EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 11(3) (2018) 148-160
DOI 10.14456/ea.2018.44
ISSN 1906-1714; ONLINE ISSN: 2586-8861

# Effect of *Holothuria scabra* (Jaeger) on Particle Size Composition and Components of Sediments

Margarita de la Torre-de la Cruz[1, 2*], Janine L. Villamor [2] and Rose Jane P. Diodoco[2]

[1] *University of the Philippines Visayas Tacloban College*
[2] *Guiuan Development Foundation, Inc.*

*Corresponding Author: cruzmarge2003@yahoo.com
Received: January 21, 2018; Accepted: June 27, 2018

## ABSTRACT

To determine the effect of sea cucumbers on the sediment components and particle composition, hatchery produced juveniles of *Holothuria scabra* (sandfish) were raised in pens for one year. Four 100 m$^2$ circular experimental pens were set up in the nearshore area of Maliwaliw Island, Salcedo, Eastern Samar, Philippines. Three pens were stocked with 200 (5.46±1.96g) juveniles each and one pen served as control. Sediment samples were collected from each of the pens before stocking and every quarter thereafter. Sediment grain size, total and labile organic matter, and chlorophyll-a were determined. There was no significant difference in the changes in sediment size particle between the control and the experimental pens from the start to the end of the study. Analysis of the sediment chlorophyll-a, total and labile organic matter showed increased values at the end of the study in both the experimental pens and the control pen also showing no significant differences. In the experimental pens, only 29 individuals were recovered from pen 1 with an average weight of 341.99 g at the end of the study. In pen 2 and 3, 19 and 14 individuals were recovered with an average weight of 353.14 g and 316.04 g, respectively. With 10.33% of the stocked sandfish recovered from the experimental pens, the results indicate that the area can support sandfish sea ranching at two sandfish per m$^2$ without causing sediment degradation.

***Keywords:*** *H. scabra;* Changes in sediment; Grain-size; Organic matter; Chlorophyll-a

## 1. Introduction

Sea cucumbers, *Holothuria scabra* commonly known as sandfish, are habitually found in the sandy or muddy areas overgrown with seagrass throughout the tropical Indo-Pacific region (Mercier *et al.* 2000; Conand, 2004). They are detritus feeders (Watanabe *et al.,* 2012), deposit feeders and suspension feeders (Graham and Thompson, 2009) which move along the surface or burrow within soft sediments. They ingest sediments, and digest and assimilate some of the non-living and living organic matter in it (Lopez and Levinton, 2011). According to Mezali and Soualili (2013), an important concept in the ecology of benthic species is the particle selectivity by detritus feeders. The holothurians, as detritus feeders, utilize the organic matter that coats sediment and the detrital particles as food. It has been proposed that particle size along with niche separation is one resource axis and can be observed in the optimal foraging strategy. It was suggested in previous studies (Mercier *et al.,* 1999; Hamel *et al.,* 2001) that Holothurians can recognize sediments with high organic content but have no food preference.

Sea cucumbers play an important role in bioturbation, a process where living organisms participate in the particle-mixing of sediments (Herringshaw and Solan, 2008). As bioturbators of sediments in many marine ecosystems (Wolkenhauer *et al.,* 2010), deposit-feeding sea cucumbers can reduce the accumulation of excess organic matter in coastal sediments such as under aquaculture farms (Slater and Carton, 2009). They bioturbate and bioirrigate the upper layer of marine sediments, resulting in increased depth of the mixed layer. Furthermore, organic matter remineralisation and decomposition, recycling of nutrients, and the flux of materials from the sediment in the water column are altered (Teal *et al.,* 2008).

However, in the rapid expansion of marine aquaculture to meet food and production demand, Plotieau *et al.* (2013) speculated that large-scale farming enterprises in lagoons may result in some alterations of benthic community structure. Further, they raised the concern of high-density sea cucumber farming drastically changing the sediment components. To date, there are very few studies dealing with the impact of *H. scabra* farming on the sediments like the study conducted by Plotieau *et al.* (2013). This study assessed the changes in the sediment characteristics in terms of particle size composition, and sediment components in terms of organic matter and chlorophyll-a content over time, which could be attributed to the sandfish.

## 2. Materials and Methods

### *2.1 Study Site and Experimental Set-up*

This study employed the method developed by the Australian Centre for International Agricultural Research (ACIAR) FIS/2010/042 project. Three (3) 100 $m^2$ circular experimental pens and one (1) control pen were established in the nearshore area of Maliwaliw Island, Salcedo, Eastern Samar (Figure 1). The area is characterized by patches of seagrass dominated by *Thalassia hemprichii.* The pens were constructed using wooden poles and black D- nets. The D-net was attached to the poles up to a meter high and the lower part of the net was buried six inches deep to prevent the sandfish from escaping. Suspected predators such as brittle stars, starfish, gastropods, and crabs were collected one week before initial stocking. This was done to ensure the survival of the juveniles and so that changes in the sediment are attributable to the stocked sandfish. Five sampling stations; one station in the middle and four (4) stations on the sides around it were established in each pen for sediment sampling.

Two-month old sandfish juveniles sourced from the hatchery of the Bureau of Fisheries and Aquatic Resources (BFAR) in Guiuan, Eastern Samar were reared in floating hapa nets at the Bagonbanua Marine Reserve and Fish Sanctuary. After two months, sandfish juveniles were sand-conditioned in bottom set hapas at Maliwaliw Island for two weeks. Six hundred (600) juveniles (mean body weight, BW=5.46 ± 1.96g) were then stocked in the three experimental pens at 200 individuals per pen.

Salinity (ppt) and water temperature (°C) near the bottom were recorded at an hourly interval using a Star Oddi™ DST logger attached to the lower portion of a wooden post placed inside Pen 3. Rainfall data was obtained from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) Weather Station in Guiuan, Eastern Samar.

## 2.2 Sediment Sampling

Sediment samples were collected from each of the pens before stocking (July 2015) and every quarter thereafter until July 2016. Samples were obtained from the five sampling stations in each pen using a modified corer made from a 60 ml cut-off syringe marked at two (2) cm. The samples were obtained by plunging the syringe into the sediment, squeezing out the water and discarding sediment up to the two (2) cm mark. The top two (2) cm was used for the grain size and organic matter (n=6 cores combined), while only the top one (1) cm was used for productivity analysis (n=2). All samples were transferred to a 3x10 inch plastic bag. The samples obtained for productivity analysis were again placed in a black bag to prevent chlorophyll degradation. All samples were temporarily stored in a cooler with ice and immediately transferred to a freezer upon arrival in the laboratory.

## 2.3 Sediment Analysis

### 2.3a Particle Size Composition

Frozen sediment samples were thawed and then dried in an oven at 60° C until constant weight was obtained. Fifty (50) grams of the dried samples were weighed using a Sartorius top loading balance for dry seiving. Series of sieves with different mesh sizes (2000, 1000, 500, 250, 125, 63 and <63 μ) were used to determine the particle-size composition. Sieving was done with the aid of a mechanical shaker for 20 minutes. Fractions of sediment retained on each sieve were then transferred into pre-labelled and pre-weighed 2X3 resealable plastic bags. Weight of each plastic bag was measured using a Shimadzu analytical balance. Grain-size classes are divided into four broad categories: silt (<63 μ), fine-grained (63->125 μ); medium-grained (250–>500 μ); and coarse-grained (>1000 μ) sediments.

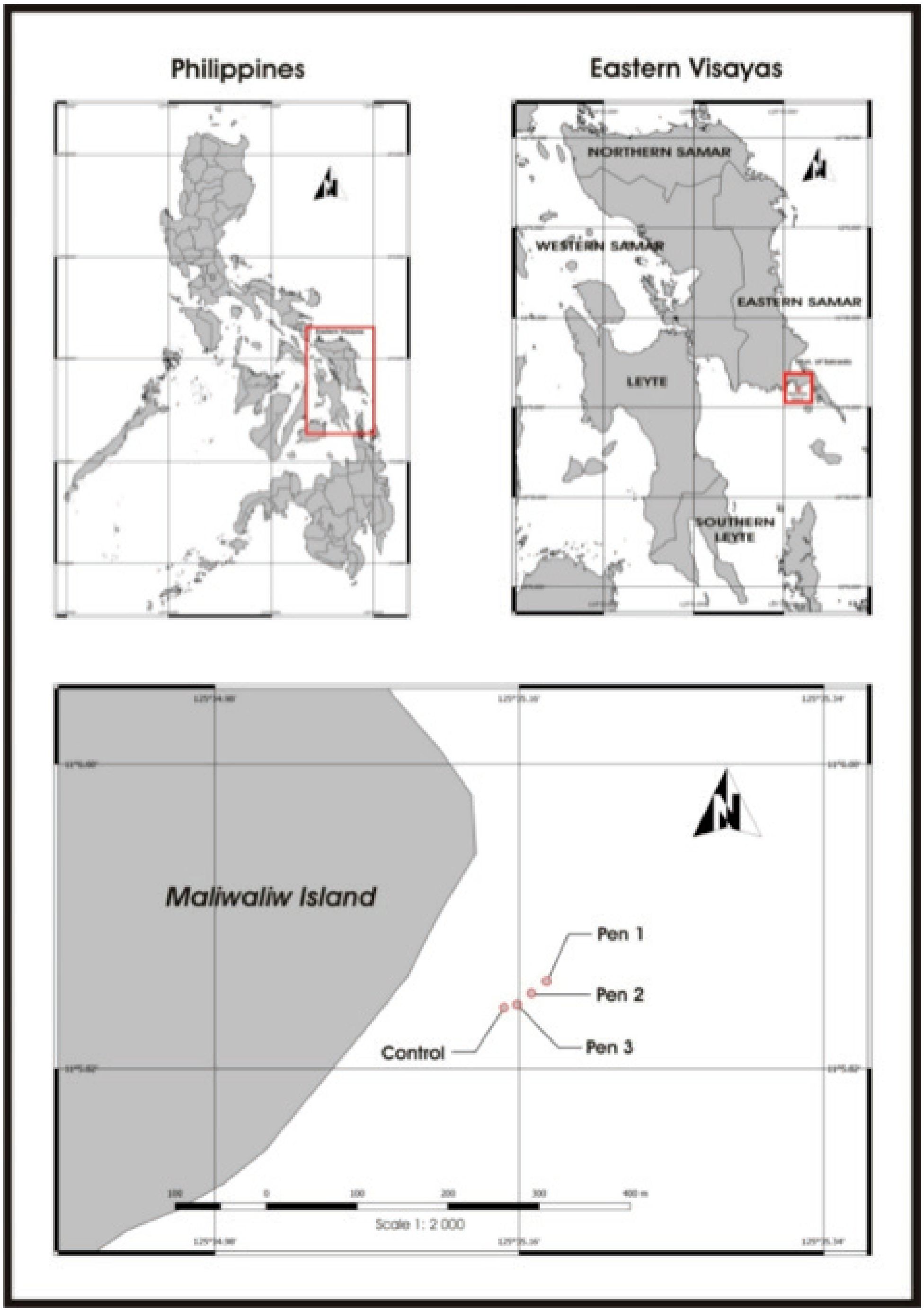


**Figure 1** Map showing the location of the four pens established in Maliwaliw Island, Salcedo, Eastern Samar, Philippines (PhilGIS, 2015).

*2.3b Organic Matter*

The loss on ignition (LOI) method was used to determine the organic matter content of the sediment. Frozen sediment samples were thawed and dried in an oven at 60° C until constant weight is attained. Pre-labelled porcelain crucibles were oven-dried at 150° C for 2 hours, then left to cool in a desiccator and weighed. Two (2) grams of the dried sediment samples (DW) were transferred into the pre-weighed crucibles and were burned at two different temperatures for 12 hours. To determine the labile OM content, samples were burned in a muffle furnace at 280° C for six hours and left to cool overnight. These were then weighed and the ash weight (AW)280 recorded. After weighing, the samples were burned again at 500° C for six (6) hours to determine the total OM content, and again left to cool overnight. The ash weight (AW)500 was again recorded. Percentage OM fractions were calculated as:

% Labile OM =(AW280/DW) x 100

% Total OM = (AW500/DW) x 100 and

% Refractory OM = (% Total OM) - (% Labile OM)

*2.3c Productivity*

Frozen sediment samples were thawed in the dark and thereafter all processes were carried out in dim light. Centrifuge tubes (15 ml) wrapped in an aluminum foil were labelled and weighed. Five grams of thawed sediment samples were transferred into a pre-weighed 15 ml centrifuge tubes. Then, 10 ml 95% ethanol was added to the centrifuge tubes; based on the recommended 1:2 sediment to solvent ratio. Samples were homogenized by mixing the solvent and the sediment using a vortex stirrer for 30 seconds. The tubes with homogenized samples were then placed in a 60°C water bath for an hour. The samples were then taken out of the water bath and left in a dark room at room temperature overnight, to extract the chlorophyll. These were then centrifuged at 4000 rpm for 8 minutes and the supernatant were placed in cuvettes. Absorbance was measured at 665 nm and 750 nm wavelengths using a spectrophotometer with 95% ethanol as reference sample. After taking all readings, one (1) drop of 0.1 N HCl was directly added to the cuvettes and agitated gently. The absorbances of the samples were measured again at 665 nm and 750 nm wavelengths. Chlorophyll-a concentration (μg/g dw) was calculated using the formula by Nusch (1980).

$$\text{Chl-a} = (E_b - E_a) \times (R/(R-1)) \times (v/(V.\,l)) \times (10^3/\alpha)$$

Where:

Chl-a = concentration of chlorophyll-a in μg/g

Eb = extinction of extract at 665 nm before acidification

Ea = extinction of extract at 665 nm after acidification (both values corrected for turbidity by subtraction of the 750 nm reading)

α = specific absorption coefficient for chlorophyll-a

V = dry weight of sediment sample, in grams (after Slater and Carton 2010)

v = volume of solvent used to extract samples, in ml

l = path length of cuvette, in cm (= 1 cm)

When the specific absorption coefficient for chlorophyll-a in 95% ethanol is taken as 82 and the maximum acid ratio is 1.7, the equation simplifies to:

$$Chl\text{-}a = 29.6(E_b - E_a) \times (v/(V \cdot l))$$

## 2.4 Statistical Analyses

Comparison of the changes over time in the sediment grain size, OM, and chlorophyll-a were analyzed using Friedman's test (P=0.05). On the other hand, comparisons between control and experimental pens were analyzed using Mann Whitney U-Test (P=0.05). Spearman rank correlation was used to measure the statistical dependence between physico-chemical parameters, OM, and chlorophyll. All analyses were performed using the R statistical software.

# 3. Results and Discussion

## 3.1 Particle Size Composition

Particle size analysis of sediment samples collected from July 2015 to July 2016 showed consistent ratio in the differently- sized grains (Figure 2). The area where the pens were located has predominantly medium grains (54.21%) known to favor the growth of *H. scabra.* (Mercier *et al.* 1999), because it retains sufficient load of organic material, and is easy for the animal to ingest and burrow in. Altamirano *et al.* (2016) hypothesized that sandfish may prefer a certain mix of particle sizes, rather than selecting uniform grain size. Results of their study showed that sandfish juveniles (5.07 + 0.05 g body weight) preferred to bury in and feed on sandy-mud substrate characterized with C14:M25:F56:S5, referring to coarse (C), medium (M), fine (F), and silt (S) percent composition.

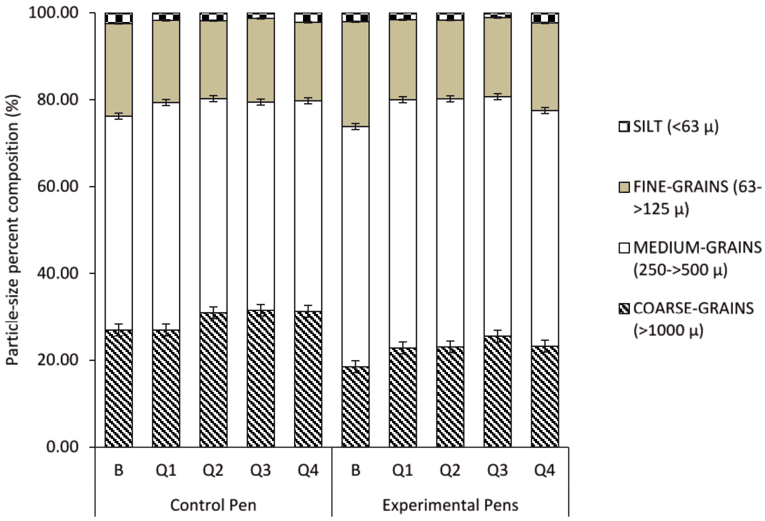


**Figure 2** Sediment grain-size (%) (mean±SD, n=5 sets of 5) within each pen combined into foure into four broad categories: Silt (<63 µ), (Fine (63->125 µ), medium (250->500 µ), and coarse (>1,000 µ) collected every quarter from July 2015 - July 2016. *Note: Baseline (B), Quarter 1 (Q1), Quarter 2 (Q2), Quarter 3 (Q3), and Quarter 4 (Q4).*

At the start of the study, the particle size ratio of the control pen has C26.98:M49.26:F21.31:S2.44 while the experimental pens had C18.52:M55.29:F24.14:S2.04 (Figure 2). At the end of the study, the particle size ratio of the control pen was measured at C31.27:M48.49:F18.06:S2.18 while that of the experimental pens was C23.24:M54.28:F20.15:S2.33. Both the control and the experimental pens showed an increase in the coarse sediment particles and decrease in the medium and fine sediments. Being deposit-feeders *H. scabra* feed on organic matter that coats the sediment. They grind the sediment into finer particles by passing it through their gut system (Bruckner *et al.*, 2003). However, similar trend in the changes of sediment particle size composition for both the experimental pens and the control pen from the start to the end of the study was observed implying no significant sediment particle-size alteration due to sandfish. The decreased in the fine sediment may also be attributed to the tidal current. According to Southard (2006), some of the sediments that are resting on the bed surface are moved when the current is sufficiently strong making the sediment surface coarser; thus, in sediment transport, the flow of selectivity entrains the finer fractions in preference to the coarser fractions. Statistical analysis did not show significant differences ($P$>0.05) in the changes of the sediment particle-size composition in both the control and the experimental pens.

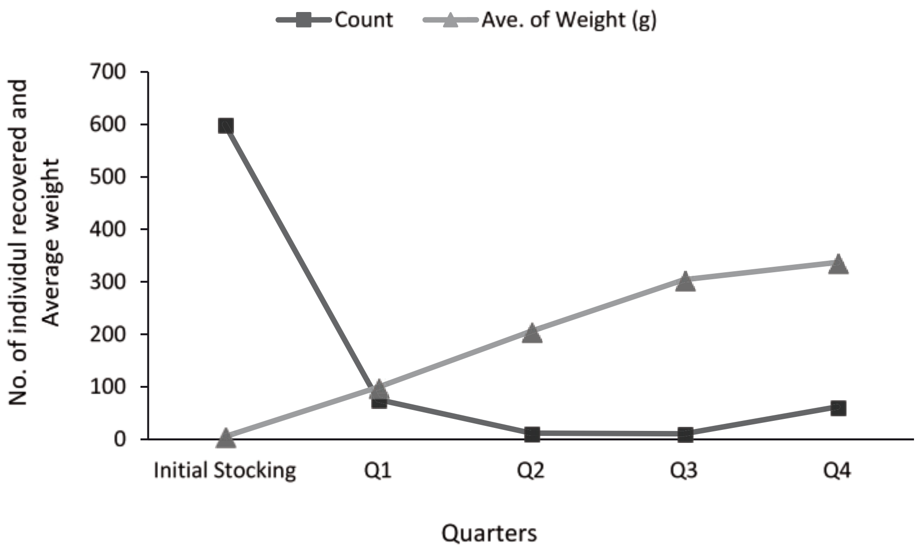


**Figure 3** Mean weight (g) and number of sandfish recovered from the experimental pens from the Jule 2015 (initial stocking) to July 2016 (Quarter 4).

Out of the 600 sandfish juveniles (5.46±1.96g) stocked in the pens, only 62 individuals or 10.33% with an average weight of 340 g were recovered at the end of the study (Figure 3). The strong tidal current over time may have caused the buried lower part of the net to loosen, that may have enabled some animals to escape from the pens. Some predators may have also been able to get into the pens and devour some of the sandfish. The sandfish recovered from the pens may not have been sufficient to significantly alter the sediment particle-size composition. In the experimental pens, only 29 individuals were recovered from pen 1 gaining an average weight of 341.99g. In pen 2 and 3, 19 and 14 individuals were recovered with an average weight of 353.14g and 316.04g, respectively.

### *3.2 Sediment Components*

The total organic matter (TOM) content and labile organic matter (LOM) content showed a similar trend in all the pens during four quarters with Q2 (4.86±0.24%) having the highest TOM content recorded (Figures 4 and 5). The values for TOM from baseline to Q2 significantly increased over time (Conover's test, P=0.004). Figures 4 and 5 also show the number of sandfish recovered in the experimental pens for a period of one year and their mean weight over time. However, the presence of sandfish in the experimental pens did not have much effect because the TOM values between the control and experimental pens were not significantly different (Kruskal Wallis, $P > 0.05$). For LOM, all values did not significantly change over time (Friedman test, $P > 0.05$) and were not significantly different in both pens (Kruskal Wallis, $P > 0.05$).

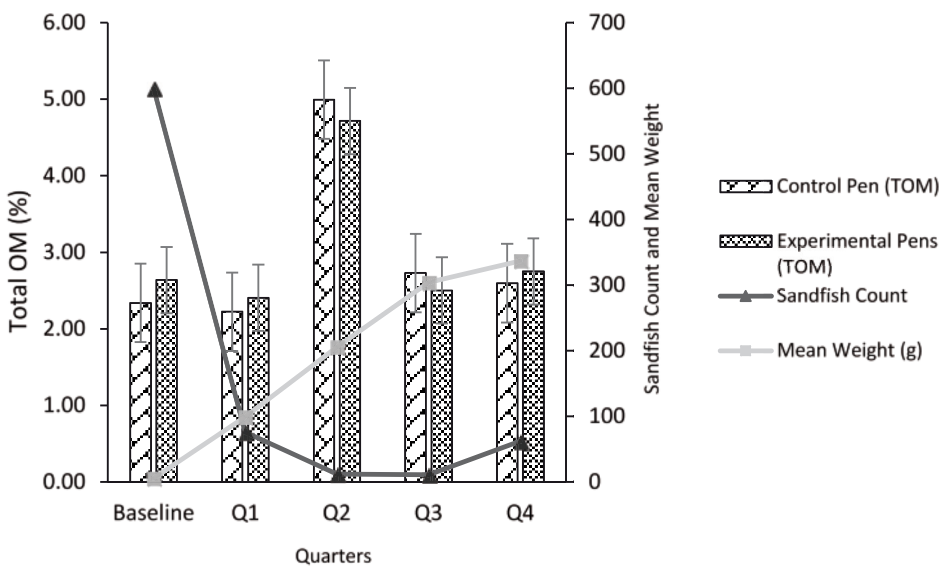


**Figure 4** Mean total organic matter (%TOM) (mean±SD, n=5 sets of 5) collected within each pen and the mean weight and sandfish count recovered from the experimental pens every quarter from July 2015 - July 2016.

Holothurians, both juvenile and adult, fed on detritus or decaying organic matter (Al Rashdi *et al.,* 2012). All organic matter (OM) values recorded from Baseline to Q4 were low. This agrees with the results of the study of Lavitra *et al.* (2010), and Tsiresy *et al.* (2011) in Toliara, SW Madagascar where quantity of recorded OM was no more than 5%. The increased TOM values recorded in Q2 (4.86 ± 0.24%) may be due to strong wave action caused by Typhoon Nona (International name, Melor), wherein debris made up of cellulose and lignin may have been deposited inside the pens. In the present study, TOM and LOM between the experimental pens and the control pen were not significantly different (Figures 3 and 4). According to Twilley *et al.* (1999), LOM has low total organic carbon (TOC) and total nitrogen (TN) ratios that breaks down easily whereas refractory organic compounds have very high TOC:TN ratios and are highly resistant to degradation. Labile includes phytoplankton while refractory includes woody debris made of lignin and cellulose. The TOM is the labile and refractory OM combined. In a study by MacTavish et al. (2012), it was revealed that deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments. Most sea cucumbers are omnivorous deposit-feeders that not only compete with but also feed directly upon benthic bacteria (HilleRisLambers *et al.,* 2012). Even if sea cucumbers are found to reduce accumulation of organic carbon (Slater and Carton, 2009) and enhance nutrient efflux from the sediment by bioturbation (Lohrer *et al.,* 2004), they also elevate nutrient concentrations through excretion (Uthicke, 2001). Experiment in tanks conducted by Altamirano *et al.* (2016) showed that growth of sandfish juveniles in the first two weeks of rearing was significantly greater on sediment where OM content remains almost unchanged. In the field, the result of the study conducted by Plotieau *et al.* (2013) on the impact of *H. scabra* intensive farming on sediment in Madagascar, also agrees with this study. They did not observe any difference in TOM content from the sediment taken both inside (with *H. scabra*) and outside their pens. Hence, it is also possible that natural inputs compensate the OM uptake by the sandfish.

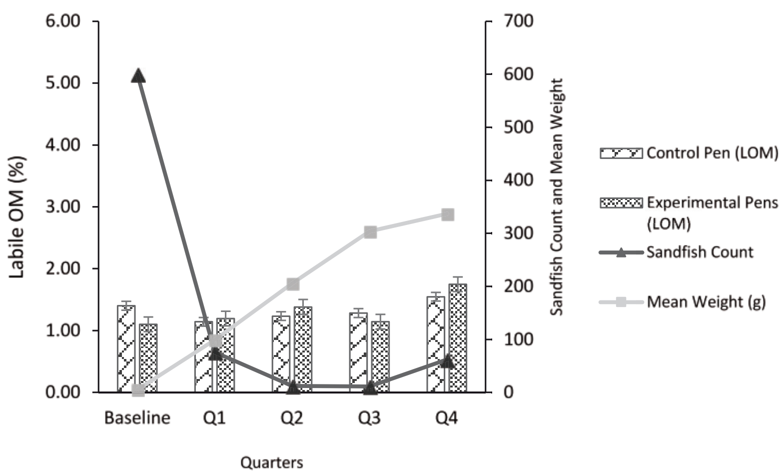


**Figure 5** Mean labile organic matter (%OM) (mean±SD), n=5 sets of 5) collected within each pen and the mean weight and sandfish count recovered from the experimental pens every quarter from July 2015 - July 2016.

Results also show that the fluctuations on the trend of chlorophyll-a content from baseline to Q4 was significant ($P < 0.05$), based on Friedman's test (Figure 6). Concentrations of chlorophyll-a recorded in Q1 (1.43 ± 0.43 µg g-1dw), Q2 (1.44 ± 0.59 µg g-1dw), and Q4 (1.01 ± 0.28 µg g-1dw) were higher compared to Baseline (0.39 ± 0.12 µg g-1dw) and Q3 (0.42 ± 0.16 µg g-1dw). This suggests that photosynthetic microorganisms in the sediment were more concentrated during these quarters. Sedimentary pigments such as chlorophylls and carotenoids are biomarkers that provide a more complete picture of the phototropic community (Reuss, 2005). They also reflect changes due to a wide variety of forcing factors: nutrient load, changes in grazing pressure and acidification (Leavitt and Hodgson, 2001). However, a similar trend in the changes on sediment chlorophyll-a content was observed in both the control and experimental pens. Similar to the OM, the presence of sandfish in the experimental pens did not have much effect because the values of chlorophyll-a concentration between both pens were not significantly different (Kruskal Wallis' test, P > 0.05) (Figure 6). This result does not conform to the findings of Plotieau *et al.* (2013), wherein photosynthetic microorganisms is depleted in pens through rearing, as holothurians ingest parts of the sediment (Lopez and Levinton, 2011) which comprise bacteria, diatoms protozoan and cyanophyceans (Massin 1982; Moriarity 1982). Relative to the organic material, Wakeham et al. (1997) assigned overall reactivity of different biochemical process and found chlorophylls to be most labile, followed by lipids, amino acids, and finally carbohydrates as the most refractory group. Like the OM, this may also suggest that, the natural input is sufficient to sustain the productivity (measured by cholorophyll-a) of the area. This may also imply that the number of sandfish recovered at the end of the study (1 individual per 4.8 $m^2$) were not sufficient to alter the sediment components.

Highest rainfall was recorded on Q4 (905.8 mm) while highest reading for temperature (29.3 °C) and salinity (32.8 ppt.) was recorded on Quarter 2 (Table 1). The correlation between the physico-chemical parameters, OM content, and chlorophyll concentration is shown in Table 2. Rainfall and salinity were significantly correlated ($P< 0.05$) to total OM and labile OM while temperature was significantly correlated to total OM only (Table 2). Results show that rainfall is positively correlated to TOM and LOM, which means that when amount of rainfall increases, percentage of total OM and labile OM also increase. Rainfall is also positively correlated to chlorophyll-a but not significant (Table 2 and Figure 6). Conversely, salinity and temperature are negatively correlated to total OM and labile OM, which means when values of salinity and temperature increases, the percentage of total OM and labile OM decreases. Chlorophyll-a is also negatively correlated to salinity and temperature but again not significantly.

Based on the results of this study, we can say that sandfish sea ranching can be done in shallow coastal waters at stocking density of two (2) sandfish juveniles per $m^2$ without causing significant changes in sediment particle composition and components.

**Table 1** Readings for physico-chemical parameters: sum of rainfall and averages of water temperature and salinity.

| Time | Rainfall (mm) | Temperature (ºC) | Salinity (ppt.) |
|---|---|---|---|
| July - Oct 2015 | 389.6 | 29.3 | 32.8 |
| Oct 2015-Jan 2016 | 529.3 | 28.0 | 30.5 |
| Jan-Apr 2016 | 504.1 | 28.0 | 30.5 |
| Apr-July 2016 | 905.8 | 28.5 | 30.4 |

**Table 2** Spearman rank correlation between physico-chem, organic matter (OM), and chlorophyll-a.

| Characteristic-a | $r_s$ | P-value |
|---|---|---|
| Rainfall | | |
| *Total OM* | 0.5801 | 0.0008 |
| *Labile OM* | 0.4916 | 0.0058 |
| *Chlorophyll A* | 0.1649 | 0.3840 |
| Temperature | | |
| *Total OM* | -0.7149 | 0.0000 |
| *Labile OM* | -0.1049 | 0.5813 |
| *Chlorophyll A* | -0.0804 | 0.6728 |
| Salinity | | |
| *Total OM* | -0.5801 | 0.0008 |
| *Labile OM* | -0.4916 | 0.0058 |
| *Chlorophyll A* | -0.1649 | 0.3840 |

## Acknowledgements

This study was funded by the Australian Centre for International Agricultural Research (ACIAR) through the project FIS/2010/042 “Expansion and Diversification of Production and Management Systems for Sea Cucumbers in the Philippines, Vietnam, and Northern Australia” in partnership with UP-Marine Science Institute (UP-MSI), Mindanao State University (MSU)- Naawan, Southeast Asian Fisheries Development Center (SEAFDEC), Bureau of Fisheries and Aquatic Resources Region VIII- Guiuan Marine Fisheries Development Center (BFAR VIII-GMFDC), Maliwaliw Multi-purpose Association (MMA), and Local Government Unit (LGU)- Salcedo. We also appreciate the assistance of Rosabella Montes for the statistical analyses of this study.

## References

Al Rashdi KM, Eeckhaut I, Claereboudt MR. A manual on hatchery of sea cucumber *Holothuria scabra* in the Sultanate of Oman. Ministry of Agriculture and Fisheries Wealth. 2012.

Altamirano JP, Recente CP, Rodriguez Jr, JC. Substrate preference for burying and feeding of sandfish *Holothuria scabra* juveniles. Fisheries Research 2017; 186: 514-523.

Baskar BK. Some observations on the biology of the holothurian: *Holothuria* (Metriatyla) *scabra* (Jaeger). Central Marine Fisheries Research Institute Bulletin 1994; 46: 39–43.

Bruckner AW, Johnson KA, Field JD. Conservation Strategies for a Sea Cucumbers: Can a CITES Apendix II. Listing Promote Sustainable International Trade? SPC Beche-De-Mer Information Bulletin. 2003; 18: 24-32.

Conand C. Sea cucumber biology, taxonomy, distribution and conservation status. In A.W. Buckner (Eds.) 2006. Proceedings of the CITES workshop on the conservation of sea cucumbers in the families' Holothuriidae and Stichopodiidae. NOAA Technical Memorandum NMFS-OPR 34 Silver spring, MD. 2004.

Graham ER, Thompson JT. Deposit- and suspension -feeding sea cucumbers (echinodermata) ingest plastic fragments. Journal of Experimental Marine Biology and Ecology. 2009; 368: 22–29.

Hamel JF, Conand C, Pawson DL, Mercier A. The sea cucumber Holothuria scabra (Holothuroidea: echinodermata): its biology and exploitation as beche-de-mer. Advances in Marine Biology 2001; 41: 129–223.

Herringshaw LG, Solan M. Benthic bioturbation in the past, present and future. Aquatic Biology 2008; 2: 201–205.

HilleRisLambers R, van de Koppel J, Herman PMJ. Persistence despite omnivory: benthic communities and the discrepancy between theory and observation. – OIKOS 2006; 113: 23-32.

Lavitra T, Rasolofonirina R, Eeckhaut L. The effect of sediment quality and stocking density on survival and growth of the sea cucumber *Holothuria scabra* reared in nursery ponds and sea pens. Western Indian Ocean Journal of Marine Science 2010; 9: 153-164.

Leavitt PR, Hodgson DA. Sedimentary pigments. In: Smol JP, Birks HJB, Last WM (eds) Tracking environmental change using lake sediments: Terrestrial, algal, and siliceous indicators. Kluwer Academic Publishers, Dordrecht, The Netherlands 2001; 3: 295-325

Lohrer AM, Thrush SF, Gibbs MM. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 2004; 431: 1092–1095.

Lopez GR, Levinton JS. Particulate organic detritus and detritus feeders in coastal food webs. In: Wolanski E and McLusky DS (eds.) Treatise on Estuarine and Coastal Science. 2011; 6: 5–21.

MacTavish T, Stenton-Dozey J, Vopel K, Savage C. Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically- Enriched Coastal Sediments. Public Library of Science ONE 2012; 7(11): 1-11.

Massin C. Effects of feeding on the environment: Holothuroidea. In: Echinoderm Nutrition (M. Jangoux and J.M. Lawrence eds). 1982; 493-497.

McVean AR, Hemcry G, Walker RCJ, Ralisaoua BLR, Fanning E. Traditional sea cucumber fisheries in southwest Madagascar: A case-study of two villages in 2002. Bêche-de-mer 2005; 21:15-18.

Mercier A, Battaglene SC, Hamel JF. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology 1999; 239 (1):125–156.

Mercier A, Battaglene SC, Hamel JF. Periodic movement, recruitment and size-related distribution of the sea cucumber Holothuria scabra in Solomon Islands. Hydrobiologia. 2000; 440: 81–100.

Mezali K, Soualili DL. The ability of holothurians to select sediment particles and organic matter. SPC Beche-de-mer Information Bulletin 2013; 33: 38-43.

Moriarty D. Feeding of Holothuria atra and Stichopus chloronotus on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. Australian Journal of Marine Freshwater Research 1982; 33 (2): 255–263.

Nusch EA. Comparison of different methods for chlorophyll and phaeopigment determination. Arch Hydrobiol. Beih. Ergebn. Limnol. 1980; 14: 14–36.

Plotieau T, Baele JM, Vaucher R, Hasler A, Kounad D, Eeckhaut I. Analysis of the impact of *Holothuria scabra* intensive farming on sediment. Cahiers De Biologie Marine 2013; 54 (4): 703–711.

Reuss N. Sediment pigments as biomarkers of environmental change. National Environmental Research Institute, Ministry of Environment, Roskilde, Denmark. 2005

Slater MJ, Carton AG. Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. Marine Pollution Bulletin 2009; 58: 1123–1129.

Southard J. Introduction to Fluid Motions, Sediment Transport, and Current-Generated Sedimentary Structures. Massachusetts Institute of Technology. 2006.

Teal LR, Bulling MT, Parker ER, Solan M. Global patterns of bioturbation intensity and the mixed depth of marine soft sediments. Aquatic Biology 2008; 2:207–218

Tsiresy G, Pascal B, Plotieau, T. An assessment of *Holothuria scabra* growth in marine micro-farms in southwestern Madagasacar. Bêche-de-mer 2011; 31: 17-22.

Twilley, RR, Cowan, J, Miller-Way T, Montagna PA, Mortazavi, B. Benthic nutrient fluxes in selected estuaries in the Gulf of Mexico. 1999; 163-209 In Bianchi, T.S., Pennock, J.R. and Twilley, R.R. (Eds), Biogeochemistry of Gulf of Mexico Estuaries, John Wiley & Sons, Inc.

Uthicke S. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology 2001; 265: 153–170.

Wakeham SG, Lee C, Hedges JI, Hernes PJ, Peterson ML. Molecular indicators of diagenitic status in marine organic matter. Geochimica et Cosmochimica Acta 1997; 61(24):5363-5369

Watanabe S, Kodama M, Zarate JM, Lebata-Ramos MJH, Nievales MFJ. Ability of sandfish (Holothuria scabra) to utilise organic matter in black tiger shrimp ponds. In: Hair, C.A., Pickering, T.D., Mills, D.J. (Eds.), Asia-Pacific Tropical Sea Cucumber Aquaculture, ACIAR Proceedings No. 136. Australian Centre for International Agricultural Research, Canberra, New Caledonia 2012; 113–120.

Wolkenhauer SM, Uthicke S, Burridge C, Skewes T, Pitcher R. The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. Journal of Marine Biology Association 2010; 90 (2): 215–223.