

①  
S/A  
✓  
3/14

## Preliminary study on broodstock rearing, induced breeding and grow-out culture of the sea cucumber *Holothuria scabra* in Sri Lanka

Ajith Kumara<sup>1</sup> & Chamari Dissanayake<sup>1,2</sup>

<sup>1</sup>National Aquatic Resources Research and Development Agency (NARA), Colombo 15, Sri Lanka

<sup>2</sup>Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

**Correspondence:** C Dissanayake, Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.  
E-mails: chamari@sjp.ac.lk; chamld4@gmail.com

### Abstract

Experiments were conducted to identify suitable methods for broodstock rearing, induced breeding and grow-out culture of *Holothuria scabra* in Sri Lanka. Two hundred and seventy-two brooders (500–600 g) collected from off Mannar were individually packed in oxygen-filled polythene bags with and without sea water and transported to a sea cucumber hatchery at Kalpitiya. Lagoon pens, sand-filled fibreglass tanks and bare tanks were used in triplicates to maintain brooders. Spawning was initiated using air dry, water jet and thermal-stimulation methods. Hatchery produced juveniles with an average weight of  $11 \pm 5$  g were reared (2 individuals  $m^{-2}$ ) in lagoon pens, mud ponds and fibreglass tanks in triplicates. The significantly high evisceration rate was observed when brooders were transported without sea water (*t*-test,  $P < 0.05$ ). Brooders maintained in bare tanks showed a significant weight reduction than the brooders in sand-filled tanks and lagoon pens (ANOVA,  $P < 0.05$ , d.f. = 2). Thermal stimulation (ambient temperature  $\pm 3$ – $5^\circ\text{C}$ ) was found to be the most successful method of spawning initiation of *H. scabra*. The mean ( $\pm$ SD) percentage males and females participated for spawning per trial was  $9.2 (\pm 10)$  and  $4.6 (\pm 5.6)$  respectively. On an average, 1.16 millions of eggs ( $\pm 1.03$  SD,  $n = 5$ ) were obtained per spawning trial. *H. scabra* juveniles reared in tanks showed significantly lower growth rate than the juveniles in pens and ponds (ANOVA,  $P < 0.05$ ). Lagoon pens and sand-filled tanks are suitable to maintain brooders and lagoon pens can be successfully used for mass rearing of juveniles.

**Keywords:** *Holothuria scabra*, broodstock, spawning initiation, lagoon pens, Sri Lanka

### Introduction

Increasing market demand for bêche-de-mer which is one of the luxury seafood commodities in Asia, has led to overexploitation of wild sea cucumber stocks throughout the world (Conand 2001; Hamel, Conand, Pawson & Mercier 2001; Ferdouse 2004; Purcell 2010; Purcell, Cathy & David 2012). Although more than 50 tropical sea cucumber species are commercially exploited and traded, population densities of high-value sea cucumber species are reported to be extremely low in many parts of the world (Conand 2004; Choo 2008; Purcell *et al.* 2012). This is particularly true for sandfish, *Holothuria scabra*; the most valuable sea cucumber species exploited in the tropical areas (Purcell 2010; Purcell *et al.* 2012).

*Holothuria scabra* are found from northern Africa to the central Pacific. They are vulnerable to heavy fishing as they mainly inhabit seagrass beds and other easily accessible inshore habitats (Conand, De San, Refeno, Razafintseho, Mara & Andriajatovo 1998; Ferdouse 1999). It has been recorded that population densities of *H. scabra* have reduced to critically low levels ( $<1$  individual  $ha^{-1}$ ) in many parts of the world, including Milne Bay Province, Papua New Guinea (To & Shea 2012), the Red Sea (Clarke 2004) and Sri Lanka (Dissanayake & Stefansson 2012). Previous studies have shown that it can take more than 50 years to recover heavily exploited sea cucumber stocks (Preston 1993; Hasan 2005; Friedman, Eriksson, Tardy & Pakoa 2011).

Depletion of stocks and increasing demand for the processed product have triggered the interest to develop aquaculture techniques for *H. scabra* as it may in turn to reduce fishing pressure on wild stocks and enhance the commercial production (James, Rajapandian, Gopinathan & Baskar 1994; Battaglione 1999; Hamel *et al.* 2001; Pitt & Duy 2003; Mercier, Ycaza & Hamel 2004; Giraspy & Ivy 2005; Purcell *et al.* 2012). Hatchery production of *H. scabra* was first started in India in 1988 (James 2004) and subsequently carried out by various countries across the globe, including the Solomon Islands (Battaglione *et al.* 1999; Battaglione *et al.* 1996), Australia (Agudo 2006, 2012; Ivy & Giraspy 2006), New Caledonia (Bell, Agudo, Purcell, Blazer, Simutoga, Phamb & Della 2007), Vietnam (Pitt, Thu, Minh & Phuc 2001; Pitt, Duy, Du & Long 2004; Duy 2010, 2012), the Philippines (Gamboa & Juinio-Meñez 2003; Olavides, Rodriguez & Juinio-Meñez 2011; Juinio-Meñez, Peralta, Dumalan, Edullantes & Catbagan 2012; Juinio-Meñez, Evangelio & Miralao 2014), Madagascar (Lavitra, Rasolofonirina, Jangoux & Eeckhaut 2009; Eeckhaut, Lavitra, Léonet, Jangoux & Rasolofonirina 2012), Fiji (Hair, Kaure, Southgate & Pickering 2011), Iran (Dabbagh & Sedaghat 2012), Maldives (Azari & Walsalam 2012) and Saudi Arabia (Hasan 2009).

In Sri Lanka, commercial sea cucumber culture activities have not been attempted so far, except the growing of wild collected *H. scabra* juveniles in sea pens to a marketable size. Although the grow-out culture of wild collected *H. scabra* to a larger size (~500 g) in sea pens is a useful practice to restore damaged fisheries (Bell, Purcell & Nash 2008), this practice created huge conflicts among sea cucumber resource users in Sri Lanka. Therefore, the fattening of wild collected small sea cucumbers was banned in 2011 and artificial breeding and larval rearing of *H. scabra* were initiated by the National Aquatic Resources Research and Development Agency (NARA) of Sri Lanka during that period.

The aims of this study were in threefold. Firstly, to identify a suitable method/s for broodstock transportation and broodstock rearing. Secondly, to identify suitable local conditions for artificial breeding and larval rearing of *H. scabra* using established methodologies. Finally, to identify suitable grow-out culture method/s for mass rearing of hatchery produced *H. scabra* juveniles and understand the factors influencing their survival and growth.

## Materials and methods

### Broodstock collection and transportation

A total of 272 brooders were collected from off Mannar through skin diving from August 2011 to September 2012. These brooders were collected and transported as six batches and each batch consisted of  $45 \pm 6$  individuals. Healthy specimens with weight ranges from 500 to 600 g were selected as preferred brooders and care was taken not to select damaged or eviscerated individuals.

Collected brooders were immediately transported to the sea cucumber hatchery at NARA Regional Research Centre, Kalpitiya. Two different packing methods were used to transport brooders; (1) packed individually in oxygen-filled double layered polythene bags without sea water (2) packed individually in oxygen-filled double layered polythene bags containing 1 L of sea water. Each bag was sealed and 4–6 bags were packed into an insulated rigid foam box. Brooders were only transported at night and the average transportation time was around 5 h. During transportation, temperature was maintained within the range of 26–27°C by placing ice bags or wet papers among the polythene bags. Each bag was refilled with oxygen in 2-h time interval. Upon arrival to the hatchery, number of eviscerated individuals were recorded with respect to the packing method.

### Broodstock maintenance

In order to investigate a suitable method/s for broodstock rearing, wild collected brooders were maintained under three different rearing systems namely; sand-filled fibreglass tanks, bare fibreglass tanks (without sand layer) and lagoon pens for a period of 6 weeks.

Six fibreglass tanks, each with 4 tonne capacity and ~25.0 m<sup>2</sup> bottom area were filled with lagoon water. A layer of sand (10–15 cm thick) was added to three fibreglass tanks (sand-filled tanks) before adding water and the rest were kept without adding sand (bare tanks) and these tanks were maintained inside the hatchery. Three lagoon pens each with 5 m × 5 m (25.0 m<sup>2</sup>) were constructed at Puttlam lagoon next to the hatchery premises. These three rearing systems were used to rear brooders.

Thirty brooders with an average weight (mean ± SD) of  $558 \pm 86$  g were maintained in

bare tanks and another thirty brooders ( $555 \pm 92$  g) were maintained in sand-filled tanks. Ten brooders were placed into each tank and three replicates were maintained for each rearing system. These brooders were fed two times per day with grounded shrimp feed and pasted eel grass. Commercial shrimp feed (starter 3) purchased from the Ceylon Grain Elevators PLC., Colombo 15, Sri Lanka was grounded and equally mixed with pasted eel grass and evenly scattered on to the bottom of each tank at a rate of  $50 \text{ g day}^{-1}$  (Agudo 2006). The decaying dark coloured eel grass leaves and muddy sediments collected from Puttlam lagoon were used to prepare the pasted eel grass. Both decaying eel grass leaves and sediments were dried at  $65^\circ\text{C}$ , ground and mixed with water to form a paste. Brooders in the hatchery tanks were maintained at ambient temperature and one-third volume of water was exchanged every day to maintain good water quality. Excess food and faecal matter were siphoned out daily. Weight of each individual was measured at 2-week intervals. Prior to weighing, the animals were taken out of the water, allowed to drain approximately 2 min and blotted dry (Battaglene 1999). Average weight increment of brooders reared in each tank was determined from the difference between the average weight at the end and the start of the experiment.

Thirty brooders with an average weight of  $553 \pm 91$  were equally placed into three lagoon pens and weight of each individual was taken every 2 weeks. These brooders were not fed any external source of food and weight increment per pen was determined.

#### Spawning initiation

Spawning initiations were attempted using three different methods namely; thermal stimulation, administering a powerful jet of water and dry treatment (Agudo 2006). When giving thermal stimulation, water temperature was increased  $3\text{--}5^\circ\text{C}$  by adding warm sea water into a spawning tank and this temperature was maintained within the system. *H. scabra* brooders were introduced into the spawning tank and they were kept around 45–60 min at that temperature. Then the water in the spawning tank was replaced with fresh sea water at ambient temperature. When ambient temperature of sea water was higher than  $32^\circ\text{C}$ , a cold shock was used for spawning

initiation. During the cold shock treatment, ice bags were used to lower the water temperature in a spawning tank. In some instances, a combination of cold and heat shocks were used to initiate spawning.

When applying a powerful jet of water, brooders were air-dried for about 45 min and subjected to a powerful jet of sea water for a period of 15–20 min. Then they were returned to a spawning tank where water was at ambient temperature. In dry treatment method, brooders were kept completely dry for about 45–60 min in a tank and the tank was refilled with fresh sea water at ambient temperature. All these breeding trials were carried out in a full moon day or 2–3 days later.

Number of males and females spawned during each trial as well as the time duration between releasing of gametes by first male and female were recorded. Fertilized eggs were collected into an 80- $\mu\text{m}$  sieve and washed with filtered sea water to remove excess sperms. Then the eggs were introduced into a 10-L plastic bucket containing filtered sea water and stirred well to distribute the eggs uniformly. Three sub samples, each with 1 mL was taken from the bucket and egg density ( $\text{nos mL}^{-1}$ ) of each sample was estimated using a counting chamber. The average egg density was used to estimate the total number of eggs produced in each spawning trial. Then, the fertilized eggs were introduced into 1000-L capacity fibreglass tanks at a rate of 1 egg per mL. Ultraviolet-sterilized sea water was used in larval rearing tanks and seawater conditions were maintained as; pH 7–8; salinity 30–32 ppt; dissolved oxygen above  $6.0 \text{ mg L}^{-1}$ . Development stages of eggs and the survival percentage of larvae in each development stage were monitored and recorded continuously. Larvae were fed 2 days after hatching and they were fed two times per day with *Chaetoceros* spp. ( $20\,000\text{--}40\,000 \text{ cells mL}^{-1}$ ). A volume of 30% of water was changed daily in larval rearing tanks. Eight to 10 days after hatching, larvae were fed with a mixture of Algamac 2000 ( $0.1 \text{ g m}^{-3} \text{ day}^{-1}$ ), fine paste of sieved ( $50 \mu\text{m}$ ) Sargassum and grounded fine grade shrimp strata ( $0.5 \text{ g m}^{-3} \text{ day}^{-1}$ ). Plastic sheets (PVC) were introduced into the larval rearing tanks by 16–17 days culture period. After 1 month, *H. scabra* juveniles were transferred into indoor nursery tanks and reared until they reached 1 g in weight ( $\sim 3 \text{ cm}$ ). Then they were nursed in sand-filled outdoor fibreglass tanks (4 tonne capacity).

### Grow-out culture trials

Three systems; lagoon pens, fibreglass tanks and mud ponds were used to rear hatchery produced *H. scabra* juveniles. Pens each with 25 m × 20 m were constructed at Puttlam lagoon close to the NARA Regional Research Centre, Kalpitiya and existing mud ponds (30 m × 20 m) and flat bottom fibreglass tanks each with 4 tonne capacity (25 m<sup>2</sup> bottom area) were used as other two rearing systems. Mud ponds have been constructed to be filled with lagoon water through tidal fluctuations and lagoon water was directly pumped to the fibreglass tanks that were used to rear *H. scabra* juveniles. Three replicates were maintained for each grow-out culture system and hatchery produced *H. scabra* juveniles with an average weight of 11 ± 5 g were stocked (2 individuals m<sup>-2</sup>) into each rearing facility in early December 2011. Before stocking, a thick layer of sand collected from the lagoon pen area was added into each fibreglass tank. Sand in lagoon pens and mud ponds, mainly consisted of coarse sand ( $\phi = 1.32$ ) and average particle size included 79.9% of coarse sand (1 mm), 16.3% of medium sand (~0.5 mm) and 2.6% of fine sand (~ 0.25 mm). The percentage organic content of sand and mud samples collected from the lagoon pen area was 1.69 and 5.36 respectively. Flow through system was maintained in fibreglass tanks throughout the culture cycle.

Stocked individuals were not fed with artificial feeds and their foods were limited to naturally occurring detritus materials contained in the sand. As naturally occurring detritus materials were limited to tanks reared individuals, detritus rich mud collected from the lagoon pen area was added into the fibreglass tanks at 2-day intervals.

Weight of *H. scabra* juveniles that were reared in three different culture systems was measured at monthly interval while water quality parameters were measured biweekly. Percentage survival of juveniles in each system was measured during as well as at the end of culture cycle.

### Statistical analysis

All the statistical analysis tests were performed using R version 2.8.1 (R Development Core Team 2009 <http://www.rproject.org>). Prior to analysis, data were examined for homogeneity of variances (*F* test). Differences were considered significant at a probability level of 0.05.

## Results

### Broodstock transportation and maintenance

The mean (±SD) percentage evisceration rate of *H. scabra* brooders that were transported without sea water was found to be 36.5% (±4.1). However, only 5.1% (±4.6) of brooders eviscerated when they were transported in oxygenated sea water. Statistical analysis proved that significantly high rate of evisceration can be occurred when *H. scabra* brooders were transported without sea water (*t*-test, *P* < 0.05, *n* = 5).

Average weight (± SD) of brooders that were reared in sand-filled tanks, bare tanks and lagoon pens were compared. At the end of the 6-week rearing period, slight increase in the average weight of brooders reared in sand-filled tanks (561 ± 80 g) and lagoon pens (560 ± 74 g) were observed. However, the average weight of brooders in bare tanks was reported as 461 (± 79 g). There was a significant weight reduction in *H. scabra* brooders that were reared in bare tanks than the brooders in sand-filled tanks and lagoon pens (ANOVA, *P* < 0.05, d.f. = 2).

### Spawning and larval survival

Among the three methods used for initiation of spawning of *H. scabra*, thermal stimulation (ambient temperature ± 3–5°C) was found to be the most successful method. A combination of both cold and heat shocks was more effective than applying cold or heat shock alone (Table 1). None of the brooders responded for dry method and a male released sperms in response to water jet method in one trial.

Eight breeding trials were carried out using thermal stimulation method and five were successful. Around 0.72, 0.38, 0.24, 1.88 and 2.60 million of eggs were produced in first, second, third, fifth and eight trials respectively. Only males released gametes in fourth and sixth trials, but none was induced to spawn in trial seven. The average (±SD) percentage of males participated for spawning per trial was 9.2 (±10.0, *n* = 23) and for females this value was 4.6 (±5.6, *n* = 12). On an average, 1.16 million of eggs (±1.03, *n* = 5) were obtained per spawning trial (Table 1).

During larval development, the early auricularia stage was observed 2 days after spawning and late auricularia were found in between 5 and 6 days.

**Table 1** Information on spawning trials of *H. scabra* including number of brooders used, method of spawning initiation, percentage males and females participated for spawning, number of eggs produced and percentage survival after 30 days of hatching

Trial No	Date	Number of brooders	Method used for spawning induction	Conditions used during spawning induction			Spawning		No. of eggs produced (million)	Time between first spawning male & female (minutes)	% survival after 30 days of hatching
				Ambient (°C)	± change during thermal stimulation (°C)	Period (minutes)	% Males	% Females			
1	11.10.2011	36	Thermal	29	-3 & +3	60 & 50	7	7	0.72	40	1.7
	11.10.2011	32	Water jet	29	-	15	0	0	-	-	-
	11.02.2011	33	Air dry	29	-	40	0	0	-	-	-
2	16.12.2011	32	Thermal	24	+5	50	8	4	0.38	55	2.1
	16.12.2011	32	Water jet	24	-	20	33	0	-	-	-
	16.12.2011	32	Air dry	24	-	45	0	0	-	-	-
3	12.01.2012	34	Thermal	28	+4	60	0	4	0.24	42	0.9
	12.01.2012	32	Water jet	28	-	-	-	-	-	-	-
	12.01.2012	34	Air dry	28	-	-	-	-	-	-	-
4	08.02.2012	36	Thermal	29	-4 & +3	60 & 60	11	0	-	-	-
	08.02.2012	36	Water jet	29	-	15	3	0	-	-	-
	08.02.2012	36	Air dry	29	-	45	0	0	-	-	-
5	07.03.2012	36	Thermal	31	-3 & +4	45 & 60	26	10	1.88	50	3.4
6	08.04.2012	30	Thermal	32	-3	60	6	0	-	-	-
7	29.09.2012	28	Thermal	33	-4	50	0	0	-	-	-
8	27.11.2012	28	Thermal	32	+4	60	25	16	2.6	45	5.2

This stage remained till 10–13 days and they became doliolaria within day 14–16 of their life cycle. Pentactula larvae were observed in day 16 and they became early juveniles around day 30.

Percentage larval survival after 30 days of hatching ranged from 0.9% to 5.2% (Table 1). Gradual improvement of percentage larval survival could be observed with time except during January.

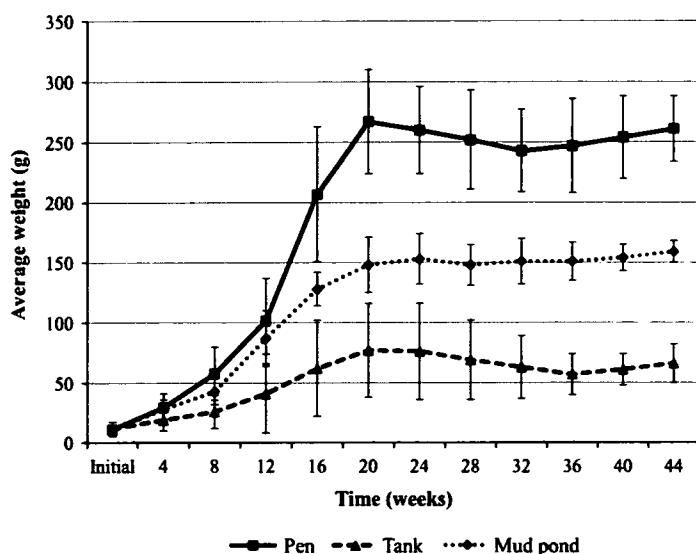
#### Grow-out culture trials

In all the culture systems, average weight ( $\pm$ SD) of cultured juveniles increased up to 20 weeks (from December to May) and showed slight weight fluctuations thereafter. *H. scabra* juveniles reared in pens had the highest average weight ( $267 \pm 47$  g) in 20 weeks culture period while individuals reared in fibreglass tanks ( $77 \pm 23$  g) and mud ponds ( $153 \pm 40$  g) gained the highest average weight within 20 and 44 weeks respectively (Fig. 1). The reported maximum weight of *H. scabra* juveniles reared in pens, fibreglass tanks and mud ponds were 456.7 g, 108.3 g and 218.4 g respectively. In all three culture systems, maximum weight was reported in between 20 and 24 weeks rearing period. At the end of the 1-year culture period, significantly higher average weight was reported for *H. scabra* juveniles reared in pens ( $182.7 \pm 101.0$  g) than the juveniles reared in mud ponds ( $113.4 \pm 55.7$  g) and fibreglass tanks ( $52.3 \pm 22.5$  g) (ANOVA,  $P < 0.05$ , d.f. = 2). The highest sea cucumber density (in terms of weight,  $\text{g m}^{-2}$ ) was

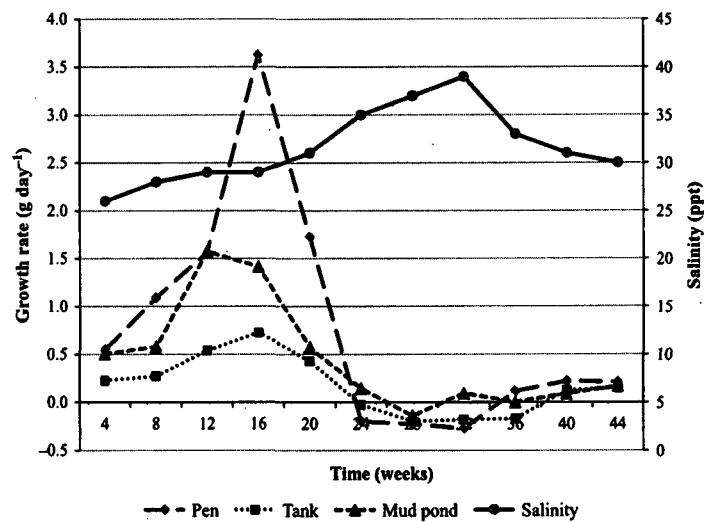
observed for juveniles reared in pens ( $534 \text{ g m}^{-2}$ ) and tanks ( $154 \text{ g m}^{-2}$ ) in 16 weeks and it took 20 weeks to reach the highest density in mud ponds ( $306 \text{ g m}^{-2}$ ).

There were differences in growth rate ( $\text{g day}^{-1}$ ) of *H. scabra* juveniles with respect to grow-out culture systems and time (Fig. 2). *H. scabra* juveniles that were cultured in pens ( $3.6 \text{ g day}^{-1}$ ) and tanks ( $0.7 \text{ g day}^{-1}$ ) reported the highest growth rate in 16 weeks, but juveniles reared in mud ponds showed the highest growth rate in 12 weeks ( $1.6 \text{ g day}^{-1}$ ). Negative or very low growth rates were observed from 24 to 36 weeks in all three culture systems. Statistical analysis proved that, there were significant differences in growth rate within each culture system with respect to time (ANOVA,  $P < 0.05$ , d.f. = 11). At the end of 1-year culture period, *H. scabra* cultured in pens showed an average growth rate of  $0.75 \text{ g day}^{-1}$  while individuals cultured in fibreglass tanks and mud ponds resulted  $0.15 \text{ g day}^{-1}$ ,  $0.46 \text{ g day}^{-1}$  respectively. Further, *H. scabra* juveniles cultured in fibreglass tanks reported significantly lower growth rate than the juveniles in lagoon pens and mud ponds (ANOVA,  $P < 0.05$ , d.f. = 2). At the end of culture cycle, significantly lower survival rate was reported for mud ponds reared individuals (3%) than the individuals reared in tanks (95%) and lagoon pens (83%, Fig. 3) (ANOVA,  $P < 0.05$ , d.f. = 2).

A sudden drop of pond water level was observed in 28 weeks and as a result, Dissolved Oxygen (DO) level decreased to  $4.2 \text{ mg L}^{-1}$  and pond



**Figure 1** Fluctuations of average weight ( $\pm$  SD) of *H. scabra* reared in three different grow-out systems at Kalpitiya, Sri Lanka.



**Figure 2** Fluctuations of average salinity (ppt) in lagoon water and average growth rate of *H. scabra* reared in three different grow-out systems at Kalpitiya, Sri Lanka.

water salinity increased up to 42 ppt. During that period, a high percentage (~81%) of eviscerated and dead animals was observed along the banks of mud ponds. However, pond water level and salinity were taken back to normal immediately by pumping lagoon water into the ponds.

The average salinity of culture sites ranged from 26‰ to 39‰ during the culture period and reached to a peak at 32 weeks (Fig. 2). As lagoon was the water source for all three culture systems, salinity of three culture systems remained the same except in mud ponds during few days of 28 weeks. Dissolved Oxygen (DO) in the pen and tank culture systems were within the range of 7.2–9.1 ppm, while in mud ponds DO levels varied from 4.2 to 7.8 ppm. The monthly average water temperature in three culture systems fluctuated from  $25.6 \pm 0.2^\circ\text{C}$  to  $35.2 \pm 0.5^\circ\text{C}$ .

## Discussion

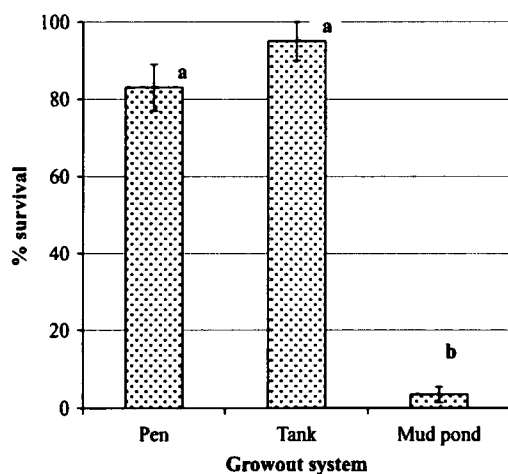
It has been established from previous studies that *H. scabra* attain sexual maturity within the size range of 15–18 cm in the tropical region (Long & Skewes 1997; Battaglione & Bell 1998). Hence, individuals larger than 22 cm (500–600 g) were selected as preferred brooders to make sure that all individuals were sexually mature.

Many holothurian species eviscerate in response to adverse environmental conditions as well as handling stress (Battaglione, Seymour, Ramofafia & Lane 2002). It has been reported that *H. scabra* can live without water for some time period and they can tolerate low dissolved oxygen and high

temperatures for a long time (Mercier, Battaglione & Hamel 1999; Agudo 2006). Thus, the significantly high evisceration rate observed in *H. scabra* brooders that were transported without sea water is inexplicable. As brooders were transported under controlled temperature, high temperature fluctuations could not be expected during transportation. However, *H. scabra* brooders that were transported without sea water were out of water more than 5–6 h and probably they were under stress than the brooders who were transported with sea water. Further, they could be stressed due to rough handling during selection, packing and transportation. Some brooders eviscerated even in the sea water medium and this may be a result of fluctuations of pH due to accumulation of excretory products and the stress mediated by rough handling (Sanni & Forsberg 1996).

Most commonly, sea cucumber broodstocks are reared and conditioned in ponds, pens or tanks prior to induce spawning in hatcheries (Morgan 2000; Agudo 2006). Sometimes they spawn spontaneously in response to the collection and transportation stress (Tanaka 1958; Smiley *et al.* 1991; Yanagisawa 1998; Morgan 2000; Eeckhaut *et al.* 2012). However, in this study, induced spawning due to transportation stress was not evident.

During this study, wild collected brooders were maintained under three different rearing conditions. Most of holothuroids including *H. scabra* are deposit feeders and they normally take food by swallowing the top layer of their surrounding substrate or ingest materials on the surface of the substrate (Roberts, Gebruk, Levin & Manship 2000).



**Figure 3** Percentage survival ( $\pm$  SD) of *H. scabra* reared in lagoon pens, fibreglass tanks and mud ponds at the end of the 1-year culture cycle at Kalpitiya, Sri Lanka (ANOVA,  $P < 0.05$ ).

Brooders in the bare tanks have to obtain their food by ingesting materials on the bottom of the tank, but others were able to swallow the sand substrate to gain required food. Previous studies have shown that sand provides not only a potential source of foods but also shelter from adverse environmental conditions and predation (Wiedemeyer 1992; Battaglione, Seymour & Ramofafia 1999; Mercier, Battaglione & Hamel 2000). Further, *H. scabra* can perform their usual daily burrowing cycles when they are reared in sandy bottom (Battaglione *et al.* 1999). Hence, the observed significant weight reduction in brooders that were reared in the bare tanks could be due to interruption of their usual feeding habit and changes in daily behavioural pattern. The findings of this study revealed that both lagoon pens and sand-filled indoor fibreglass tanks can be successfully used to maintain *H. scabra* brooders prior to initiation of spawning. However, several problems such as sea cucumber poaching and conflicts with other resource users are reported to be very common when sea cucumbers are reared in lagoon pens. In such circumstances, indoor fibreglass tanks filled with sand can be used to rear *H. scabra* brooders as they did not show significant weight reduction under rearing condition.

Thermal stress is a well-known practice used to stimulate spawning of sea cucumbers (James, Rajapandian, Baskar & Gopinathan 1988; Battaglione *et al.* 1999, 2002; Morgan 2000; Giraspy & Ivy 2005; Eeckhaut *et al.* 2012) and this method

was successful in spawning initiation of *H. scabra* in the present study. In most occasions, both male and female released their gametes in response to thermal stress, however, males were found to be more responsive to thermal shock than females. In some instances small proportion of male sandfish spawned, but no females did. Although, Battaglione (1999) reported that, *H. scabra* could be induced to spawn throughout the year in tropical region by increasing water temperature by 2–4°C, none of the brooders responded to thermal shock during February, April and September. Some studies have highlighted that spawning of *H. scabra* can be seasonally limited even in the tropical waters. For example, in Vietnam, broodstock could be induced to spawn year round, but egg yields have been shown to be best from December to April (Pitt *et al.* 2004). According to James (2004), successful spawning of *H. scabra* was obtained from October to March in the Gulf of Mannar in India. As we collected brooders off Mannar in Sri Lanka, probably they may have similar reproductive seasonality recorded by James (2004). Hence, observed unsuccessful breeding trials in April and September could be due to this spawning seasonality. According to Battaglione *et al.* (2002), spawning initiation in *H. scabra* is easiest during the dry season and hardest during the monsoon season. The present study also supported for these findings, as most of the successful breeding trials were evident during the non-monsoon period (October to March) while the failures were during the monsoon period (April to September). However, spawning seasonality was concluded based on limited experimental trials carried out in this study and warrants further spawning trials to confirm this observation.

A few number of males and females spawned during each breeding trial. This may be due to several reasons such as the use of a small number of brooders, difficulties in proper conditioning under captivity, lack of understanding of sex and gonad maturity stages of brooders. Countries like Japan use more than 200 brooders to ensure successful spawning (Yanagisawa 1998). However, maintenance of such a large number of brooders may not be possible in most hatcheries particularly, in developing nations. Further it will be very costly. Proper broodstock conditioning, determination of sex and maturity stage of each individual before initiating spawning would be useful to achieve more successful results in the future.



**Table 2** Average growth rate of *H. scabra* reared in different grow-out systems in different localities

Country	Types of grow-out system	Average growth rate	Reference
Australia	Pond	0.33 g day <sup>-1</sup> (~100 g after 10 months culture period)	Purcell <i>et al.</i> (2012)
India	Pond	1.44 g day <sup>-1</sup> (~270 g after 6 months culture period)	James (1999); Purcell <i>et al.</i> (2012)
Iran	Pond	0.06 g day <sup>-1</sup>	Dabbagh and Sedaghat (2012)
New Caledonia	Pond	0.6–0.8 g day <sup>-1</sup>	Agudo (2012); Bell <i>et al.</i> (2007)
Vietnam	Pond	2.2–3.2 g day <sup>-1</sup>	Agudo (2012); Pitt <i>et al.</i> (2004)
Fiji	Pen	0.5 g day <sup>-1</sup> (~160 g after 11 months culture period)	Hair (2012)
Philippines	Pen	1.7 g day <sup>-1</sup> (~200 g after 4 months culture period)	Gamboa, Aurelio, Ganad, Concepcion and Abreo (2012) Purcell <i>et al.</i> (2012)
Madagascar	Pen	1.9 g day <sup>-1</sup> (~405 g after 7 months culture period)	Robinson and Pascal (2012)
New Caledonia	Pen	0.4–0.7 g day <sup>-1</sup>	Purcell and Simutoga (2008)
Vietnam	Pen	1.7 g day <sup>-1</sup>	Pitt <i>et al.</i> (2004)
Southwestern Madagascar	Pens	1.8 g day <sup>-1</sup>	Tsiresy, Pascal and Plotieau (2011)
Solomon Islands	Pens	0.7 g day <sup>-1</sup>	Battaglione (1999)

Tropical sea cucumbers are often highly fecund and absolute fecundity of *H. scabra* was estimated to be within the range from 9 to 17 million eggs (Conand, 1988). However, female spawns a fraction of mature gametes when they are induced (Battaglione *et al.* 2002). This may be the possible reason for an observed low number of eggs during each spawning trial. All the breeding trials were carried out during or slightly later in the full moon period as a number of studies have reported that *H. scabra* can be successfully induced to spawn around the full moon phase (McEuen 1988; Babcock, Mundy, Keesing & Oliver 1992; Mercier *et al.* 2000).

It was observed that larval development stages of *H. scabra* were similar to other aspidochirote holothurians. Observed length of *H. scabra* larval cycle was very similar to the observations made by James *et al.* (1988). In each trial, <5% of larval survival was recorded at the end of the 1-month culture period and similar observation has been made by other countries like the Philippines, New Caledonia and Madagascar (Purcell *et al.* 2012). Lack of technical expertise on *H. scabra* larval rearing, inappropriate hatchery conditions, poor larval feed and larval handling could be some possible reasons for this observation. However, percentage larval survival increased when increasing the number of breeding and larval rearing trials. This may be due to gradual improvement of

hatchery conditions and hand on experiences gained by hatchery staff on larval rearing.

Earthen ponds and sea pens have been widely used to grow hatchery produced *H. scabra* juveniles in many countries (Purcell *et al.* 2012). Differences in growth rate of *H. scabra* juveniles reared in these grow-out facilities have been discussed by various authors and some of their findings are summarized in table 2.

This study reveals that *H. scabra* juveniles reared in lagoon pens have a significantly higher growth rate than the juveniles reared in earthen ponds and fibreglass tanks. However, the observed growth rate was lower than the values reported for same system in the Philippines, Madagascar and Vietnam. Similarly, pond reared *H. scabra* juveniles in India and New Caledonia have shown higher growth rate than the present observation (Table 2). The reasons for the slow growth rate of juveniles reared in ponds are not very clear, but it is assumed that the accumulation of a thick layer of fine mud in the pond bottom has made some effect on their growth. Low water exchange and lack of nutrients may be other environmental factors which were not favourable for the growth and survival of these individuals.

Although it was reported that survival and growth is higher in earthen ponds than lagoon pens (Purcell *et al.* 2012), it is difficult to conclude which system is more favourable as it depends on

several factors, including climatic conditions, water quality parameters, bottom conditions, and availability of food etc.

The slow growth rate of juveniles that were reared in fibreglass tanks may be due to lack of food as they were not fed artificial feeds. Though, it was expected to provide natural food through adding detritus rich mud into the tanks, it did not appear to be very effective. As information on growth and survival of *H. scabra* juveniles cultured in fibreglass tanks are limited, it is difficult to make comprehensive comparison. Hence, further culture trials with artificial feeds may be useful in future.

In all the culture systems, weight loss was apparent after 4–5 months culture period. As explained by Robinson and Pascal (2012), this may be an indication of existence of carrying capacity ( $>250 \text{ g m}^{-2}$ ) in lagoon pens and mud ponds. The observed slow growth rate of sea cucumber juveniles that were reared in fibreglass tanks may probably be due to unfavourable rearing conditions or lack of adequate food. But further experiments are needed to find out the exact reason/s for this observation.

The observed high mortalities among the juveniles reared in mud ponds during 28 weeks could be due to sudden drop of pond water level. Accumulation of the large number of eviscerated and dead animals at the edges of ponds during that period indicated that they were under stress. As there was neither predation nor adverse environmental conditions, high survival rate was observed in fibreglass tanks.

*H. scabra* juveniles can tolerate salinity up to 40 ppt, however, their growth will stop or slow down beyond 35 ppt (Asha, Rajagopalan & Diwakar 2011). This may be another possible reason for observed very low or negative growth rate during high salinity period. However, further experiments are needed to confirm this hypothesis.

This is the first attempt of broodstock rearing, induced breeding and grow-out culture trials of any sea cucumber species in Sri Lanka. The findings of this study will help to update the existing knowledge of artificial breeding and larval rearing of *H. scabra* in the regional and global context. It seems that the most favourable conditions for spawning initiation and larval rearing of *H. scabra* can be varied with geographical locations, hence it is necessary to find out most appropriate local conditions for successful culture practices.

## Acknowledgments

The authors would like to thank Indika Wijesinghe, Hashan Tharurange and T.H. Sunil Shantha for their enormous support throughout this study. Assistance given by staff members of NARA Regional Research Centre at Kalpitiya is greatly acknowledged. NARA management is thanked for providing financial assistance and necessary facilities to carry out this study successfully. The reviewers are thanked for their useful comments.

## References

- Agudo N.S. (2006) *Sandfish Hatchery Techniques*. Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and The World-Fish Center. Nouméa, New Caledonia, pp. 1–136.
- Agudo N.S. (2012) Pond grow-out trials for sandfish (*Holothuria scabra*) in New Caledonia. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills), *ACIAR Proceedings* **136**, 104–112.
- Asha P., Rajagopalan M. & 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**, 218–224.
- Azari B.G.D. & Walsalam G.I. (2012) Maldives sea cucumber farming experience. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills), *ACIAR Proceedings* **136**, 128.
- Babcock R., Mundy C., Keesing J. & Oliver J. (1992) Predictable and unpredictable spawning events: in situ behavioural data from free-spawning coral reef invertebrates. *Invertebrate Reproduction Development* **22**, 213–222.
- Battaglene S.C. (1999) Culture of tropical sea cucumbers for the purposes of stock restoration and enhancement. *Naga* **22**, 4–11.
- Battaglene S.C. & Bell J.D. (1998) Potential of the tropical Indo-Pacific sea cucumber, *Holothuria scabra*, for stock enhancement. Chap. 33. In: *Stock Enhancement and Sea Ranching*. Proceedings First International Symposium on Stock Enhancement and Sea Ranching, 8–11 September 1997, Bergen, Norway (ed. by B.R. Howell, E. Moksness & T. Svasand), pp. 478–490. Blackwell, Oxford.
- Battaglene S.C., Seymour J.E. & Ramofafia C. (1999) Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. *Aquaculture* **178**, 293–322.
- Battaglene C.S., Seymour E.J., Ramofafia C. & Lane I. (2002) Spawning induction of three tropical sea cucumbers, *Holothuria scabra*, *H. fuscogilva* and *Actinopyga mauritiana*. *Aquaculture* **207**, 20–47.
- Bell J., Agudo N., Purcell S., Blazer P., Simutoga M., Phamb D. & Della P.L. (2007) Grow-out of sandfish

- Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. *Aquaculture* **273**, 509–519.
- Bell J.D., Purcell S.W. & Nash W.J. (2008) Restoring small-scale fisheries for tropical sea cucumbers. *Ocean and Coastal Management* **51**, 589–593.
- Choo P.S. (2008) Population status, fisheries and trade of sea cucumbers in Asia. In: *Sea Cucumbers. A Global Review of Fisheries and Trade* (ed. by V. Toral-Granda, A. Lovatelli & M. Vasconcellos). *FAO Fisheries and Aquaculture Technical Paper* **516**, 81–118.
- Clarke S. (2004) Understanding pressures on fishery resources through trade statistics: a pilot study of four products in the Chinese dried seafood market. *Fish and Fisheries* **5**, 53–74.
- Conand C. (1998) Holothurians. In: *FAO Species Identification Guide: The Marine Living Resources of the Western Central Pacific*, Vol. 2 (ed. by K. Carpenter & V. Niemi), pp. 1158–1189. Food and Agriculture Organization of the United Nations, Rome.
- Conand C. (2001) Overview over the last decade of sea cucumber fisheries - what possibilities for a durable management? In: *Echinoderms 2000: Proceedings of the 10th International Conference* (ed. by M. Barker), pp. 339–344. Dunedin, New Zealand.
- Conand C. (2004) Present status of world sea cucumber resources and utilisation: an international overview. *FAO Fisheries Technical Paper* **463**, 13–23.
- Conand C., De San M., Refeno G., Razafintseho G., Mara E. & Andriajatovo S. (1998) Sustainable management of sea cucumber fishery sector in Madagascar. *SPC Beche-de-Mer Information Bulletin* **10**, 7–9.
- Dabbagh A.R. & Sedaghat M.R. (2012) Breeding and rearing of the sea cucumber *Holothuria scabra* in Iran. *SPC Beche-de-mer Information Bulletin* **32**, 49–52.
- Dissanayake D.C.T. & Stefansson G. (2012) Present status of the commercial sea cucumber fishery in the coastal waters of Sri Lanka. *Journal of the Marine Biological Association of the United Kingdom*, **92**, 831–841.
- Duy N.D.Q. (2010) Seed production of sandfish (*Holothuria scabra*) in Vietnam. *Aquaculture Extension Manual* 48. Southeast Asian Fisheries Development Center, Iloilo, Philippines.
- Duy N. D. Q. (2012) Large-scale sandfish production from pond culture in Vietnam. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C. Hair, T. Pickering & D. Mills) *ACIAR Proceedings* **136**, 34–39.
- Eckhaut I., Lavitra T., Léonet A., Jangoux M. & Rasolonirina R. (2012) In-vitro fertilisation: a simple, efficient method for obtaining sea cucumber larvae year round. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (C.A. Hair, T.D. Pickering & D.J. Mills). *ACIAR Proceedings* **136**, 40–49.
- Ferdouse F (1999) Be'che-de-mer markets and utilisation. *SPC Be'che-de-mer Information Bulletin* **11**, 3–9.
- Ferdouse F. (2004) World markets and trade flows of sea cucumber/bêche-de-mer. In: *Advances in Sea Cucumber Aquaculture and Management* (ed. by A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J.-F. Hamel & A. Mercier). *FAO Fisheries Technical Paper* **463**, 101–117.
- Friedman K., Eriksson H., Tardy E. & Pakoa K. (2011) Management of sea cucumber stocks: patterns of vulnerability and recovery of sea cucumber stocks impacted by fishing. *Fish and Fisheries* **12**, 75–93.
- Gamboa R. & Juinio-Meñez M.A. (2003) Pilot-testing the laboratory production of the sea cucumber, *Holothuria scabra* (Jaeger) in the Philippines. *The Philippine Scientist* **40**, 111–121.
- Gamboa R.U., Aurelio R.A., Ganad D.A., Concepcion L.B. & Abreo N.A.S. (2012) Small-scale hatcheries and simple technologies for sandfish (*Holothuria scabra*) production. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills), *CIAR Proceedings*, **136**, 63–74.
- Giraspy D.A.B. & Ivy G. (2005) The influence of commercial diets on growth and survival in the commercially important sea cucumber *Holothuria scabra* var. *versicolor*. *SPC Beche-de-mer Information Bulletin* **28**, 46–52.
- Hair C.A. (2012) Sandfish (*Holothuria scabra*) production and sea-ranching trial in Fiji. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills). *ACIAR Proceedings* **136**, 129–141.
- Hair C., Kaure T., Southgate P. & Pickering T. (2011) Potential breakthrough in hatchery culture of sandfish *Holothuria scabra* by using algal concentrate as food. *SPC Beche-Demer Information Bulletin* **31**, 60–61.
- Hamel J.F., Conand C., Pawson D.L. & Mercier A. (2001) The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer. *Advances in Marine Biology* **41**, 129–223.
- Hasan M.H. (2005) Destruction of a *Holothuria scabra* population by overfishing at Abu Rhamada Island in the Red Sea. *Marine Environmental Research* **60**, 489–511.
- Hasan M.H. (2009) Stock assessment of holothuroid populations in the Red Sea waters of Saudi Arabia. *SPC Beche-de-mer Information Bulletin* **29**, 31–37.
- Ivy G. & Giraspy D.A.B. (2006) Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) in Queensland. *Australia. SPC Beche-de-mer Information Bulletin* **24**, 28–34.
- James D.B. (1999) Hatchery and culture for the sea cucumber *Holothuria scabra* Jaeger in India. *Naga* **22**, 12–16.
- James D.B. (2004) Captive breeding of the sea cucumber, *Holothuria scabra*, from India. *Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper* **463**, 385–395.
- James D.B., Rajapandian M.E., Baskar B.K. & Gopinathan C.P. (1988) Successful induced spawning and rearing of holothurian, *Holothuria* (Metriatyla) *scabra*, Jaegar at Tuticorin Marine Fisheries Information Service. *Technical and Extension Service* **87**, 30–33.

- James D.B., Rajapandian M.E., Gopinathan C.P. & Baskar B.K. (1994) Breakthrough in induced breeding and rearing of the larvae and juveniles of *Holothuria (Metriatyla) scabra* Jaeger at Tuticorin. *CMFRI Bulletin* **46**, 66–70.
- Juinio-Meñez M.A., dePeralta G.M., Dumalan R.J.P., Edulantes C.M. & Catbagan T.O. (2012) Ocean nursery systems for scaling up juvenile sandfish (*Holothuria scabra*) production: ensuring opportunities for small fishers. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills), *ACIAR Proceedings*, **136**, 57–62.
- Juinio-Meñez M.A., Evangelio J.C. & Miralao S.J.A. (2014) Trial grow-out culture of sea cucumber *Holothuria scabra* in sea cages and pens. *Aquaculture Research* **45**, 1332–1340.
- Lavitra T., Rasolofonirina R., Jangoux M. & Eeckhaut I. (2009) Problems related to the farming of *Holothuria scabra* (Jaeger, 1833). *SPC Beche-de-mer Information Bulletin* **29**, 20–30.
- Long B. & Skewes T. (1997) Distribution and abundance of beche-de-mer on Torres Strait reefs. *SPC Beche-de-mer Information Bulletin* **9**, 17–22.
- McEuen F.S. (1988) Spawning behaviour of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Marine Biology* **98**, 565–585.
- Mercier A., Battaglene S. & Hamel J.F. (1999) Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *Journal of Experimental Marine Biology and Ecology* **239**, 125–156.
- Mercier A., Battaglene S.C. & Hamel J.F. (2000) Periodic movement, recruitment, and size related distribution of the sea cucumbers *Holothuria scabra* in Solomon Islands. *Hydrobiologia* **440**, 81–100.
- Mercier A., Ycaza R.H. & Hamel J.-F. (2004) Aquaculture of the Galapagos sea cucumber, *Isostichopus fuscus*. In: *Advances in Sea Cucumber Aquaculture and Management* (ed. by A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J.F. Hamel & A. Mercier), *FAO Fisheries Technical Paper*, **463**, 347–358.
- Morgan A.D. (2000) Induction of spawning in the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea). *Journal of the World Aquaculture Society* **31**, 186–194.
- Olavides R.D.D., Rodriguez B.D.R. & Juinio-Meñez M.A. (2011) Simultaneous mass spawning of *Holothuria scabra* in sea ranching sites in Bolinao and Anda municipalities, Philippines. *SPC Beche-de-mer Information Bulletin* **31**, 23–24.
- Pitt R. & Duy N.D.Q. (2003) To produce 100 tonnes of sandfish. *Secretariat of the Pacific Community Beche-de-mer Information Bulletin* **18**, 15–17.
- Pitt R., Thu N.T.X., Minh M.D. & Phuc H.N. (2001) Preliminary sandfish growth trials in tanks, ponds and pens in Vietnam. *SPC Beche-de-mer Information Bulletin* **15**, 17–27.
- Pitt R., Duy N.D.Q., Du T.V. & Long H.T.C. (2004) Sandfish (*Holothuria scabra*) with shrimp (*Penaeus monodon*) co-culture tank trials. *SPC Beche-de-mer Information Bulletin* **20**, 12–22.
- Preston G.L. (1993) Beche-de-mer. In: *Nearshore Marine Resources of the Southern Pacific: Information for Fisheries Development and Management* (ed. by A. Wright & L. Hills), pp. 371–407. Institute of Pacific Studies, Fiji: Forum Fisheries Agency, Solomon Islands: International Centre for Ocean Development.
- Purcell S.W. (2010) Managing sea cucumber fisheries with an ecosystem approach. Edited/compiled by Lovatelli A., Vasconcellos M. and Yimin Y. *FAO Fisheries and Aquaculture Technical Paper* **520**, 134–157.
- Purcell S.W. & Simutoga M. (2008) Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild. *Reviews in Fisheries Science* **16**, 204–214.
- Purcell W., Cathy A.H. & David J.M. (2012) Sea cucumber culture, farming and sea ranching in the tropics: progress, problems and opportunities. *Aquaculture* **368–369**, 68–81.
- R Development Core Team. (2009) Available at: <http://www.rproject.org> (accessed 27 October 2010).
- Roberts D., Gebruk A., Levin V. & Manship B.A.D. (2000) Feeding and digestive strategies in deposit-feeding holothurians. *Oceanography and Marine Biology Annual Review* **38**, 257–310.
- Robinson G. & Pascal B. (2012) Sea cucumber farming experiences in south-western Madagascar. In: *Asia-Pacific Tropical Sea Cucumber Aquaculture* (ed. by C.A. Hair, T.D. Pickering & D.J. Mills), *ACIAR Proceedings* **136**, 142–154.
- Sanni S. & Forsberg O.I. (1996) Modelling pH and carbon dioxide in single-pass sea-water aquaculture systems. *Aquacultural Engineering* **15**, 91–110.
- Smiley S., McEuen F.S., Chaffee C. & Krishnen S. (1991) Echinoderms and Lophophorats. *Reproduction of Marine Invertebrates* **4**, 663–750.
- Tanaka Y. (1958) Seasonal changes occurring in the gonad of *Stichopus japonicus* Bulletin of Fish Hokkaido University **9**, 29–36.
- To A.W.L. & Shea S.K.H. (2012) Patterns and dynamics of beche-de-mer trade in Hong Kong and mainland China. *Traffic Bulletin* **24**, 65–76.
- Tsiresy G., Pascal B. & Plotieau T. (2011) An assessment of *Holothuria scabra* growth in marine micro-farms in southwestern Madagascar. *SPC Beche-de-mer Information Bulletin* **31**, 17–22.
- Wiedemeyer W.L. (1992) Feeding behaviour of two tropical holothurians, *Holothuria (Metriatyla) scabra* and *H. (Halo-deima) atra* from Okinawa, Japan. In: *Proceeding of 7th International Coral Reef Symposium* (ed. by R.H. Richmond), pp. 853–860. University of Guam Press, Guam, USA.
- Yanagisawa T. (1998) Aspects of the biology and culture of the sea cucumber. In: *Tropical mariculture* (ed. by S.S. De Silva), pp. 292–308. Academic Press, London.