Marine and Fishery Sciences 36 (2): xxx-xxx (2023)
https://doi.org/10.47193/mafis.3622023010504

TECHNICAL PAPER

Can commercially available mosquito nets be used for rearing sandfish (*Holothuria scabra*) juveniles in floating ocean nursery?

Margarita de la Torre-de la Cruz*, Jonh Rey L. Gacura, Racelle R. Rescordado and Janine L. Villamor

Guiuan Development Foundation, Inc., Guimbaolibot Avenue, Poblacion Brgy. 10, Guiuan, Eastern Samar, 6809, Philippines. ORCID *Margarita de la Torre-de la Cruz* https://orcid.org/0000-0002-6947-5632, *Jonh Rey L. Gacura* https://orcid.org/0000-0002-3265-0150, *Racelle R. Rescordado* https://orcid.org/0000-0002-8290-7268, *Janine L. Villamor* https://orcid.org/0000-0003-3821-4488

ABSTRACT. This study investigates the potential of locally available mosquito nets as an alternative material for sandfish ocean nurseries. Mosquito nets (~ 2 mm mesh) were designed into single (SL), double (DL), and triple-layered (TL) to approximate the conventional hapa (CH) with ~ 1 mm mesh normally used in sandfish ocean nurseries. The study was conducted in Maliwaliw island and in a cove in Buyayawon both located in Eastern Samar, Philippines. Results showed that TL had the highest juvenile survival (35.93 ± 10.56%) in Maliwaliw, which was significantly different ($p < 0.05$) from SL and DL. The DL design showed the highest survival (25.23 ± 17.15%) in Buyayawon by day 60, followed by TL (21.37 ± 3.11%), although not statistically significant. On the other hand, average growth rate was highest in SL in both sites where survival was also the lowest. Biomass, however, was the highest in DL in Buyayawon (1,014 ± 266 g m^{-2}) followed by TL in Maliwaliw (925 ± 107 g m^{-2}). Sandfish juvenile survival in DL and TL were higher than those in the CH in both sites suggesting that mosquito nets can indeed be used as an alternative material for rearing early-stage sandfish juveniles in floating ocean nurseries.

Key words: Ocean nursery, post-settled sandfish, growth, survival, Philippines.

¿Se pueden utilizar redes mosquiteras disponibles en el mercado para criar juveniles del pepino de mar (*Holothuria scabra*) en jaulas oceánicas flotantes?

RESUMEN. Este estudio investiga el potencial de los mosquiteros disponibles localmente como un material alternativo para los criaderos de pepinos de mar en el océano. Los mosquiteros (malla de ~ 2 mm) se diseñaron en una sola (SL), doble (DL) y triple capa (TL), para aproximarse al hapa convencional (CH) con malla de ~ 1 mm que normalmente se usa en los criaderos de pepinos de mar en el océano. El estudio se realizó en la Isla de Maliwaliw y en una cala en Buyayawon, ambas ubicadas al Este de Samar, Filipinas. Los resultados mostraron que el TL tuvo la supervivencia de juveniles más alta (35,93 ± 10,56%) en Maliwaliw, significativamente diferente ($p < 0,05$) de los diseños SL y DL. La DL obtuvo la mayor supervivencia (25,23 ± 17,15%) en Buyayawon al día 60, seguido de TL (21,37 ± 3,11%), aunque no fue estadísticamente significativa. Por otro lado, la tasa de crecimiento promedio fue más alta en SL en ambos sitios, en donde la supervivencia también fue la más baja. La biomasa, sin embargo, fue más alta en DL en Buyayawon (1.014 ± 266 g m^{-2}) seguida de TL en Maliwaliw (925 ± 107 g m^{-2}). La supervivencia de juveniles de pepinos de mar en DL y TL fue mayor que la de CH en ambos sitios, lo que sugiere que las redes mosquiteras pueden usarse como un material alternativo para criar juveniles de pepinos de mar en etapa temprana en jaulas oceánicas flotantes.

Palabras clave: Criadero oceánico, pepino de mar bentónico, crecimiento, supervivencia, Filipinas.

*Correspondence:
mtdelacruz1909@gmail.com

Received: 16 January 2023
Accepted: 2 March 2023

ISSN 2683-7595 (print)
ISSN 2683-7951 (online)

https://ojs.inidep.edu.ar

Journal of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)

INTRODUCTION

Holothuria scabra, commonly known as sandfish, are high-value sea cucumber species cultured in the Philippines and other countries. The success of sandfish grow-out culture largely depends on the supply of sandfish juveniles. Fortunately, the technology for juvenile sandfish production in land-based hatcheries are well established (Agudo 2006; Gamboa et al. 2012; Juinio-Meñez et al. 2012). Post-settled hatchery-produced sandfish juveniles (< 5 mm in length) are transferred to ocean nursery systems (Agudo 2006; Gamboa et al. 2012; Juinio-Meñez et al. 2012; Mills et al. 2012) for further rearing. Sandfish culture techniques in nursery systems vary in different countries. In Vietnam, and northern Australia, pond nursery systems employ hapa nets stocked with sandfish juveniles measuring 1-2 mm (Pitt and Duy 2004; Bowman 2012). In New Caledonia, nursery systems follow two phases (Agudo 2006). Early-stage juveniles (< 5 mm) are maintained in bare (without sand) tanks until they reach a size of about 10-20 mm (1 g). Then, they are transferred to tanks lined with a thin layer of sand enriched with mud or food supplement. While this hatchery-based nursery system has shown good results, this is not practical in the Philippines because of the high operating cost and space limitations (Juinio-Meñez et al. 2012). Marine ponds with sandy substrate are also not common in the country. To address these bottlenecks in sandfish juvenile production, the floating ocean nursery system was developed (Juinio-Meñez et al. 2012).

Ocean nursery rearing of early-stage sandfish juveniles (~ 3 mm) uses a conventional hapa made from fine nylon net with ~ 1 mm mesh size (Gamboa et al. 2012; Sinsona and Juinio-Meñez 2019; Altamirano and Noran-Baylon 2020). Juveniles are reared for 30-60 d before released to sea ranch or enhancement sites.

A study conducted in Bolinao (Pangasinan province) comparing the growth and survival of sandfish in ocean floating hapas and hapas in ponds found that survival rates of juveniles in both hapa systems were similar albeit with lower average growth rates in ocean nursery (Juinio-Meñez et al. 2012). Likewise, a study by Cabacaba and Campo (2019) in Guiuan, Eastern Samar, comparing the growth and survival of sandfish juveniles in floating hapas and those in rearing tanks, showed better growth of juveniles in the floating hapas but lower survival rate. However, the difference in the survival rate was not significant. Same authors recorded 64.88% survival of 1,000 early sandfish juveniles stocked per hapa in floating ocean nursery after 45 d of rearing, while Altamirano and Noran-Baylon (2020) recorded 70.5% survival after 60 d. According to Juinio-Meñez et al. (2012), ocean nurseries using floating hapas are cost-effective and strategic for community-based grow-out and sea ranching. However, the fine nylon mesh net (~ 1 mm) used in ocean nursery systems is not readily available in local stores in Eastern Samar nor in Eastern Visayas. They came from Western Visayas and are rather expensive for small-scale fishers who want to go into sandfish rearing. One piece of locally available mosquito net costs only about USD 3.00, whereas fine mesh nets cost USD 30.00 a piece, including handling and delivery costs. Hence, this study was conducted to investigate the potential of using locally available mosquito nets as an alternative material for rearing early-stage sandfish juveniles in floating ocean nurseries.

MATERIALS AND METHODS

Field experiments were conducted from March to May 2021 in the nearshore waters of Barangay (smallest political unit in the Philippines) Maliwaliw, Salcedo (Site 1), and in Barangay Buyayawon, Mercedes (Site 2) in Eastern Samar, Philippines (Figure 1). Site 1 (11.10513° N-125.58088° E) is adjacent to a mangrove and seagrass bed and exposed to moderate to strong wave action. Site 2 (11.10886° N-125.69852° E) is a cove with minimal wave exposure, and water flow/circulation is constrained by a provincial road cutting through and made possible only through a short bridge. Some houses are situated along the coastline of the cove which could be a potential source of nutrient inputs into the water.

Mosquito nets used has a dimension of 1.7 m × 0.9 m × 1.2 m (length × width × height) and do not have uniform mesh size. Because of the mesh size, three designs were tried: single-layer (SL), double-layer (DL), and triple-layer (TL) alternating bigger and smaller mesh sizes. The DL and TL were made by overlaying 2 and 3 mosquito nets, respectively. To prevent flapping of the overlayed nets while at sea, the bottom and sides of nets were sewed horizontally and vertically (Figure 2). For each site, 12 hapas were installed consisting of triplicate of each mosquito hapa net design, and the conventional hapa. Each hapa net is attached to a rectangular frame (2 m × 1 m) made from PVC pipe (4" diameter). To maintain the rectangular shape of the bottom, a rectangular perforated (2 m × 1 m) frame made from 3/4" blue PVC pipe were also attached to each hapa net. Improvised sinkers made from re-used plastic bottles (~ 1 l) filled with sand were also attached to the four corners of the hapa net.

Prior to stocking juveniles, nets were pre-conditioned for three days, following nursery culture

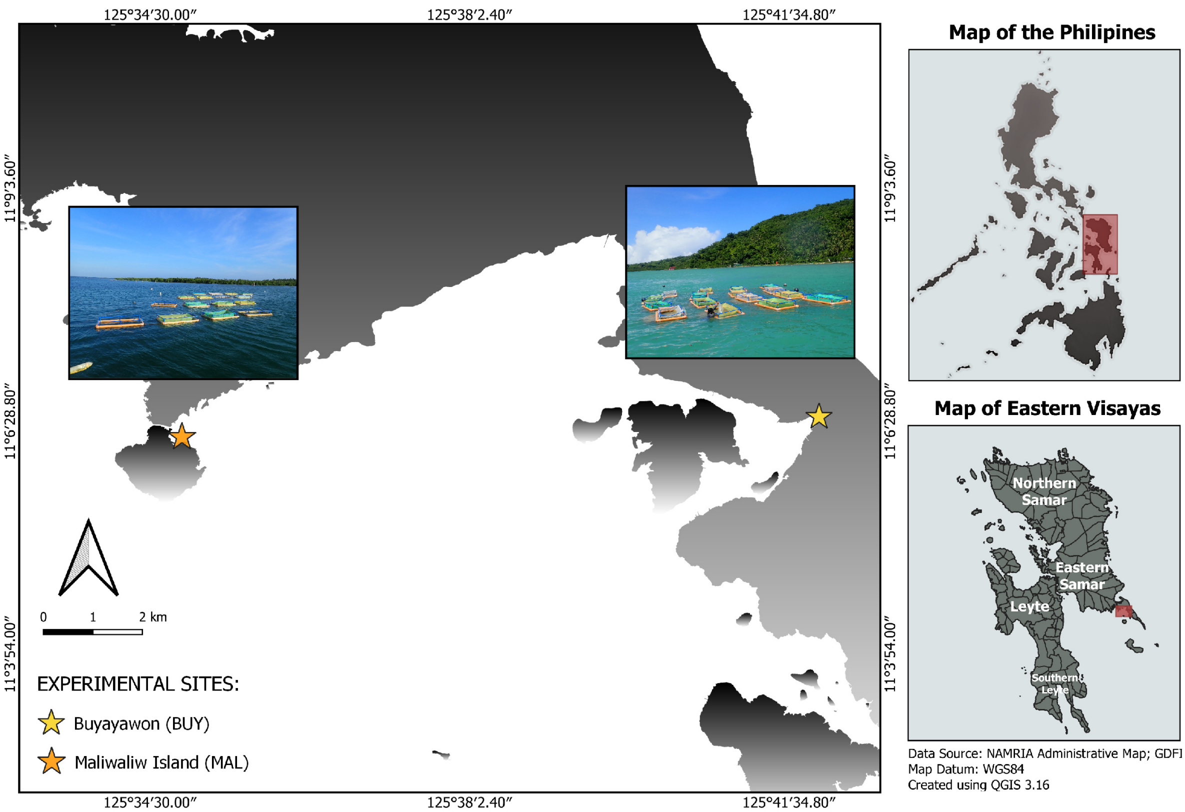

Figure 1. Location of experimental sites in Eastern Samar, Philippines. Data Source: NAMRIA Administrative Map, GDFI 2022.WGS84. QGIS 3.16.

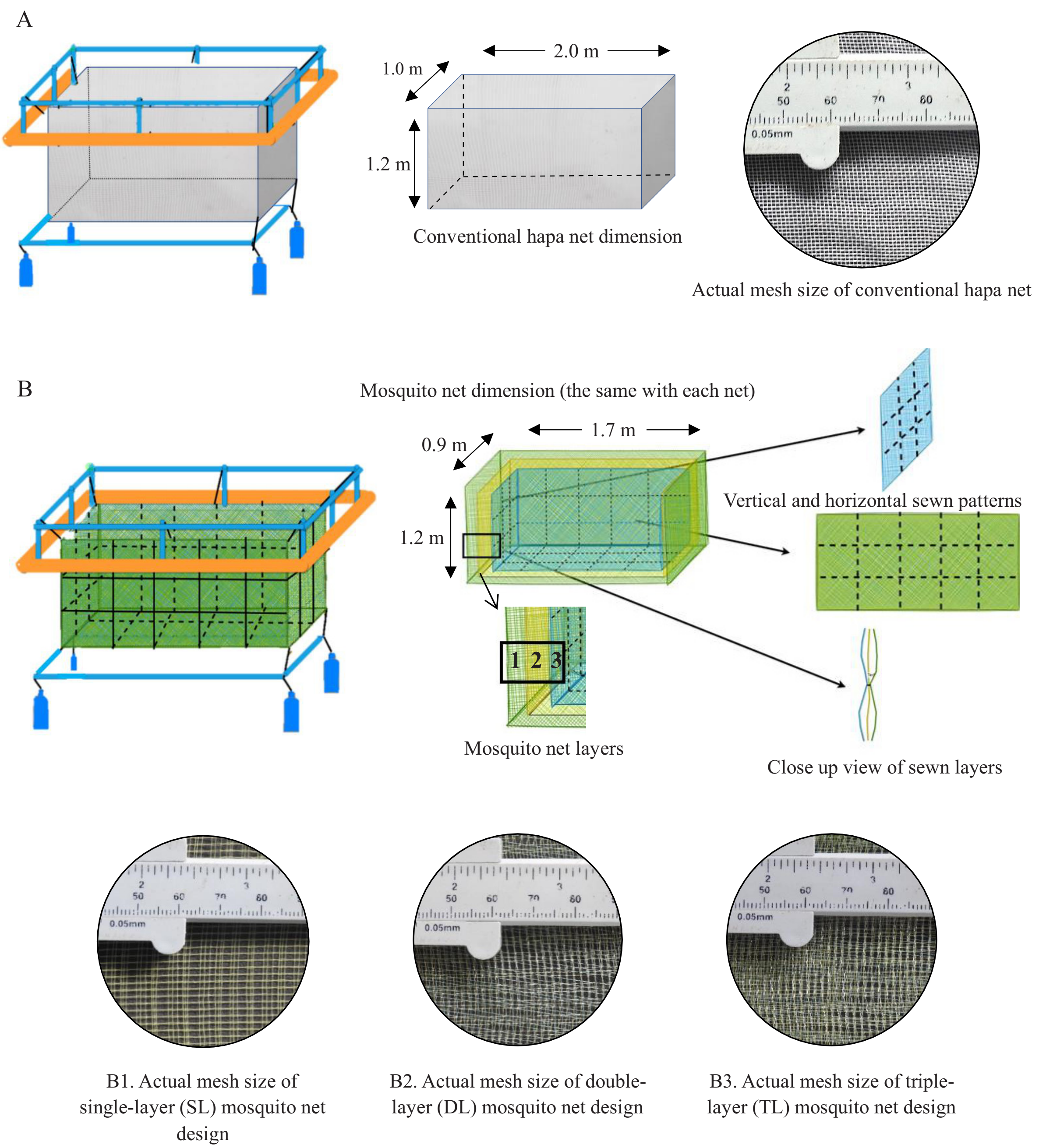

Figure 2. Diagram of experimental set-up: conventional hapa, dimension, and actual image of mesh size (A); and mosquito net design, dimension, layering, sewn patterns, and actual image of mesh sizes of different layers (B).

protocols (Gorospe et al. 2019; Sinsona and Juinio-Meñez 2019; Altamirano and Noran-Baylon 2020). Pre-conditioning allows colonization of periphyton biofilm, which will serve as food source for sandfish juveniles. Twenty-four thousand early stage (50 d post settlement) sandfish juveniles were obtained from the Bureau of Fisheries and Aquatic Resources-Guiuan Marine Fisheries Development Center (BFAR-GMFDC) sea cucumber hatchery facility. To get the initial weight, juveniles were photographed with scale reference and length was measured using CPCE V4.1 software. Body weight values were then derived using the equation, $W = 0.000614*L^{2.407}$ (Purcell and Agudo 2013). Juveniles were then packed in transparent polyethylene plastic bags filled with seawater at 1,000 juveniles per bag. Oxygen was pumped into each bag before closing it tightly with a rubber band, and then transported in a boat to the study sites in Maliwaliw Island, and Buyayawon. Maliwaliw and Buyayawon are approximately 8.74 and 6.15 nm away from the BFAR-GMFDC hatchery, respectively. Juveniles were acclimatized *in situ* for 5 min before they were slowly poured out of the plastic bags into each hapa net. Each net was stocked with 1,000 juveniles.

Mean weight, average growth rate (AGR), and survival of stocked juveniles were monitored on day 30 (D30) and 60 (D60). Average growth rate (AGR) was calculated as the difference in the final average weight and initial average weight divided by the number of rearing days (Altamirano and Noran-Baylon 2020). Biomass was computed as the ratio of the product of the number of survival and mean weight divided by the bottom area of the hapa. Survival of juveniles was monitored by counting all surviving juveniles on D30 and D60. To avoid biofouling, nets were cleaned every 15 d. Nets were replaced with preconditioned nets on D30.

Growth and survival data among net designs were analyzed using IBM* SPSS* Statistics Version 21. Prior to analysis, all measurements and computed data (e.g. AGR, biomass) were tested for normality using Shapiro-Wilk Test ($p > 0.05$). The effect of site and hapa net layers on sandfish mean weight, AGR, survival, and biomass on D30 and D60 were analyzed using two-way ANOVA. Where significant differences were found, Tukey HSD was used as post-hoc test. Where there is a statistically significant interaction effect found ($p < 0.05$), a simple main effects analysis was carried out using the SPSS Statistics syntax. Data on the conventional hapa for both sites were excluded from the analysis since Maliwaliw has missing hapa replicates due to Typhoon Surigae.

RESULTS

On D30, DL had the highest mean wet weight at Site 1 (2.38 ± 0.69 g) and Site 2 (1.01 ± 0.02 g) (Figure 3). In contrast, SL recorded the lowest mean weight at Site 1 (1.84 ± 0.17g) and in TL at Site 2 (0.89 ± 0.11 g). However, results did not significantly differ among net designs ($p = 0.458$). At D60, SL recorded the highest mean weight in both Sites 1 (11.68 ± 4.78 g) and 2 (9.87 ± 9.15 g), while TL recorded the least in both Sites 1 (4.23 ± 1.53 g) and 2 (4.71 ± 0.55 g). Statistical analysis did not show significant differences among net designs (two-way ANOVA, $p > 0.05$). The mean weight of the surviving juveniles in CH was 2.02 g, but from 1 hapa net only as two of the CH nets were overturned during typhoon Surigae (*Bising*) prior to D30 monitoring. At Site 2, the mean weight of juveniles in CH (0.93 ± 0.14 g) was second highest to DL, but differences were not significant to all mosquito net designs. With respect to location, Site 1 had significantly bigger sandfish (2.04 ± 0.46 g) on D30 regardless of net design ($p < 0.05$), but did not significantly differ from Site 2 on D60.

Similar to the mean weight, significant differences of AGR between sites were found on D30 only. At Site 1, AGR of juveniles was highest in DL followed by CH, while SL and TL had same values. At Site 2, all mosquito net designs including CH had the same AGR values (0.03 g day^{-1}). At D60, SL and DL had the highest AGR (0.19 g day^{-1}), while TL (0.07 g day^{-1}) recorded the low-

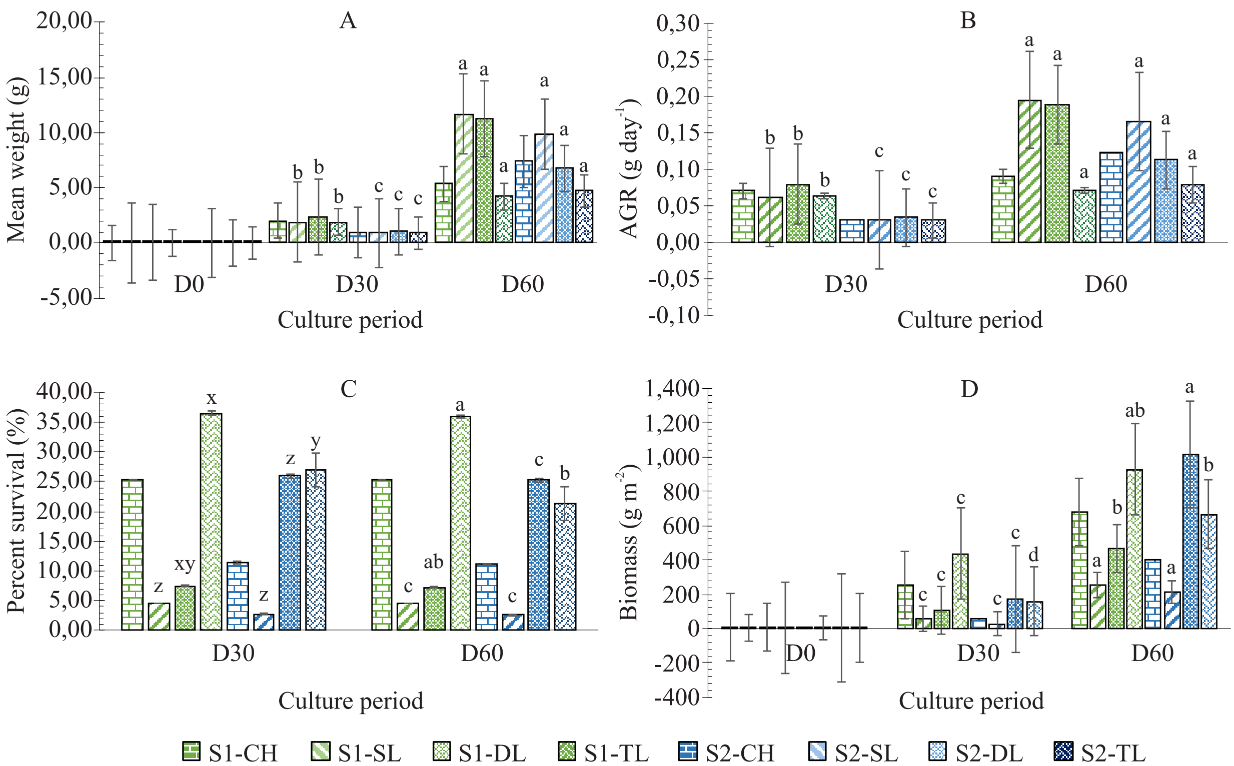

Figure 3. Mean weight (A), average growth rate (B), percent survival (C), and biomass (D) of sandfish (*Holothuria scabra*) reared in different sites (S1 = Maliwaliw, Salcedo, Eastern Samar; S2 = Buyayawon, Mercedes, Eastern Samar) using different net designs (CH = conventional hapa; SL = single layer mosquito net; DL = double layer mosquito net; and TL = triple layer mosquito net) from March to May 2021. Means with the same lower case indicate homogeneous groups as determined by two-way ANOVA and Tukey HSD post-hoc test ($p < 0.05$).

est at Site 1. Similarly, SL (0.16 g day^{-1}) had the highest AGR at Site 2 followed by CH (0.12 g day^{-1}), while TL (0.08 g day^{-1}) had the lowest. However, values were not statistically different between sites nor among net designs ($p > 0.05$).

Significantly differing results in survival of sandfish juveniles between sites and among net designs were found during the culture period ($p < 0.05$). At D30, highest survival was recorded in TL in both sites (36.53% and 27.07%, respectively), while SL consistently had the lowest. At D60, best survival was still observed in TL (35.93%) at Site 1 while DL (25.23%) surpassed the TL (21.37%) at Site 2, albeit not significantly ($p = 0.607$). The juvenile survival in DL was significantly higher ($p < 0.05$) at Site 2 than Site 1 at D30 and D60. Interestingly, sandfish juveniles in CH had low percentage survival with 25.30% for one net only at Site1 and 11.13% at Site 2 on D60.

Biomass (g m^{-2}) were significantly different ($p < 0.05$) between the SL, DL, and TL ($p = 0.001$). Moreover, there is a statistically significant difference between the biomass of sandfish reared at Site 1 and Site 2 for TL ($p = 0.002$). At D30, TL biomass at Site 1 was highest (433.88 ± 126.01 g m^{-2}) among all net designs and between sites. At Site 2, DL had the highest biomass at (172.77 ± 123.34 g m^{-2}) but did not significantly differ from TL and SL at 159.18 ± 41.77 g m^{-2} and 28.22 ± 43.20 g m^{-2}, respectively. At D60, biomass in DL (1014.17 ± 455.14 g m^{-2}) at Site 2 surpassed TL (924.66 ± 107.13g m^{-2}) at Site 1, albeit not significantly ($p > 0.05$). Similar to growth and survival, biomass produced in CH on D60 at Sites 1 and 2 (679.31 g m^{-2} and 398.20 g m^{-2}, respectively) was lower than that of TL in both sites.

DISCUSSION

This is a preliminary study on the potential of using mosquito nets as an alternative material for rearing early-stage sandfish juveniles in floating ocean nurseries. Sandfish growth performance on D30 at Site 2 was lower compared to those reared at Site 1. Though food plays a vital role in the growth and development of any organism, food availability may not be a problem as Site 2 had more food available indicated by the high chlorophyll-*a*, and ash-free dry weight (unpublished data) of periphyton, which measures microalgae and total biomass, respectively. Factors that may have affected the growth performance of juveniles might be related to environmental parameters in each site. According to Magcanta et al. (2021), water salinity affects the growth of sandfish juvenile. High precipitation brought by heavy rains and typhoon Surigae during the first 30 d of rearing could have lowered the water salinity at Site 2 which has restricted water movement. During this period, low surface water salinity ranging from 20.95 to 25.40 was recorded for Site 2. Altamirano et al. (2021) also noted poor growth performance in sandfish reared in protected coves with less water movement than those reared at sites exposed to relatively high-water movements. However, this was more related to the effects of flow velocity on biofilm colonization in hapa nets rather than on water salinity.

Survival of sandfish juveniles in mosquito net hapas during the first 30 d of rearing greatly depended on the design (i.e. SL, DL, TL) which may be due to the juvenile-size and mesh-size relationship. Since sandfish juveniles stocked in the hapas on D0 were only 1-5 mm in length, many juveniles could have easily escaped from the SL (~ 2 mm mesh) set-up. On the other hand, some juveniles in DL and TL were found trapped in between the net layers. In contrast, there is nowhere to go for juveniles in SL but out to the sea if they escape from the relatively big mesh size of the mosquito net. Thus, the significantly higher juvenile survival especially in TL can be attributed to the net design.

The low survival in DL at Site 1 compared to Site 2 may be due to higher exposure to wave action. In addition, the impact of typhoon Surigae may have caused juveniles to be 'squeezed' out of the outer net. Conversely, survival of sandfish juveniles in CH at Site 2 was lower compared to the CH (remaining unit) at Site 1. This could be due to the fouling of hapa nets at Site 2 which has minimal water disturbance unlike Site 1. Such condition allows faster biofilm accumulation and subsequent fouling particularly as CH has very fine mesh. Other factors such as handling, predation (Cabacaba and Campo 2019; Altamirano and Noran-Baylon 2020), and salinity fluctuations (Magcanta et al. 2021) during heavy rains also affect growth and survival of sandfish juveniles.

While sandfish juveniles from SL had higher mean weight at both sites, the juvenile survival was incredibly low, and the resulting biomass is also low (251 ± 184 g m^{-2} and 214 ± 455 g m^{-2} for Site 1 and Site 2, respectively). This suggests that SL is not suitable for rearing early-stage sandfish juveniles. On the other hand, TL showed better growth and survival performance in both sites. Additionally, the survival of sandfish juveniles from D31-D60 was high (98.63%) after restocking, which was observed on D60. Unfortunately, it is not possible to compare the growth and survival performance of TL and CH at Site 1, since juveniles were lost when two replicate CH nets got overturned during typhoon Surigae before the D30 monitoring.

Results of this study demonstrated the potential of using mosquito nets in rearing early-stage sandfish juveniles in ocean nursery systems. In particular, the DL and TL designs can substitute the CH. The sandfish biomass at Site 2 on D60 for DL and TL was much higher than that from CH. Likewise, the biomass produced at Site 1 for TL was also much higher than CH. Moreover, the cost of the DL (USD 6.00) and TL (USD 9.00) per unit is significantly much lower than that of the CH (USD 30.00). To validate the results and improve ocean nursery systems of sandfish with the use of mosquito nets, studies on the effect of a) wet and dry seasons, b) cleaning frequency, c) predation, d) stocking density, and e) food availability on the growth and survival of sandfish juveniles using DL and TL are recommended.

ACKNOWLEDGMENTS

This work was supported by the Australian Center for International Agricultural Research (ACIAR) through the (FIS/2016/122) 'Increasing technical skills supporting community-based sea cucumber production in Vietnam and the Philippines' Sincere thanks to community partners in Maliwaliw and Buyayawon, and to Mr Roy Francis M. Abuda for their assistance during the conduct of the study. The help of Mr Cristan Campo in processing our data is also much appreciated.

Disclosure statement

All authors have read and approved the final manuscript and declare that they have no conflicts of interest.

Data availability statement

The authors confirm that all relevant data and its supporting information are within the manuscript.

REFERENCES

Agudo N. 2006. Sandfish hatchery techniques. Noumea: Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and, WorldFish Center. 44 p.

Altamirano JP, 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. Aquaculture. 526: 735379. DOI: https://doi.org/10.1016/j.aquaculture.2020.735379

Altamirano JP, Sinsona MJ, Caasi OJC, de la Torre-de la Cruz M, Uy WH, Noran-Baylon R, Juinio-Meñez MA. 2021. Factors affecting the spatio-temporal variability in the production of sandfish *Holothuria scabra* juveniles in floating hapa ocean nursery systems. Aquaculture. 541: 736743. DOI: https://doi.org/10.1016/j.aquaculture.2021.736743

Bowman WM. 2012. Sandfish production and development of sea ranching in northern Australia. In: Hair CA, Pickering TD, Mills DJ, editors. Asia-Pacific tropical sea cucumber aquaculture, Proceedings of an international symposium held in Noumea, New Caledonia, 15-17 February 2011. ACIAR Proceedings No. 136. Canberra: Australian Centre for International Agricultural Research. p. 75-78.

Cabacaba NS, Campo CJM. 2019. First-phase juvenile rearing of the sea cucumber *Holothuria scabra* in Eastern Samar, Philippines. Philippine J Fish. 26 (2): 72-81. DOI: https://doi.org/10.31398/tpjf/26.2.2019A0009

Gamboa RU, Aurelio RM, Ganad DA, Conception LB, Abreo NAS. 2012. Ocean nursery systems for scaling up juvenile sandfish (*Holothuria scabra*) production: ensuring opportunities for small fishers. In: Hair CA, Pickering TD, Mills DJ, editors. Asia-Pacific tropical sea cucumber aquaculture. Proceedings of an international symposium held in Noumea, New Caledonia, 15-17 February 2011. ACIAR Proceedings No. 136. Canberra: Australian Centre for International Agricultural Research. p. 63-74.

Gorospe JR, Juinio-Meñez MA, Southgate PC. 2019. Effects of shading on periphyton characteristics and performance of sandfish, *Holothuria scabra* Jaeger 1833 juveniles. Aquaculture. 512: 734307. DOI: https://doi.org./10.1016/j.aquaculture.2019.734307

Juinio-Meñez MA, de Peralta GM, Dumalan RJP, Edullantes CMA, Catbagan TO. 2012. Ocean nursery systems for scaling up juvenile sandfish (*Holothuria scabra*) production: ensuring opportunities for small fishers. In: Hair CA, Pickering TD, Mills DJ, editors. Asia-Pacific tropical sea cucumber aquaculture. Proceedings of an international symposium held in Noumea, New Caledonia, 15-17 February 2011. ACIAR Proceedings No. 136. Canberra: Australian Centre for International Agricultural Research. p. 57-62.

Magcanta ML, Sornito MB, Espadero AD, Bacosa HP, Uy WH. 2021. Growth, survival, and behavior of early juvenile sandfish *Holothuria scabra* (Jaeger, 1883) in response to feed types and salinity levels under laboratory conditions. Philippine J Sci. 150 (5): 901-913.

Mills DJ, Duy NDG, Juinio-Meñez MA, Raison CM, Zarate JM. 2012. Overview of sea cucumber aquaculture and sea-ranching research in the South-East Asian region. In: Hair CA, Pickering TD, Mills DJ, editors. Asia-Pacific tropical sea cucumber aquaculture. Proceedings of an international symposium held in Noumea, New Caledonia, 15-17 February 2011. ACIAR Proceedings No. 136. Canberra: Australian Centre for International Agricultural Research. p. 22-31.

Sinsona MJ, Juinio-Meñez MA. 2019. Periphyton characteristics influence the growth and survival of *Holothuria scabra* early juveniles in an ocean nursery system. Aquacult Res. 50 (9): 2655-2665. DOI: https://doi.org/10.1111/are.14223

Pitt R, Duy NDQ. 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A, editors. Advances in sea cucumber aquaculture and management. FAO Fish Tech Pap. 463: 333-346.

Purcell SW, Agudo NS. 2013. Optimisation of mesh enclosures for nursery rearing of juvenile sea cucumbers. PLoS ONE. 8 (5): e64103. DOI: https://doi.org/10.1371/journal.pone.0064103