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Ocean nursery rearing of sea cucumber *Holothuria scabra* early juveniles in *hapa* nets at different stocking densities

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Abstract
Sea cucumbers are high value marine species with very high potential in aquaculture as profitable industry. Depletion of this species in the past years prompted researchers to indulge into various researches to maximize aquaculture opportunities of this species. In this study, sea cucumbers were subjected to ocean rearing to compare the survival and growth performance at different stocking densities arranged following the Randomized Complete Blocked Design. This focused on the growth performance and survival of Sea cucumber *Holothuria scabra* early juveniles reared in *hapa* nets at 250 ind m $^{-3}$, 500 ind m -3, and 750 ind m -3, respectively.
Results of the study revealed that Treatment I (250 ind m-3) obtained the highest growth and survival showing significantly difference ($p<0.05$) at the end of the culture (Day 42). Water parameters were recorded suggest that the early juveniles were exposed at the same environmental conditions while species composition and densities of food present were the same in each sampling period suggesting that the early juveniles were exposed to the same quantity and quality of food. Cost and benefit analysis showed that rearing of early juveniles in *hapa* nets reduced the cost and labor compared to rearing in tanks. Results further suggest that in ocean rearing of *Holothuria scabra,* the lower the stocking density the higher the growth rates of the species. It further shows that ocean rearing using hapa nets are profitable way of growing sea cucumbers compared in tanks. Thus, this study promotes *hapa* net rearing as an adaptable way of aquaculture technology.

Keywords: Sea cucumber, *Holothuria scabra*, nursery rearing, *hapa* nets, ocean rearing

1. Introduction
Sea cucumbers are viable candidates for sea farming, restocking and stock enhancement [1] which offer livelihood especially in coastal communities. Among commercial species of sea cucumber, sandfish (*Holothuria scabra*) is the most commercially valued tropical species [2]. Dried sandfish, *trepang* or beche-de-mer, commands high market value [3]. In the Philippines, sandfish are harvested and exported for food and medicinal purposes predominantly in China Hong Kong Special Administrative Region, Singapore, Republic of Korea, Taiwan Province of China and Japan.
Commercial exploitation of sea cucumbers in the Philippines began in the late 18th century [3] and number of species harvested has increased over the years. Collection and processing of sea cucumbers have been reported in almost all islands of the country including Bolinao, Bani and Alaminos in Pangasinan; San Fernando, La Union; San Vicente, Cagayan; Masinloc, Zambales; Polilo, Quezon; Calatagan, Batangas; Cebu; Negros Occidental; Surigao del Norte; South Cotabato with no restriction and regulation basis.
Its high value attribute contributed to the susceptibility of sandfish to overexploitation. While sandfish was an important component of beche-de-mer fisheries way back decades ago, its contribution to beche-de-mer exports is now relatively small, even trivial [2]. Its high commercial value in international market can be attributed to their pharmacological benefits and aphrodisiac properties as recognized especially in China and Malaysia [4]. It is remarkably known that sea cucumber fisheries had rapidly grown and expanded due to growing demand in the international market and various researches [4]. Aside from its high value in the market and research tools, these are also ecologically important because they help in the enhancement of ocean's productivity as biofilters [5]. They also help in the recycling of sediments and bioturbation.

However, the declining population of commercial sea cucumbers attracted global interest for mariculture and other multi-trophic culture systems due to its high market value and ability to thrive on the waste products of fish and shellfish [6]. Hatchery produced sea cucumbers are cultured in tanks at a recommended stocking density of 500-700 juveniles per m2 [2] until they reach stocking size of 1-20 grams for pond culture, sea ranching, stock restoration or farming in sea pens. A lack of published technology on nursery rearing systems has hindered cost-effective commercial production of juveniles [6].

Rearing of juveniles in hatchery usually offers space competition making it the most significant constraint in growing juveniles to stocking size of 1g above [6]. It was found out that there is a behavioral change in cultured sandfish upon reaching 1 g in weight [7]. At this weight, sandfish are growing better on sandy or muddy-sand substrates. To compensate for long grow-out cycles and potentially low survival, juveniles must be produced cheaply to competent size for stocking [8, 9]. Hatchery system for nursery rearing of juveniles are relatively labor intensive [10, 2] and cost expensive. Thus, studies have been conducted using *hapa* nets in pond system using early juveniles (15-25mm size) in New Caledonia [2]; other related studies in Central Pacific by [7]; and ocean nursery systems of early juveniles in the Philippines [11]. Trial grow-out culture of *Holothuria scabra* has been conducted [12] in Bolinao, Pangasinan from hatchery-produced juveniles with various sizes of >4g, 1-4g, and <1 g, respectively. The use of enclosure mesh (*hapa)* in the sea or earthen ponds was deemed necessary for scaling up production of juveniles because the cost of using of *hapa* and water exchange is relatively low.

Hatchery systems have been observed to be labor expensive [10, 2] and cost expensive since it uses continuous source of water and aeration system. A suitable size of 1-g juveniles is recommended for grow-out culture of sea cucumber however, space competition in tanks constraints growing of this species up to this size [6]. Since hatchery-produced sea cucumbers are cost and labor-intensive, studies to compensate long grow-out and low survival rate of sea cucumbers using cheap but competent way were tested [8,9]. Few studies on alternating hatchery-produced juveniles were conducted in New Caledonia using *hapa nets* in pond system [2], in Central Pacific [7] and in the Philippines [11]. Use of enclosure net or *hapa nets* in the sea or pond system was deemed with great potential for scaling up production of juveniles because the use of *hapa* is low and water exchange is minimized if not eliminated.

While studies and researches have been conducted on sea cucumber, there is still paucity of published data on manipulating stocking density through *hapa*-based rearing to attain highest possible survival of juveniles for grow-out purposes. It was further recommended that experimentation with floating systems is needed [6]. Growth rates and survival of sandfish in ponds to market size are also favourable, and should improve via studies on stocking density, feeding regimes and pond management [12]. Results of the trial grow-out of Purcell et al. of 2012 showed that growth and survival of *H. scabra* are affected by period and grow-out site. Mercier and Hamel of 2013 further stated that some species have been successfully reared to the juvenile stage however cost-effectiveness is not always achieved especially in *H. scabra* where grow-out techniques and global survival rates need to be improved.

Hence, this study was conducted to provide information on the optimum stocking density in the ocean nursery rearing of sea cucumber early juveniles while promoting a cost-effective way of producing juveniles for grow-out in the wild environment.

2. Materials and Methods

This study employed experimental method of research which aimed to assess the growth and survival of sandfish *(Holothuria scabra)* cultured at different stocking densities: 250 ind m-3, 500 ind m-3, and 750 ind m-3, respectively using Randomized Complete Block Design (RCBD). Hatchery-produced early juveniles of sea cucumber *H. scabra* measuring 5mm-10mm and an average weight of 0.0089g were secured and transported in the study site for almost an hour. These were left floating inside the *hapa* nets for 20 minutes for acclimatization. Biometric sampling including weight and length of the stocks was conducted every 2 weeks to monitor the growth and survival. Water parameters including temperature, salinity, pH, Total Suspended Solids (TSS), Dissolved Oxygen (DO), phosphate, ammonia, nitrite and chlorophyll were monitored during the study.

Plankton samples were collected from each hapa and added with formaldehyde to preserve the organisms. The samples were brought to the laboratory and subjected under the microscope. Plankton identification and quantification were done using Shirota (1966) and Newell and Newell (1963), respectively.

While, data on growth included weight gain, length gained and daily growth rate were computed and subjected into data analysis For initial weight, early juveniles with 0.089 g were used in the study. For the initial length, the median which was 0.75 cm was used for the computation.

Furthermore, percentage survival per treatment was calculated using the following formula

$$\text{Survival \%} = \frac{N_I - N_F}{N_I} \times 100$$

Where NI is the Initial number of specimens and NF is the final number of specimens.

For Cost and Benefit Analysis, it was computed using the equation below:

Return on Investment= Gross sale- Total Production Cost

3. Results and Discussion

3.1 Weight gain

Result (Fig 1) shows early juveniles increased in weight in every sampling period. After 42 days, Treatment I (250 ind m-3) obtained the highest mean weight gain of 1.9111 grams followed by Treatment II (500 ind m-3) and Treatment III (750 ind m-3) with mean weight gains of 1.0518 grams and 0.9213 grams, respectively. AOVA indicated significant differences ($p<0.05$) among stocking densities, sampling periods and their interactions. DMRT results suggest that weight gain at 250 ind m-3 during day 42 was significantly higher ($p<0.05$) among all treatments.

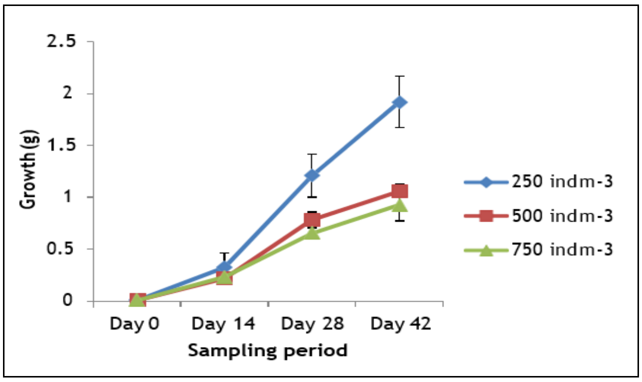

Fig 1: Weight gain trends of sea cucumber *H. scabra* early juveniles reared in *hapa* nets at different stocking densities

Results suggest that stocking density and weight gain are inversely proportional. It shows that as stocking density decreases, weight gain increases. It also goes true with other studies. Growth rates of *H. scabra* juveniles were highest at lower stocking density using nursery tanks [16].

3.2 Length gain

Results (Fig 2) show that all treatments have increasing length patterns in every sampling period. After 42 days of culture, Treatment I (250 ind m-3) attained highest mean length gain of 2.6233 cm followed by Treatment II with 1.599 cm and Treatment III with 1.5811 cm, respectively. Results showed significant differences ($p<0.05$) among stocking densities and sampling periods. However, no interaction ($p>0.05$) between the predictors was observed. It appeared that length gain at 250 ind m-3 stocking density during day 42 was significantly higher ($p<0.05$) among all treatments.

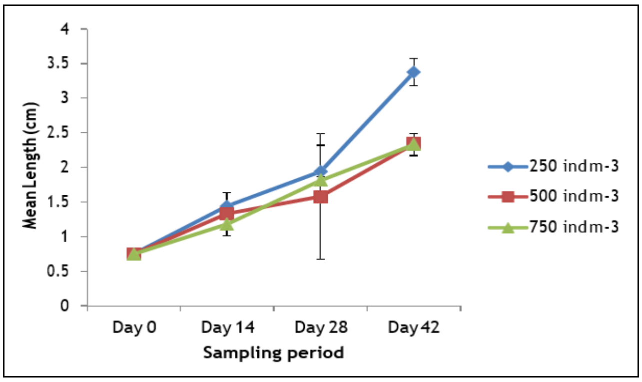

Fig 2: Mean length of sea cucumber *H. scabra* early juveniles reared in *hapa* nets at different stocking densities

Results showed that as stocking density decreased, length gained by the *H. scabra* early juveniles increased. Reference [16, 2] Have Agudo (2006) and Lavitra *et al.* (2010) derived the same results with their studies where sea cucumber juveniles attained highest growth rates at low stocking densities.

Reference [17] also Liu (2004) also suggested that, overcrowding induces a great variability in body length because it reduces available space that slows size of the species. Likewise, Slater and Carton of 2007 also found in their study on sea cucumber that growth was density-dependent and at highest densities showed lowest growth which appeared to be constrained by food limitation. However, in this study, food was abundant in all culture sites suggesting that variation in growth could be deemed with the space competition.

3.3 Daily Growth Rate

Results present an increasing trends of growth rate of sea cucumber *H. scabra* early juveniles reared in *hapa* nets at different stocking densities for 42 days. Treatment I (250 ind m-3) showed the highest daily growth rates in all sampling periods showing 0.023g, 0.086g and 0.137 g from Day 0, Day 28 and day 42, respectively. Daily growth rates of Treatment II showed 0.016g, 0.056g and 0.076g, while Treatment III obtained 0.017g, 0.047g and 0.066g for Day 0, 28 and Day 42, respectively. Two-way ANOVA result indicated significant differences ($p<0.05$) among stocking densities, sampling periods and their interactions. DMRT results suggest that weight gain at 250 ind m-3 during day 42 was significantly higher ($p<0.05$) among all treatments.

Results state that stocking density affects daily growth rate of sea cucumber early juveniles. It shows that the lower the stocking density the higher the daily growth rate of the juveniles. *H. scabra* juveniles were highest at lower stocking density using nursery tanks [16].

Lower growth rate of Treatment II and Treatment III can also be attributed to overcrowding. Overcrowding induces slow growth rates because it reduces available space that slows down growth of the species [17].

3.4 Survival Rate

Figure 3 shows the survival rate trends of sea cucumber *H. scabra* early juveniles reared in *hapa* nets at different stocking densities. A decreasing pattern in survival rates of all treatments was observed during the culture period. Highest survival rate was observed in Treatment I (250 ind m-3) with a mean survival rate of 58.27% after 42 days of culture in *hapa* nets as shown in figure below. Treatment II (500 ind m-3) obtained an average survival rate of 32.33% while Treatment III (750 ind m-3) has the lowest mean survival rate with 27.78%. Two-way ANOVA result indicated significant differences ($p<0.05$) among stocking densities, sampling periods and their interactions

DMRT results showed significantly higher ($p<0.05$) survival rate at 250 ind m-3 stocking density at the end of the culture period (Day 42).

Results of the study showed an inversely proportional relationship between stocking density and survival rate. It states that as stocking density decreases, survival rate increases. Similar results were also observed in the study on the growth of *H. scabra juveniles* [13, 2].

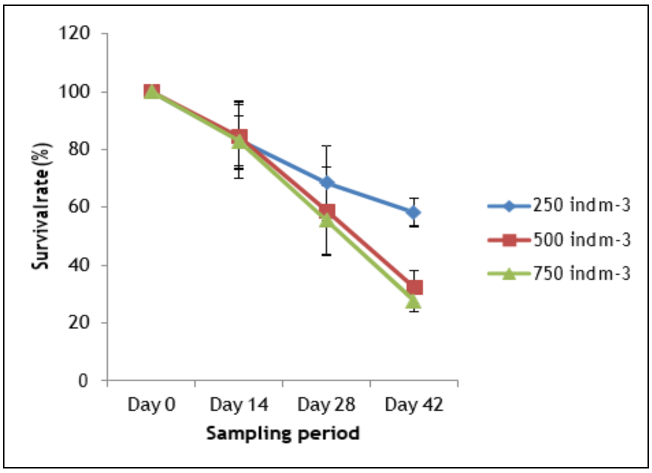

Fig 3: Survival rate trends of sea cucumber *H. scabra* early juveniles reared in *hapa* nets at different stocking densities

High mortality rates could also be attributed to the faeces settling on the *hapa* nets. High stocking densities increase potential of diseases [13]. High density in sea cucumber causes high mortality rates, low Dissolved oxygen, more faeces settling in the substrates and competition for space [18].

3.5 Physico-chemical parameters

Results present that physico-chemical parameters monitored during the implementation of the study were within the ideal range required for the rearing of sea cucumber juveniles [2].

Two-way ANOVA results showed that variation of all parameters have significant difference (p<0.05) at different sampling periods except salinity, ammonia and chlorophyll-a, respectively. DMRT results further showed that temperature was significantly higher (p<0.05) during Day 0. Consequently, pH and TSS were found to be significantly higher in all treatments during Day 14 and DO was significantly higher in Treatment I (250 ind m-3) during Day 28.

Although there were significant differences in the environmental parameter at different sampling periods, results showed that *H. scabra* early juveniles used in the study were all exposed in the same environmental conditions. Hence, confounding effects that may affect purpose of this study was minimized if not eliminated. This further suggests that space contributed to the growth and survival of the juveniles as that competition for space becomes the most significant constraint for grow-out of sea cucumber juveniles to a stocking size in tanks [2,7].

3.6 Plankton composition and density

Planktons identified from the experimental site were grouped into baciliariaceae (diatoms), mastigophora (dinoflagellates), ciliate (oligotricha) and crustacean (copepods). Lowest plankton density (850, 026) was observed in Treatment I (250 ind m-3) during the initial stocking. On the other hand, highest plankton density (4, 200,794) was recorded during Day 42 in Treatment I (250 ind m-3). Two-way ANOVA result showed that there were significant differences in the plankton density during sampling periods. DMRT further indicated that plankton densities appeared to be higher in Day 42 in all treatments compared to the previous samplings. DMRT revealed no significant difference in the species composition and plankton densities present in each *hapa* (p>0.05). It further shows that the sea cucumber *H. scabra early* juveniles were exposed to the same quantity and quality of food.

High densities during Day 42 can be attributed to the decreasing survival rate of *H. scabra* early juveniles in all treatments showing only 27.78%-58.27% at the end of the study. Thus, it shows that there was lesser number of individuals consuming the plankton compared to the previous sampling periods with higher number of individuals. It further suggests that the juveniles were exposed with same condition during sampling periods.

Moreover, results showed that the area is plankton was abundant during the conduct of the study which suggests that the culture area can support sea cucumber culture regardless of the stocking density. It can be inferred that food was not a factor that may have affected the growth and survival of the *H. scabra* early juveniles in this experiment. Hence, the growth and survival of the *H. scabra* early juveniles may be attributed to the space competition.

Stocking densities influence survival, feeding, growth and general health of sea cucumber juveniles. High stocking densities increase the incidence of diseases [13].

3.7 Cost and benefit analysis

The cost and benefit analysis for rearing sea cucumber early juveniles in tanks and in *hapa* was compared. It reveals that *hapa*-based rearing is more economical compared to rearing in tanks.

Results presented that production cost in tanks was a higher compared to rearing in *hapa* nets. Nursery system in tanks requires machines and other equipment to ensure good health of the juveniles. Nursery system in tanks also required daily water management which caused additional labor. On the other hand, nursery system in marine waters using *hapa* nets requires no water management and no equipment which suggests a cost and labor effective system.

4. Conclusions

Growth and survival of sea cucumber *H. scabra early* juveniles and stocking density are inversely proportional. It states that as stocking density decreases, growth and survival of sea cucumber juveniles increase. The stocks were exposed with the same environmental conditions during the conduct of the study suggesting that space competition contributed in the growth response and survival of the stocks. Plankton was abundant in the area especially diatoms which serve as nutrition for sea cucumber *H. scabra* early juveniles. Use of *hapa nets* in the nursery rearing of sea cucumber *H. scabra* early juveniles is more economical compared to the culture system using tanks. Sea cucumber culture offers a great potential for livelihood. Sea cucumbers play significant role in the environment and offers potentials to many researches.

5. Recommendations

Proper agencies should prelude the development of ocean nursery technology for *H. scabra*. Natural resources including sea cucumbers must be managed and utilized with regulations. Ocean nursery using *hapa* nets can be used for scaling up production of sea cucumber juveniles because it requires lower expenses compared to culture system in tanks. Use of *hapa* nets should be properly disseminated to enhance small fishers in the involvement of culture.

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