



Stocking Density, Growth and Survival Rate of Post-Settled Juveniles of *Holothuria scabra* (Jaeger 1833) Reared in an Ocean-Based Floating Hapa

Yussuf S Yussuf^{1, 2*} and Saleh AS Yahya¹

¹Institute of Marine Sciences, University of Dar es Salaam, Mizingani Road, P.O. Box 668, Zanzibar, Tanzania. E-mail: yussufyams@hotmail.com

²Department of Biology, College of Natural and Mathematical Sciences, University of Dodoma, P. O. Box 338, Dodoma, Tanzania. E-mail: saleh.yahya@udsm.ac.tz

* Corresponding author.

Received 24 Oct 2020, Revised 2 Jul 2021, Accepted 20 Jul 2021, Published Aug 2021

DOI: <https://dx.doi.org/10.4314/tjs.v47i3.15>

Abstract

Ocean-based nursery system using floating hapa have proved to be the best option for raising early sandfish juveniles. However, the nursery system can be further improved by providing key information that will support their application. The present study provides information on the effect of stocking density on growth and survival of sandfish juveniles reared in floating hapa (1 x 0.5 x 1 m). Three densities were tested, 100, 200 and 400 early juveniles (5 mm; 0.093 g) per hapa net in a randomized set-up. Initial density of 100 and 200 showed significant higher growth rate and final size (0.082–0.074 g/day; 3.76–3.42 g) compared to 400 (0.045 ± 0.002 g/day; 2.11 ± 0.07 g). Moreover, at 100 and 200 densities, > 95% of all juveniles attained release size (2 g) and only 74% for 400 density after 45 days of rearing. Sandfish juveniles showed negative allometric growth and their survival did not display any pattern (100 = 91%; 200 = 35%; 400 = 51.3%). The coefficient of variation for weight of juveniles showed strong positive relationship with initial density ($R^2 = 0.99$, $p = 0.039$) with CV values of (100 = 48.6%; 200 = 49.8%; 400 = 50.1%). It is therefore concluded that, 100 stocking density should be opted for optimum results, however, 200 can also be considered provided that, the survival is improved.

Keywords: Sea cucumber, Ocean-based Nursery, Stocking density, Survival rate, Growth rate.

Introduction

Hatchery method for high value sea cucumber species has been established over the recent decades (Pitt and Duy 2004, Agudo 2006, Miltz et al. 2018) and the transfer of technology has been always done by different countries in their effort to restore their depleted sea cucumber stock and increase aquaculture production (Bell et al. 2008, Purcell et al. 2012, Juinio-Meñez et al. 2017). *Holothuria scabra* commonly known as sandfish is one of the more commercially important species and most threatened tropical sea cucumber (Purcell 2014) whose hatchery methods have been well

documented and adopted in many Indo-Pacific countries (Purcell et al. 2012). However, a conventional hatchery method for this species has been frequently modified and simplified at different production phases in order to optimize their production and minimize costs of raising the juveniles (Gamboa et al. 2012).

Previously, nursery phase for raising early sandfish juveniles to a release size was carried out in fibreglass and land-based concrete tanks (Battaglione 1999, Pitt and Duy 2004, Agudo 2006). However, these nursery systems are associated with high production costs (e.g., purchasing food, electricity and additional

manpower) (Juinio-Meñez et al. 2012), long duration for rising the juveniles (low growth rate) (Juinio-Meñez et al. 2012, Mills et al. 2012) and great size variability among juveniles (Battaglione et al. 1999, Pitt and Duy 2004, Gamboa et al. 2012, Juinio-Meñez et al. 2012, Gorospe et al. 2017). Moreover, this was a major constraint for small hatcheries with limited space since they require large area (Juinio-Meñez et al. 2012, Mills et al. 2012, Purcell et al. 2012, Juinio-Meñez et al. 2017). Consequently, various forms of nursery systems have been developed to overcome the shortfalls accompanied with land-based nursery system.

The introduction of periphyton-based nursery system as an alternative way for rearing early sandfish juveniles was one of the major hatchery developments which has contributed to significant reduction of production costs, shortened the production cycle and at the same time increased juveniles production (Juinio-Meñez et al. 2012). The system was first developed in Vietnam where fine-mesh net “hapa” was used in marine pond which showed promising results with good economic returns (Pitt and Duy 2004, Duy 2012). However, the availability of suitable ponds with proper salinity and adequate water exchange (Juinio-Meñez et al. 2012) and high pond management costs have become limiting factors for the applications of this system in some countries like Philippines (Mills et al. 2012, Altamirano et al. 2017, Altermirano and Noran-Baylon 2020) and perhaps in Zanzibar (Tanzania). Therefore, the only option available for such countries is to adopt a similar system but in ocean or seas using floating hapa (Pitt and Duy 2004, Juinio-Meñez et al. 2012, Juinio-Meñez et al. 2017) or bottom-set cages and tray (Gorospe et al. 2017, Juinio-Meñez et al. 2017).

Ocean-based nursery system using floating hapa was pioneered in the Philippines and delivered similar performance with those in marine ponds (Pitt and Duy 2004, Juinio-Meñez et al. 2012, Juinio-Meñez et al. 2017). Moreover, the system has been reported to

have added advantages of being cost-effective and easily adopted by small-scale sea cucumber farmers (Juinio-Meñez et al. 2012, Gorospe et al. 2017, Juinio-Meñez et al. 2017). However, despite promising results reported from the systems, limited numbers of studies (only in western Pacific and Southeast Asia (Altermirano and Noran-Baylon 2020)) have been conducted that provide key information that supports their applications (Damaso and Argente 2019). Therefore, more studies from other regions are required to support its applications in juveniles’ production (Purcell et al. 2012, Purcell and Agudo 2013).

Rearing of sandfish juveniles in nursery system can yield individuals of variable final release sizes, ranging from 1 g (Agudo 2006, Purcell and Agudo 2013, Damaso and Argente 2019), 2 g (Duy 2010, Altermirano and Noran-Baylon 2020) to 3 g (Purcell and Simutoga 2008), depending on different factors such as stocking density. Various nursery systems have reported an inverse relationship between stocking density and juveniles’ growth (Battaglione et al. 1999, Pitt and Duy 2004, Bell et al. 2008, Gorospe et al. 2017) and rearing duration (Altermirano and Noran-Baylon 2020). However, information on the effects of initial stocking density for sandfish juveniles reared in ocean-based nursery systems using floating hapa is very scarce. Altermirano and Noran-Baylon (2020) reported long rearing duration (up to 3 months) for sandfish juveniles stocked at high density (≥ 1000) in floating hapa. Though, the periphyton (biofilm) is the primary food source for early juveniles reared in floating hapa, prolonged rearing period can cause excessive growth of biofouls on the hapa nets which can impede water flow, while extra weight added may cause drag and damage the nets (Fitridge et al. 2012). Moreover, high initial stocking density requires size grading of juveniles and periodic change or cleaning of floating hapa net (Altermirano and Noran-Baylon 2020). Size grading and net cleaning might be detrimental to fragile juveniles and too technical for small-scale sea cucumber farmers, while periodic change of floating net

could not be economically viable. Therefore, optimum density that will offer juveniles with the right size ready to be released without involving size grading, net cleaning or changing should be determined so that the system can be easily adopted by small-scale sea cucumber farmers. Furthermore, no prior study has reported growth pattern (length-weight relationship) of sandfish juveniles reared in floating hapa.

Sandfish hatchery has just been introduced in Zanzibar, Tanzania aimed at restoring dwindling populations of high value tropical species *H. scabra* and at the same time increase aquaculture production through farming. However, the nursery stage has become a major challenge that hinders mass production of sandfish juveniles in the hatchery. Therefore, the present study reports the overall performance of ocean-based nursery system in Zanzibar, Tanzania, effects of initial stocking densities and length-weight relationship of sandfish juveniles reared in floating ocean-

based nursery system. The findings of the present study will make a major contribution towards sandfish production in Zanzibar, Tanzania.

Materials and Methods

Experimental site

This study was conducted at Pete Inlet (Uzi-Muongoni) located in the south district of Unguja Island at 6°16'39"S and 39°24'41"E (Figure 1) between February and April 2019. The area is well protected from strong waves and winds which makes it ideal for floating ocean-based nursery system. Physical parameters such as salinity (32–35 ppt), temperature (26.2–33.8 °C) and DO (4.3–6.5 mg/L) of the area were within optimum ranges for the growth of sea cucumbers. The water depth is > 1 m during lowest low tides and characterized by a number of seagrass species dominated by *Thalassia hemprichii*, *Enhalus arcoroides* and *Cymodocea serrulata* making the area potential for sea cucumber farming.

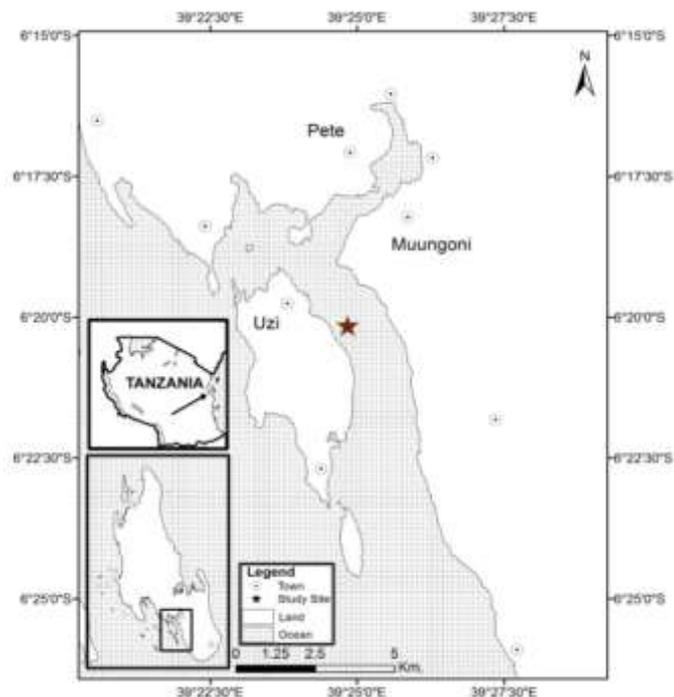


Figure 1: The study site (Uzi-Muongoni) Zanzibar, Tanzania.

Source of experimental juveniles

Post-settled sandfish juveniles were sourced from a single batch of larvae which were produced in Zanzibar marine multispecies hatchery using a combination of dry treatment, thermal shock and food stimulation as explained by Agudo (2006). The larvae were reared in larvae tanks for 45 days post fertilization and fed with mixed micro-algae species. Thereafter, juveniles of an average size of 5 mm and 0.093 g were randomly selected from the batch by detaching them from the settlement plates. The juveniles were then packed in twenty litre plastic bags half filled with water and the remaining half filled with oxygen. They were then transported in an ice box to the experimental site. Upon arrival, the juveniles were acclimatized to the new environmental conditions for 30 minutes before being stocked in a hapa net.

Construction of ocean-based nursery system and experimental set-up

The ocean-based nursery system consisted of a 6.25 cm (diameter) PVC pipe which worked as floating buoy and frame, 1 mm (mesh) mosquito net as hapa netting and 20 litre buckets filled with concrete as anchors (Figure 2). The whole system was made using

three separate PVC frames of 4.0 x 0.5 m (L x W) each attached to one another using 8 mm ropes. Three hapa nets of 1 x 0.5 x 1 m (L x W x H) were placed in each frame making a total of nine hapas attached to the main PVC frames using 6 mm rope and cable tie and leaving a gap of 0.5 m between hapas. The hapas were tied to small anchors at the bottom and extra weight was introduced inside the hapa to stretch the net. Total grazing area for the juveniles in each hapa net including the walls was 3.5 m². Because of 4.5 m tidal range, the mooring ropes were sufficiently slack (6.5 m) in order to hold the system and allow it to move up and down, and forward and backward with the tide flood and ebb. The hapa nets were conditioned for three days before stocking in order to allow the formation of biofilm. The juveniles were stocked at densities of 100, 200 and 400 hapa⁻¹ with three replicates for each treatment in a randomized way. The whole system was covered on top using fishing net to protect the juveniles from predators. A temperature logger was placed one meter deep at the centre of the system to monitor the temperature. The experiment was conducted for forty five (45) days.

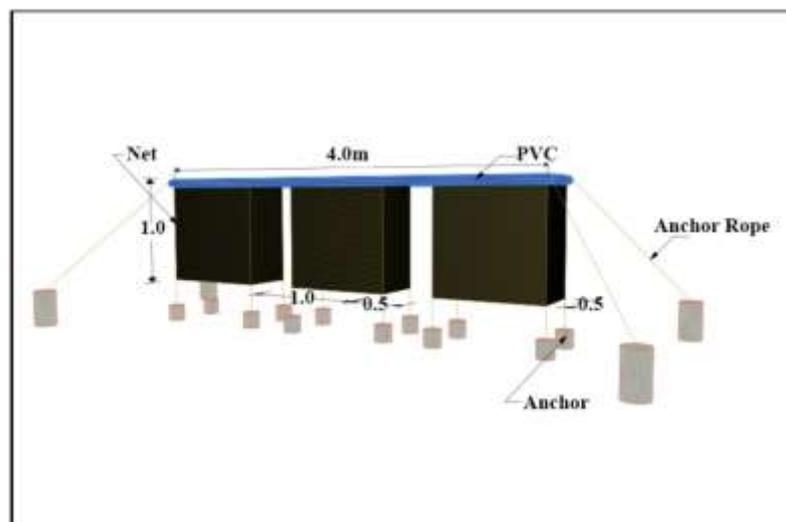


Figure 2: Schematic diagram of floating ocean-based nursery system set-up.

Measurements and length-weight relationships

After forty five (45) days, the juveniles from each hapa were collected, counted, placed in a separate container and transported back to the laboratory for measurements. Upon arrival, they were left overnight to allow them to defecate in order to get an exact weight of the juveniles after gut evacuation. Wet weight and total length of each individual were measured using a meter ruler and a weighing balance to the nearest of 0.1 mm and 0.1 g, respectively. Juveniles from different hapas were measured and recorded separately. Basic descriptive measures of growth performance were calculated, which included; coefficient of variation (CV), absolute growth rate (AGR), specific growth rate (SGR) and survival rate and were computed as shown below.

Coefficient of variation (CV) for weight and length: $CV (\%) (\text{Weight}) = (SD_{BW}/X_{BW}) \times 100$, or $CV (\%) (\text{Length}) = (SD_{BL}/X_{BL}) \times 100$, where SD_{BW} and SD_{BL} are the standard deviations of body weights (BW) and body length (BL), and X_{BW} and X_{BL} are the means of all measured BW and BL. *Absolute growth rate (AGR)* (weight and length) was estimated as the difference between the final and initial weights or length of sandfish juveniles over the number of rearing days (g/day). *Specific Growth Rate* (SGR, expressed as %/day) was determined using the formula $SGR (\text{Weight}) = 100 (\ln W_f - \ln W_i)/T$, or $SGR (\text{Length}) = 100 (\ln L_f - \ln L_i)/T$, where W_i and L_i , W_f and L_f were the initial and final weight or length of the sandfish, respectively, and T represents the number of days between initial and final weighing.

Length-weight relationship of sandfish juveniles were analysed using the power function (Pauly 1984).

$$W = a L^b$$

Where: W= Wet weight in gram, L = Total length in cm, a = Intercept, b = Slope. The value of b from each stocking density and pooled data were used to determine growth patterns of the juveniles from the nursery

system after being tested based on Pauly (1984) using the Students t-test.

Furthermore, percentage survival per treatment was calculated using the formula $\text{Survival}(\%) = ((N1 - NF)/N1) \times 100$ where NI is the initial number of juveniles and NF is the final number of juveniles.

Data analysis

All data were tested for normality and homogeneity of variances using Shapiro-Wilks and Levene's tests, respectively. The differences in lengths between individuals from different treatments were tested using one way ANOVA, while differences in weights were tested using Kruskal-Wallis test since the data were not normally distributed even after transformation. Relationship between CV and stocking density was evaluated using scatter plots.

Results and Discussion

Overall performances of ocean-based nursery system

Nursery phase is an important part of sea cucumber culture where juveniles from the hatchery are reared in hapa nets for certain period until they are ready for grow out stage. It is a common exercise in sandfish hatchery to have two nursery phases, whereby post-settled early sandfish juveniles (≤ 5 mm) are first transferred from larval rearing tanks to hapa nets until they are > 1 g. Thereafter, they are transferred to second nursery phase (sand conditioning) until they reach release a size of 3 g (Pitt and Duy 2004, Purcell et al. 2012). However, in some hatcheries, early sandfish juveniles can only be reared in hapa nets until they reach 2–3 g and released without involving second nursery phase. After forty five days of rearing in the present study, the overall sizes of all juveniles from floating hapa nets regardless of their stocking densities ranged from 0.3 to 10.8 g (mean 2.79 ± 0.08 g (SE)) and 10 mm to 56 mm (mean 29.40 ± 0.45 mm (SE)). The size distribution of all juveniles when all data were pooled together is shown in Figures 3A and 3B. Generally, 67.9% of all the

juveniles attained release size of ≥ 2 g and were ready to be transferred to the next stage. However, if we consider the juveniles' release size of 1 g as reported by Agudo (2006), Purcell and Agudo (2013) and Damaso and Argente (2019), more than 97% of all the juveniles in the present study attained the size ready for grow-out or second nursery stage.

The present findings add to the number of studies that confirmed ocean-based nursery system using floating hapa as a better and viable nursery system for rearing early sandfish juveniles than both fiberglass and concrete tanks. The ocean-based floating hapa in this study was able to produce sandfish juveniles

ready to be released for a practical period of about 45 days, contrary to hatchery tank which requires long rearing period (~120 days) to at least attain similar release size; this is based on practical experience in Zanzibar hatchery. The percentage of juveniles that attained release size reported in the present study is greater than that of 50% reported from hapa nets installed in marine ponds in Vietnam with the final size of juveniles ranging from 0.5 to 2 g (Duy 2010). The large percentage of juveniles that attained release size in this study could be contributed by lower initial density of 100-400/m² compared to that of 600-800/m² (Duy 2010).

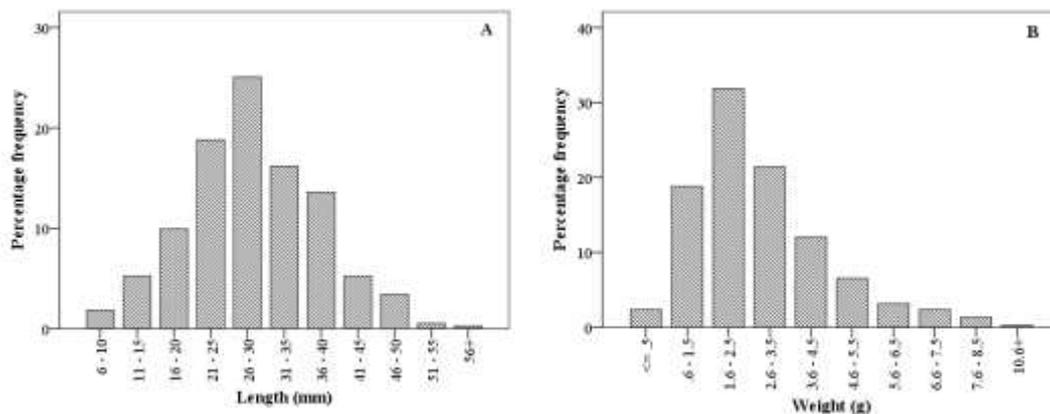


Figure 3: Size distribution of sandfish juveniles when all data were pooled together after 45 days of experiment (A = Length frequency and B = Weight frequency).

The average weight and length gained by the juveniles during the experimental period from the whole system were 2.697 ± 0.083 g (SE) and 24.15 ± 0.454 mm (SE), while the growth rate (AGR) ranged from 0.005 to 0.230 g/day (mean 0.06 ± 0.002 g/day) and 0.111-1.133 mm/day (mean 0.54 ± 0.01 mm/day), respectively. Furthermore, the overall survival rate from the whole nursery system was 52.4%. The overall mean growth rate reported in the present study is smaller compared to that of Agudo (2006) and Juinio-Meñez et al. (2012) who reported an average growth rate of 0.08-0.1 g/day and 0.6 g/day from New Caledonia and the Philippines, respectively. The floating hapa nets from these other studies were placed

in high nutrient marine earthen ponds; perhaps resulted in high concentrations of biofilms and hence had high growth rates. Moreover, high initial stocking density could also be a contributing factor to low growth rate in the present study compared to 150/m² from Agudo (2006) and Juinio-Meñez et al. (2012).

Juinio-Meñez et al. (2012) further reported survival rates of 44% and 12-30% from ocean-based floating hapas in two different set-ups. The higher survival rate in the present study may be attributed to the extra protection accorded to the juveniles from predators by covering the top of hapa nets. On the other hand, Gorospe et al. (2019) recorded survival rates of 56.9% and 34.4% for shaded hapa and

others at ambient light, respectively. Over 50% survival from shaded hapa recorded by Gorospe et al. (2019) is comparable to the findings of this study. The study used a net to cover the top which in the process provided shade and protection from predators and hence the similarity of the results. However, Altamirano and Noran-Baylon (2020) reported survival rates that ranged from 70 to 97% from floating hapa stocked with 150-500 hapa⁻¹. The low survival in the present study could have been partly contributed by the presence of small holes at the net floor of some hapa and perhaps causing some juveniles to escape from the nursery system. The system was positioned in a way that the hapa nets which were at the front of the system were absorbing the energy generated by ebbing tide which damaged the nets. This can be improved or prevented in future by either changing the orientation of the nursery or using strong netting materials that can resist the energy and force generated by the tidal movements.

Effects of initial stocking density

The results indicated that, the growth of sandfish juveniles reared in an ocean-based nursery system using floating hapa is inversely related to the initial density. This is in agreement with previous studies conducted for this species in other nursery systems, for example: Damaso and Argente (2019), Altamirano and Noran-Baylon (2020) in ocean-based nursery and Newell (1963) in hatchery tanks. Low stocking density showed higher growth rates (SGR and AGR), maximum weight and length gained and higher final sizes of sandfish juveniles. The mean final weight and length of juveniles from 100, 200 and 400 stocking densities were 3.76 ± 0.20 g and 33.29 ± 0.80 mm, 3.42 ± 0.19 g and 31.68 ± 0.88 mm and 2.11 ± 0.07 g and 26.77 ± 0.61 mm, respectively (Figure 4A and 4B). The AGR (weight and length) were 0.082 ± 0.004 g/day and 0.629 ± 0.018 mm/day, 0.074 ± 0.004

g/day and 0.593 ± 0.196 mm/day and 0.045 ± 0.002 g/day and 0.484 ± 0.014 mm/day (Figure 5A and 5B) and their corresponding SGR (weight and length) were 7.94 ± 0.121 and $4.152 \pm 0.055\%$ /day, 7.68 ± 0.143 and $4.126 \pm 0.066\%$ /day and 6.64 ± 0.086 and $3.599 \pm 0.055\%$ /day for 100, 200 and 400, respectively. Results from one way ANOVA indicated significant differences ($p < 0.05$) in final sizes for both weight and length between juveniles from different stocking densities. However, Tukey's post-hoc test (and Kruskal Wallis for weight) indicated that significant differences only existed between juveniles from the stocking densities 100 and 400, and between 200 and 400, but not between juveniles from 100 and 200 stocking densities where $p = 0.416$ (length) and $p = 0.758$ (weight).

Different initial densities resulted in juveniles of a wide range of sizes (Figure 6). The coefficients of variation (CV) values for both weight and length showed strong positive relationships with initial density of juveniles (length: $R^2 = 0.99$, $p = 0.041$; weight: $R^2 = 0.99$, $p = 0.039$) with CV values of 22.7, 25.5 and 32.9% (length) and 48.6, 49.8 and 50.1% (weight) for 100, 200 and 400 densities, respectively. This indicated that, the higher the initial stocking density of sandfish juveniles in floating hapa, the more heterogeneous the size of juveniles as indicated by increase in CV values and hence high variations among juveniles which will require size grading. Despite high values of CV from the hapa nets with initial densities of 100 and 200, more than 95% of the juveniles attained release sizes (> 2 g) (100 = 96.34% and 200 = 95.04%) which is the primary goal of a nursery system. The initial density of 400 gave 73.63% of sandfish juveniles ready to be transferred to the next stage after 45 days and the remaining ~24% required an extra rearing time to attain required release sizes.

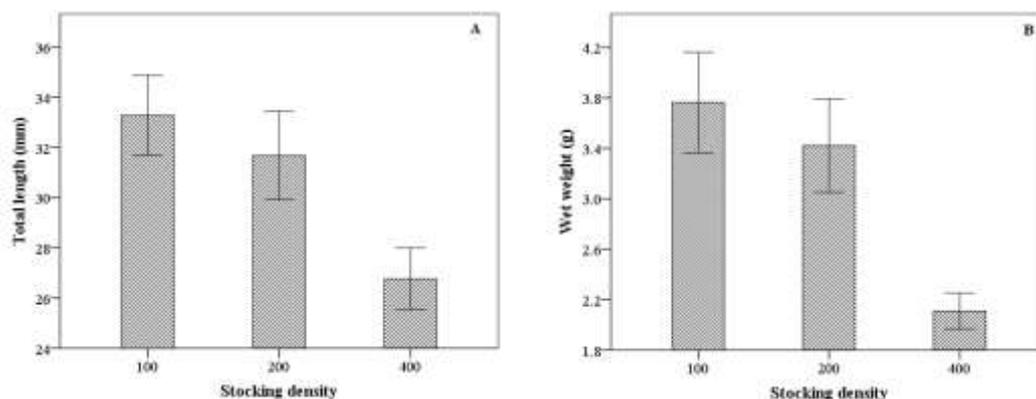


Figure 4: Average final sizes of sandfish juveniles at different stocking densities (A = Average length and B = Average weight). Error bars denote standard error of the mean (SEM).

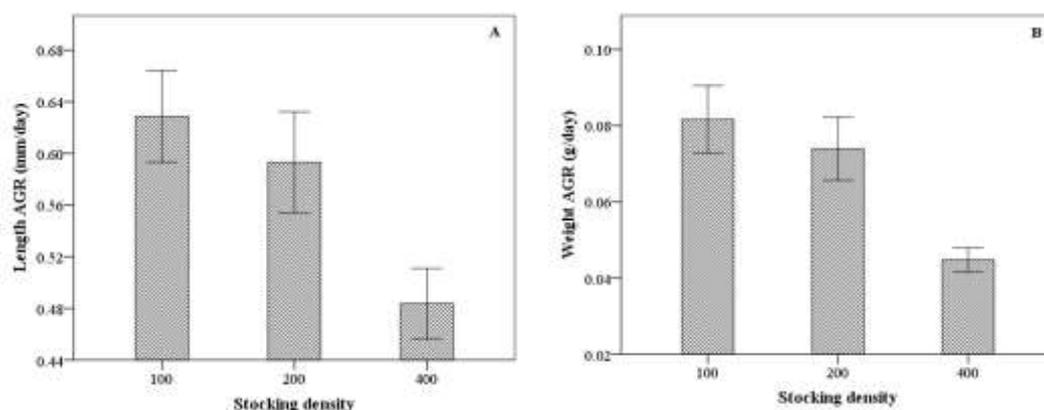


Figure 5: Average growth rate (AGR) of sandfish juveniles at different stocking densities (A = Length and B = Weight). Error bars denote standard error of the mean (SEM).

Comparable trends of higher growth rate and maximum final sizes at lower stocking densities have been reported for sandfish juveniles reared in sea pens (Purcell and Simutoga 2008), land-based tanks (Battaglione et al. 1999), nursery ponds (Pitt and Duy, 2004) and bottom-set trays (Gorospe et al. 2017). Despite similarity in general trends of sandfish juvenile growth stocked at different densities, the growth performance and survival of sandfish juveniles vary spatially and temporally even for juveniles reared in the same stocking density. These differences are caused by various factors such as environmental variables, food availability and

predation rate in respect to the natural genetic differences among individuals (Qiu et al. 2014). The weight gained and growth rates reported in the present study were higher at every stocking density than that of Damaso and Argente (2019), probably because of lower initial stocking density used in the present study.

However, the present findings are comparable with that of Altermirano and Noran-Baylon (2020) from the Phillipines who reported the growth rate (AGR) which ranged from 0.06 to 0.083 g/day at stocking density of 150–500 hapa⁻¹ and 71–97% of their juveniles attained release size of 2 g. Moreover,

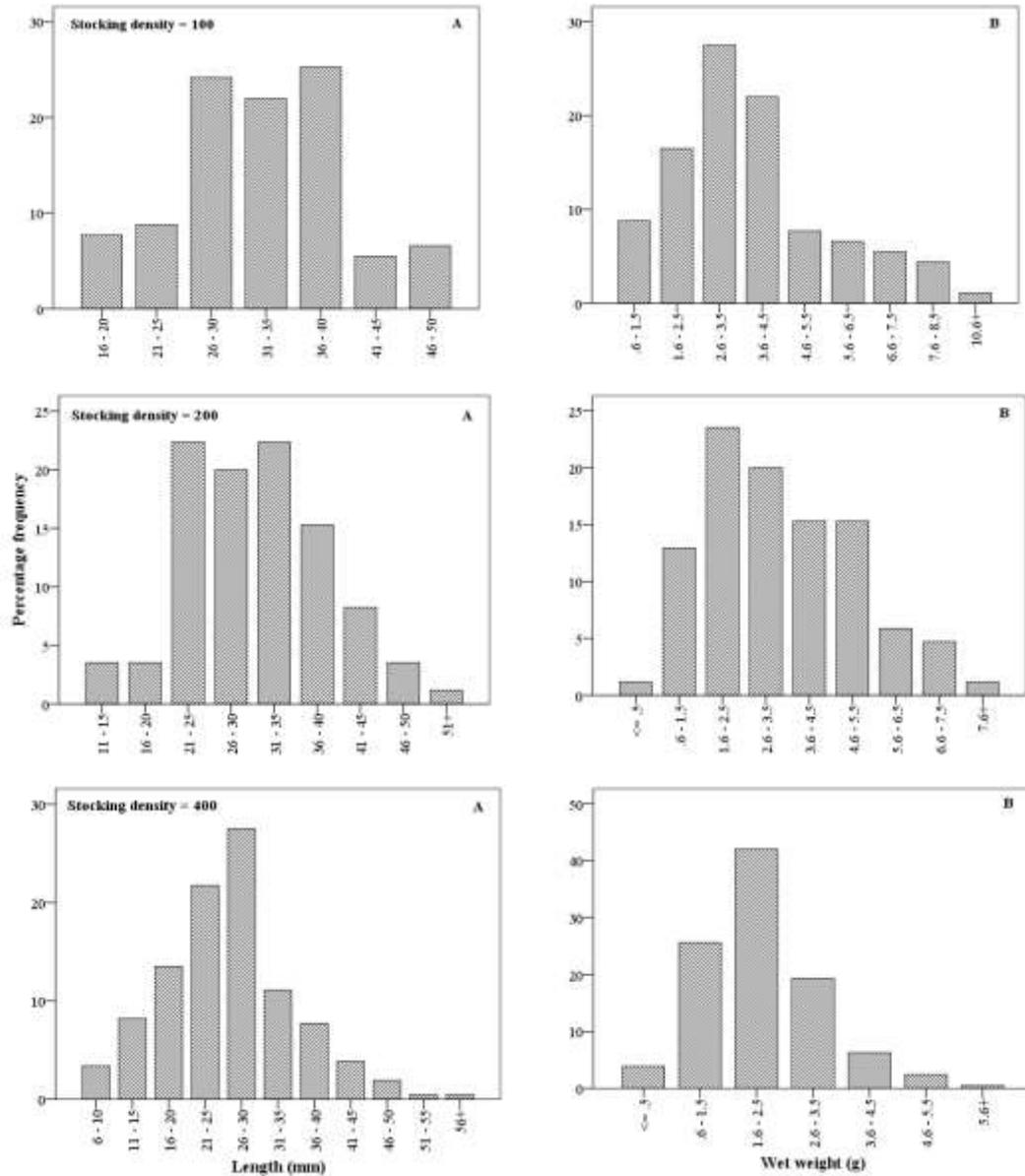


Figure 6: Size distribution of sandfish juveniles at three stocking densities (A: Length and B: Weight).

Altermirano and Noran-Baylon (2020) reported that, sandfish juveniles stocked at density $\leq 500 \text{ hapa}^{-1}$ required 34-48 rearing days to attain release size which is in agreement with the findings of the present study. The authors further reported that, at 150 hapa^{-1} density,

juveniles were able to reach release size after 34 days only; this could have also been the case in the present study for 100 and 200 stocking densities if we could have monitored the growth on a weekly basis. In the present study, 74% of juveniles attained released size

of 2 g at 400 stocking density which is also comparable with 71% (500 hapa⁻¹) of Altermirano and Noran-Baylon (2020). The CV value reported in the present study is slightly higher than that of Altermirano and Noran-Baylon (2020) (150 = 42%; 250 = 45%; 500 = 47%) indicating greater variations among juveniles in the present study.

No clear trend was observed on the effects of initial stocking density on survival rates of the sandfish juveniles reared in floating hapa. The highest and lowest survival rates were recorded at densities of 100 and 200, respectively. Survival rates ranged from 82 to 100, 0 to 70 and 34.5 to 68% for 100, 200 and 400 stocking densities, respectively. Unexpected 100% mortality was observed in one of the hapas at the stocking density of 200, probably because of predation or escaping of juveniles since the hapa net was damaged and a number of crabs were found inside despite being covered and hence lowered the mean survival rate. Several other studies have also reported non-significant effects of initial density on survival rates; for example, Battaglione et al. (1999) for newly-settled sandfish juveniles (1.5 mm) in hatchery tanks, Lavitra et al. (2010) for post metamorphic sandfish juveniles in bottom-set cages, Gorospe et al. (2017) for early juvenile sandfish (1 cm) in nursery ponds and Purcell and Simutoga (2008) for various size sandfish juveniles ranging between 1-10 g in sea pens. High

mortality rate at high density could be attributed to low DO, accumulation of faeces and competition for space amongst the individuals (Xilin 2004). Such densities are said to be above the carrying capacity of a given environment.

Length-weight relationships

The length-weight relationship results for sandfish juveniles reared in the ocean-based floating hapa are presented in Table 1 and Figure 7. The correlation coefficients (r values) for the length-weight relationships at three stocking densities ranged from 0.861 to 0.913, and when all the data were pooled together, the value of r was 0.844. The calculated r was found to be greater than the critical r values (0.131 to 0.278), which indicates high significant correlations between length and weight of sandfish juveniles from the system. Moreover, the percentage contribution of independent variables to dependent variables is described by the coefficient of determination (R²) values, which ranged from 0.763 to 0.797 from the three stocking densities and pooled data. The R² values in this study are smaller than that recorded by Al-Rashdi et al. (2007) of 0.80 in Sultanate of Oman, but greater than that of 0.43-0.68 recorded by Natan et al. (2015) from Indonesia. The differences could be contributed by the fact that, they were subjected to different environmental conditions with respect to their natural genetic differences.

Table 1: Length-weight relationship of sandfish juveniles from ocean-based floating hapa

Stocking Density	n	W = a L ^b	Log W = Log a + b x Log L	r calc	r table (p = 0.01)	t calc	t table (p = 0.01)
100	91	W = 0.004L ^{1.898}	Log W = 2.346 + 1.898LogL	0.867*	0.269	9.832*	3.403
200	85	W = 0.003L ^{1.944}	Log W = 2.419 + 1.944LogL	0.913*	0.278	9.688*	3.412
400	207	W = 0.019L ^{1.405}	Log W = 1.74 + 1.405LogL	0.861*	0.179	32.212*	3.339
Pooled	383	W = 0.009L ^{1.657}	Log W = 2.026 + 1.657LogL	0.844*	0.131	31.06*	3.316

* The difference is significant at 0.01 levels.

The values of b ranged from 1.405 to 1.944, which are out of the expected range of b = 2.5–

3.5. The b value provides information on the growth patterns of aquatic organisms (Pauly

1984) including sea cucumbers. The results of Student t test indicated that, calculated t values were greater than the tabulated (critical) values at $p = 0.01$. Therefore, it can be concluded that, the b-values significantly differed from $b = 3$ and the growth was negatively allometric. The results of this study are comparable with other studies that have been conducted for this species showing negative allometric growth;

for example, in Indonesia ($b = 1.264$ to 2.127) (Natan et al. 2015), Sultanate of Oman ($b = 2.18$) (Al-Rashdi et al. 2007), Vietnam ($b = 2.84$) (Pitt and Duy 2004) and New Caledonia ($b = 2.28$) (Conand 1990). The value of b changes depending on the environment the individual has been exposed to, physiological conditions at the time of collection, sex, gonad development and food supply (Froese 1998).

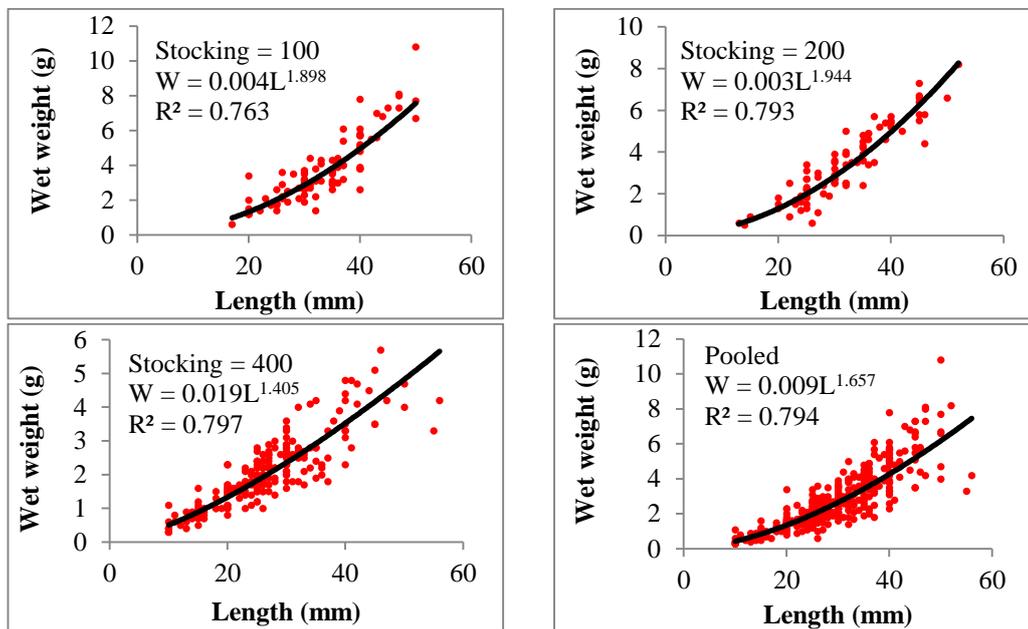


Figure 7: Length-weight relationships of sandfish juveniles at three stocking densities and when all data were pooled together.

The growth rate of sea cucumbers is closely related to the water temperature since it affects the metabolism and physiological performance of the organism (Dong et al. 2006). The water temperature during the experimental period ranged from 26.22 to 33.84 °C with an average of 30.94 ± 0.02 °C. There were significant differences in average water temperature on a

weekly basis (Figure 8) with highest temperature of 31.3 °C recorded in the third week after stocking. The average temperature in the first week which is very important for the survival of the organism was within the optimum range of 26 – 30 °C as reported for sandfish larvae and broodstock (Agudo 2006).

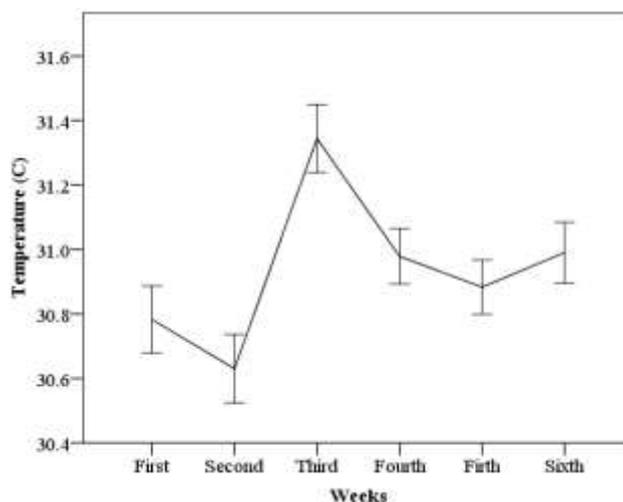


Figure 8: Weekly mean water temperature during experiment period. Error bars denote standard error of the mean (SEM).

Conclusions

The present study was designed to test the effects of initial stocking density on growth and survival of post-settled sandfish juveniles reared in an ocean-based nursery system using floating hapa. It is concluded that, initial density of 100 hapa⁻¹ (1 x 1 x 1 m) should be considered in raising sandfish juveniles since it showed maximum growth, high survival rate, and low CV value with more than 97% of all the juveniles attaining release sizes over a practical period of 45 days. However, since initial density of 200 juvenile's hapa⁻¹ showed no significant difference from 100 hapa⁻¹ in overall performance except for survival rate, this density can also be considered in rearing juveniles provided that the survival is improved. Moreover, stocking density of 400 hapa⁻¹ can be a better option for large scale sandfish hatchery production but the release size of 1 g should be considered, which is expected to produce 96% of juveniles. Monitoring of the growth and survival at shorter intervals (e.g. 2 weeks) would have been better, as at low density the juveniles might have reached release sizes earlier than high density, which was not detected in the present study. Similar kinds of experiments

should be performed at different periods (seasons) of the year since the environmental parameters are different, which might have an impact on the overall growth performance of the juveniles.

Acknowledgement

We acknowledge the Swedish International Development Agency (Sida)–Bilateral Marine Science Program (BMSP) for supporting this study. We also thank the Food and Agriculture Organization (FAO) of the United Nations through the Zanzibar Marine Multispecies Hatchery Project for their technical support and giving us permission to use their equipment during the course of this study. We specifically thank Mr Pierre Phillippe Blank, the Chief Technical Advisor of the project for his advice and technical guidance during the experiment.

Declaration of Interest

We do not have any potential competing or conflicting interests regarding publication of this paper.

References

Agudo N 2006 Sandfish Hatchery Techniques. *Australian Centre for International*

- Agricultural Research*. New Caledonia: Secretariat of the Pacific Community and the WorldFish Center, Noumea.
- Al-Rashdi KM, Claereboudt MR and Al-Busaidi SS 2007 Density and size distribution of the sea cucumber, *Holothuria scabra* (Jaeger, 1935), at six exploited sites in Mahout Bay, Sultanate of Oman. *J. Agric. Marine Sci.* 12: 43-51.
- Altamirano JP, Recente CP, Rodriguez Jr, JC 2017 Substrate preference for burying and feeding of sandfish *Holothuria scabra* juveniles. *Fish. Res.* 186 (Part 2): 514-523
- Altermirano JP and Noran-Baylon RD 2020 Nursery culture of sandfish *Holothuria scabra* in sea-based floating hapa nets: Effects of initial stocking density, size grading and net replacement frequency. *Aquaculture* 526: 735379.
- Battaglione SC, Seymour JE and Ramofafia C 1999 Survival and growth of cultured sea cucumbers, *Holothuria scabra*. *Aquaculture* 178: 293-322.
- Bell JD, Purcell SW, Nash WJ 2008 Restoring small-scale fisheries for tropical sea cucumbers. *Ocean Coast. Manag.* 51(8-9): 589-593.
- Conand C 1990 The fishery resources of Pacific Island countries. Part 2. Holothurians, FAO Fisheries Technical Papers. FAO, Rome, 143 p.
- Damaso MF and Argente FAT 2019 Ocean nursery rearing of sea cucumber *Holothuria scabra* early juveniles in hapa nets at different stocking densities. *Int. J. Fish. Aquat. Stud.* 7(4): 253-257.
- Dong Y, Dong S, Tian X, Wang F and Zhang M 2006 Effect of diel temperature fluctuations on growth, oxygen consumption and proximate body composition in the sea cucumber *Apostichopus japonicus* Selenka. *Aquaculture* 255: 514-521.
- Duy NDQ 2010 Seed production of sandfish (*Holothuria scabra*) in Vietnam. SEAFDEC Aquaculture Extension Manual No. 48. Southeast Asian Fisheries Development Centre, Iloilo.
- Duy NDQ 2012 Large scale sandfish production from pond culture in Vietnam. In: Hair CA, Pickering TD, Mills DJ (Eds). Asia-Pacific Tropical Sea cucumber Aquaculture, ACIAR Proceeding No 136. Australian center for International Agriculture Research, Canberra, pp. 34-39.
- Fitridge I, Dempster T, Guenther J and De Nys R 2012 The impact and control of biofouling in marine aquaculture: a review. *Biofouling* 28(7): 649-669.
- Froese R 1998 Length-weight relationship for 18 less-studied fish species. *J. Appl. Ichthyol.* 14:117-118.
- Gamboia RU, Remie MA, Daisy AG, Lance BC and Neil Angelo SA 2012 Small-scale hatcheries and simple technologies for sandfish (*Holothuria scabra*) production. In: Hair C, Pickering T and Mills D (Eds). Asia-Pacific Tropical Sea Cucumber Aquaculture. ACIAR Proceedings No. 136. Canberra, Australia: Australian Centre for International Agricultural Research. 210 p.
- Gorospe JC, Altamirano JP and Juinio-Meñez MA 2017 Viability of a bottom-set tray ocean nursery system for *Holothuria scabra* (Jaeger 1833). *Aquac. Res.* 48 (12): 5984-5992.
- Gorospe JRC, Juinio-Meñez MA and Southgate PC 2019 Effects of shading on periphyton characteristics and performance of sandfish, *Holothuria scabra* (Jaeger 1833), juveniles. *Aquaculture* 512: 734307.
- Juinio-Meñez MA, de Peralta GM, Dumalan RP, Edullantes CA and Catbagan TO 2012 Ocean nursery systems for scaling up juvenile sandfish (*Holothuria scabra*) production: ensuring opportunities for small fishers. Asia-Pacific Tropical Sea Cucumber Aquaculture. Proceedings of an international symposium held in Noumea, New Caledonia, 15–17 February 2011. Australian Centre for International Agricultural Research, Canberra, pp. 57-62.
- Juinio-Meñez MA, Tech ED, Ticao IP, Gorospe JRC, Edullantes CMA and Rioja RAV 2017 Adaptive and integrated culture production systems for the tropical sea

- cucumber *Holothuria scabra*. *Fish. Res.* 186: 502-513.
- Lavitra T, Rasolofanirina R and Eeckhaut I 2010 The effect of sediment quality and stocking density on survival and growth of the sea cucumber *Holothuria scabra* reared in nursery pond and sea pens. *Western Indian Ocean. J. Mar. Sc.* 9(2): 153-164.
- Militz TA, Leini E, Duy NDQ and Southgate PC 2018 Successful large-scale hatchery culture of sandfish (*Holothuria scabra*) using micro-algae concentrates as a larval food source. *Aquaculture Reports* 9: 25-30.
- Mills DJ, Duy NDQ, Menez MAJ, Raison CM, Zarate JM 2012 Overview of sea cucumber aquaculture and sea ranching research in South-East Asia Region. In: Hair CA, Pickering TD, Mills DJ (Eds). *Asia-Pacific Tropical Sea cucumber Aquaculture*, ACIAR Proceeding No 136. Australian center for International Agriculture Research, Canberra, pp. 22-31.
- Natan Y, Unepetty PA, Lewerissa YA and Pattikawa JA 2015 Species and size composition of sea cucumber in coastal waters of UN bay, Southeast Maluku, Indonesia. *Int. J. Fish. Aqua. Stud.* 3(1): 251-256.
- Newell GE and Newell RC 1963 *Marine plankton: A practical guide*, pp169-225 Hutchinson educational Ltd., London.
- Pauly D 1984 *Fish Population Dynamics in Tropical Waters: A Manual for Use with Programmable Calculators*. ICLARM, Manila, 325 p.
- Pitt R and Duy NDQ 2004 Breeding and rearing of sea cucumber *Holothuria scabra* in Vietnam. In: Lovatelli A, Conand C Purcell S, Uthicke S, Hamel JF and A. Mercier A (Eds.), *Advances in Sea Cucumber Aquaculture and Management*, *FAO Fisheries Technical Paper* 463: 333-346.
- Purcell S and Simutoga M 2008 Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild. *Rev. Fish. Sci.* 16: 204-214.
- Purcell S, Hair C and Mills D 2012 Sea cucumber culture, farming and sea ranching in the tropics: progress, problems and opportunities. *Aquaculture* 368: 68-81.
- Purcell SW 2014 Value, market preferences and trade of *Beche-de-mer* from Pacific Island sea cucumbers. *PLoS One* 9(4): 95075
- Purcell SW and Agudo NS 2013 Optimisation of mesh enclosures for nursery rearing of juvenile sea cucumbers. *PLoS One* 8 (5): 64103.
- Qiu T, Zhang L, Zhang T, Bai Y and Yang H 2014 Effect of culture methods on individual variation in the growth of sea cucumber *Apostichopus japonicus* within a cohort and family. *Chin. J. Oceanol. Limnol.* 32(4): 737-742.
- Xilin S 2004 The progress and prospects of studies on artificial propagation and culture of the sea cucumber, *Apostichopus japonicus*. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF and Mercier A (Eds). *Advances in sea cucumber aquaculture and management (ASCAM)*, Food and Agricultural Organisation (FAO) of United Nation; Rome, Technical Paper 463: 273-276.