 2009. Incidence of fish mortality in Tuticorin bay, Gulf of Mannar. *J. Mar. Biol. Ass. India*, 51: 173-177.

Battaglene, S. C. 1999. Culture of tropical sea cucumbers for stock restoration and enhancement. *Naga*, 22: 4-11.

Chellaram, C., V. D. Samuel and J. K. Patterson Edward. 2003. Status of echinoderm fishery in the Gulf of Mannar south east coast of India, *SDMRI Research Publication*, 3: 173-176.

Chen, C. P. and C. S. Chian. 1990. Short note on the larval development of the sea cucumber *Actinopyga echinites* (Holothuroidea: Echinodermata). *Bull. Inst. Zool. Academia Sinica*, 29: 127-133.

Giraspy, D. A. B. and G. Ivy. 2005. Australia's first commercial sea cucumber culture and sea ranching project in Harvey Bay, Queensland, Australia. *SPC Beche-de-mer Inform. Bull.*, 21: 29-31.

Giraspy, D. A. B. and G. Ivy. 2008. The influence of commercial diets on growth and survival in the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand). *SPC Beche-de-mer Inform. Bull.*, 28: 46-52.

Hamel, J. F., C. Conand, D. Pawson and A. Mercier. 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and its exploitation as beche-de-mer. *Adv. Mar. Biol.*, 41: 129-223.

Harder, W. 1968. Reactions of plankton organisms to water stratification. *Limnol. Oceanogr.*, 13: 156-168.

James, D. B., M. E. Rajapandian., B. K. Baskar and C. P. Gopinathan. 1988. Successful induced spawning and rearing of the holothurian *Holothuria* (Metriatyla) *scabra* Jaeger at Tuticorin. *Mar. Fish. Infor. Serv. T & E Ser.*, 87: 30-33.

Jiaxin, C. 2003. Overview of sea cucumber farming and sea ranching practices in China. *SPC Beche-de-mer Inform. Bull.*, 18: 18-23.

Kashenko, S. D. 2002. Reactions of the larvae of the sea cucumber *Apostichopus japonicus* to sharp desalination of surface water. A laboratory study. *SPC Beche-de-mer Inform. Bull.*, 16: 11-15.

Liu, X., G. Zhu., Q. Zhao., L. Wang and B. Gu. 2004. Studies on hatchery techniques of the sea cucumber, *Apostichopus japonicus*. *In:* A. Lovatelli, C. Purcell., S. Uthicke, J. F. Hamel, A. Mercier (Eds.) *Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Technical Paper 463*, FAO, United Nations, Rome, p. 287-295.

Mercier, A., S. C. Battaglene and J.F. Hamel. 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *J. Exp. Mar. Biol. Ecol.*, 23: 55-69.

Morgan, A. D. 2000. Induction of spawning in the sea cucumber *Holothuria scabra* (Echinodermata: holothuroidea). *J. World Aquacult. Soc.*, 31: 186-194.

Pitt, R., 2001. Preliminary sandfish growth trials in tanks, ponds and pens in Vietnam. *SPC Beche-de-mer Inform. Bull.*, 15: 17-27.

Pitt, R. and N. D. Q. Duy. 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Vietnam. *In:* A. Lovatelli, C. Purcell, S. Uthicke, J. F. Hamel, A. Mercier (Eds.) *Advances in Sea cucumber Aquaculture and Management. FAO Fisheries Technical Paper 463*. FAO United Nations, Rome, p. 333-346.

Pitt, R., N. D. Q. Duy, T. V. Duy and H. T. C. Long. 2004. Sandfish (*Holothuria scabra*) with shrimp (*Penaeus monodon*) co-culture in tanks. *SPC Beche-de-mer Inform. Bull.*, 20: 12-22.

Thierry, L., R. Richard, J. Michel and E. Igor. 2009. Problems related to the farming of *Holothuria scabra* (Jaeger, 1833). *SPC Beche-de-mer Inform. Bull.*, 29: 20-30.

Wang, R. and Y. Chen. 2004. Breeding and culture of the sea cucumber, *Apostichopus* japonicus, Liao. *In*: A. Lovatelli, C. Purcell, S. Uthicke, J. F. Hamel, A. Mercier. (Eds.), *Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Technical Paper 463,* FAO United Nations, Rome, p. 277-286.

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