# BIOTURBATION AND EFFICIENCY OF SEDIMENT INGESTION OF THE BLACK SEA CUCUMBER *HOLOTHURIA ATRA* BASED ON SEDIMENT SIZE

ANDY SANCHEZ ALCARAZ

*Environmental Science Policy and Management, University of California, Berkeley, California 94720 USA*

*Abstract.* Bioturbation by sea cucumbers allows for the cycling of nutrients in ocean waters which aids in sustainment of ecosystem health. Sea cucumbers have been utilized by aquaculture farms to reduce the eutrophication caused by fish waste, and so understanding their feeding capabilities and different ingestion efficiencies based on the sediment given to them can lead to more beneficial uses of these invertebrates. The reliance that zooxanthallae in coral have on bioturbation's nutrient cycling also emphasizes the importance to observe and study feeding preferences and pellet excretion on different habitat compositions. I investigated the feeding and excretion of the black sea cucumber, *Holothuria atra,* to see if there would be a significant difference in the rate of pellet excretion and therefore the rate of bioturbation of two size classes feeding on various sediment compositions. My findings from a Kruskal-Wallis test indicate a p value of .02082 for the large size class, indicating there was a difference in feeding and bioturbation for certain sediment stations. A Dunn's post hoc p value of .01157 and .004774 show there was a difference in bioturbation rates in the large size class between the unsieved Gump sand and Snack Mahana sand, and between the fine sieved Gump sand and Snack Mahana sand, respectively. These results emphasize how, for the large class of sea cucumbers, they will not efficiently feed on silty sediment sizes compared to their natural habitat and compared to coarser sediment composition. A p value of .2438 from a Kruskal-Wallis test of for the small class suggests that there is no significant difference in feeding and bioturbation efficiency based on sediment size for smaller black sea cucumbers, indicating that this size can efficiently cycle the nutrients in a broader range of sediment compositions in nature and perhaps in aquaculture.

*Key words: benthic feeders; bioturbation; deposit feeding; eutrophication; holothurians;* Holothuria atra*; micronutrient; namako; sea cucumber; sediment.*

## INTRODUCTION

Bioturbation from sea cucumbers is the process of reworking the benthic sediment in the ocean and affecting the chemical composition and accumulation of carbon, phosphorous, and nitrogen, as well as other nutrients due to their movement, feeding, and excretion (Chen 2004, Hou *et al.* 2018). The benefits of the deposit feeding activity of sea cucumbers include the contribution to ecosystem productivity due to the recycling of nutrients, including the release of nitrogenous wastes. This supports benthic algal growth and productivity of zooxanthallae in corals (Wolfe 2017). The ingestion of sediment and the feeding and excrement process allows for a healthy and productive ecosystem, where recycled nutrients are needed for high primary productivity.

Sea cucumbers belong to the class Holothuroidea and are benthic sediment feeders that feed on microorganisms, detritus, and nutrients in the sediment they live on (Yingst 1976). Their sediment uptake and deposition has an effect on nutrient cycling and the oxidation of the top sediment layer, as it lowers the amount of organic material in the sediment that, if in excess, can lead to anaerobic conditions. Studies have shown that eutrophic habitats are preferred by holothurians over oligotrophic habitats, because they provide food (Mangion 2004, Isgoren-Emiroglu and Gunay 2007). Without bioturbation and the removal of organic material, the sea floor can compact and be unfavorable for benthic and infaunal organisms (Isgoren-Emiroglu 2007). Due to the fact that muddier and siltier sediments accumulate more organic material which can cause higher eutrophication than in sandier, coarser sediment, perhaps sea cucumbers can feed more efficiently on finer sediment sizes that are more susceptible to causing eutrophic conditions and harming the environment (Martinez Garcia 2015).

The black sea cucumber *Holothuria atra* is abundant in the tropical indo Pacific waters, with two morphotypes. There is the asexual and sexual morphotypes, where the asexual morphotypes were found near the shore as they tend to prefer warm water, little wave action, and shallow water (Uthicke 2010). *H. atra* does not eviscerate, but they can go through stiffening of their bodies for self defense, as well as stopping their feeding motions.

Holothurians have been particularly wanted in aquaculture as feces, uneaten food, and metabolic waste from fish causes eutrophication when put into the oceanic waters, leading to a decline in ocean health (Talbot and Hole 1994). It has been shown that areas with higher sea cucumber densities decrease organic phosphorous and increase NaOH-P. This helps prevent eutrophication in aquaculture systems by facilitating organic phosphorous decomposition and increasing the capacity of phosphorus sorption (Hou *et al.* 2018). Aquaculture farms may rely on sandier and coarse sediments similar to those in a high organic environment as it accumulates less organic matter. Likewise, the farms can utilize holothurians to control the organic matter that accumulates at higher levels in silty sand. The ability for silty sand to accumulate more organic matter and thus be more eutrophic and potentially harmful to biodiversity and ocean health implies that sea cucumbers prefer this sediment and can feed and excrete at a higher rate due to the surplus of nutrients. Since what is ingested by sea cucumbers is ejected completely, there is next to no dissolution of sand grains along the digestive track, allowing for pellet studies to be accurate in the composition and weight of sand the sea cucumbers are actively feeding on (Yamanouti 1939, Hammond 1981). The collection of these pellets can reveal preferences in feeding and reveal direct weight of sand flowing through a population of sea cucumbers, as well as what sizes of sediment the sea cucumbers are selecting when given different habitat samples.

Keeping in mind the studies regarding differences in nutrient capacity between different sediment gradients and the importance of holothurian benthic feeding, I am curious in studying how more efficient and larger excretion rates in bioturbation correlates to sediment size. I hypothesize that there will be a significant difference in the sediment size tolerance that different size bins of *H. atra* can ingest and excrete, as well as the rate of this bioturbation process. I hypothesize that smaller sized sea cucumbers will excrete a higher weight of sediment pellets when fed their natural sediment from Snack Mahana, as well as the finer sieved sand compared to Gump Station sand and the coarse sieved sand. Regardless of what sediment station they are fed I hypothesize that they will excrete finer sediments than what is completely available.

I believe that there will not be a significant difference in feeding and excrement for the large sized sea cucumbers due to their size, as I hypothesize that this size difference will enable them to tolerate a broader range of sediment sizes. I still believe that Snack Mahana sand will be excreted most out of the feeding stations, and I believe there will not be a significant difference in the composition of what is excreted versus what was offered for the larger sea cucumbers. I believe this because of their size, that they will be able to feed, select, and tolerate more of the composition of the sand they are given.

## METHODS

### *Study Site and Study Animals*

Raw data was collected on the island of Mo'orea in French Polynesia. Specifically, the black sea cucumber *Holothuria atra* sample specimens were collected out in Gump Research Station and near Snack Mahana (see Fig. 1).

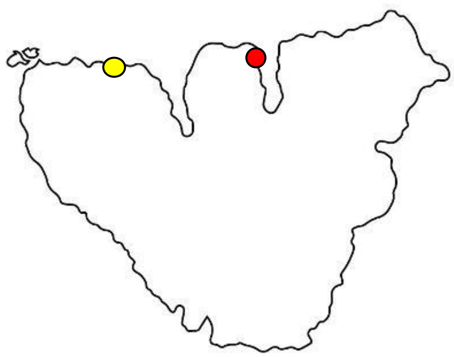

FIG. 1. The island of Moorea. Gump Station marked by the red dot and Snack Mahana marked by the yellow dot.

Both size classes of *H. atra* were locally abundant at the beach adjacent to Snack Mahana. In total, 80 sea cucumbers were collected. This allowed me to create 4 groups with 10 small individuals and 4 groups of 10 large individuals. The small size class had namakos no larger than 10 cm and the large size class had namakos of at least 10 cm. The sea cucumbers were located about 2-10 meters from the shores of Snack Mahana, and were grabbed by hand and placed in tubs of sea water for transport. Ocular observation was used to determine that the small size class would weigh less than the large size class.

The sample specimens were collected by hand at the beach adjacent to Snack Mahana. They were placed in a large enough bucket with sea water to accommodate them for the ride back at the station. Gloves were not necessary as these species do not eviscerate but gloves aided in extra protection nonetheless. 20 of each size class was collected for the first experimental run, and the last 20 of each size class was collected once the raw data from the first run was complete. The sea cucumbers were returned once the experiments were run, simply by placing them among the other namakos.

### *Sediment collection*

The sediment at Snack Mahana was composed by fine and medium sized sediments, with some shell fragments and *Padina* algae, and little coral rubble. Sand from here was collected for the control portion of the experiment, to observe the natural feeding and excretion rate of H. atra in sand size they are accustomed to.

Coarser sand samples were collected from Gump Research Station (17°29'17.09"S, 149°52'44.87"W), where the sediment was composed of a broader gradient of fine, medium, and large sediment. The sea leopard sea cucumber, *Bohadschia argus*, was found living just before the water deepened greatly, so the shallow areas before the deep ocean was the approximate location for sediment collection. The sea leopard was used as reassurance that some form of holothurians were actively feeding on this sediment and that there would be proper nutrients for the black sea cucumber.

Sediment at Gump Station was collected by kayaking out with a buddy to areas where sea leopard sea cucumbers were found living, and a glass jar was used to pick up surrounding sand into a large plastic bucket by scooping out sand by hand after diving down into the benthic layer. The bucket was filled with water to allow it to sink down and stabilize. The bucket was placed onto the kayak once half full and used the same day to fill the wet lab tubs. Kayaking was not needed to collect sediment at Snack Mahana, as the black sea cucumber lived in very shallow and nearby waters, but a plastic bucket and glass jar helped with collecting sand nearby the feeding and living sea cucumbers at this location.

Small sand samples were collected from Snack Mahana and from Gump Station, to have data for the composition of each habitat. Pellet samples from the namakos were also collected from the site itself with a metal spoon. The pellets were placed in a waterproof pouch and were collected to ensure the control pellets would match the natural pellets in terms of composition.

A sample of sea leopard Gump pellets was also used as a way to compare what was eaten by sea cucumbers naturally occurring at Gump and what was eaten by sea cucumbers who lived in a finer sediment habitat. Snorkeling in Gump Station and using a spoon to collect sea leopard pellets into a waterproof pouch or bag was done to collect pellet samples from three sea cucumbers to get variation.

*Sea cucumber feeding and pellet collections*

Methods for sea cucumber feeding, purging, and pellet collections were taken and followed by Symphonie Yu (2017).

Groups of ten sea cucumbers of the same size bin were placed in plastic tanks or tubs in the wet lab with steady seawater flow. Sediment that the sea cucumbers use to cover their bodies was removed with a gloved finger and under a flow of sea water at the wet lab so this sand would not be mixed up with the pellets and feeding trials. The sea cucumbers were left without any sediment in the flowing tubs for 24 hours to purge what they had been feeding on at Snack Mahana. Then, a 2 cm thick layer of Snack Mahana sediment was placed on the surface while the 10 sea cucumbers in each group were kept separate in the meantime in another tub. The sea cucumbers were placed on their specific tubs of sediment to feed for 24 hours.

After 24 hours of feeding, the pellets were collected from each tub by using a metal spatula or spoon and placing each pellet in a plastic cup. After this feeding collection, the sea cucumbers were purged again for 24 hours by removing the sediment from the tubs. All sediment found after 24 hours had been excreted and originated from the previous feeding cycle, allowing for easy collection. The bucket was completely away from the flow so as to not disturb the water or collection process, and the water was partly emptied out until a the sediment can be scraped into a small plastic container with a metal spatula or spoon. The same process of feeding, feeding collection, purging, and purge collection was repeated for the other size class, and for every sediment station.

To sieve the Gump Sand for the sea cucumbers, a 2 mm sieve and a 500 µm sieve were used. The 2 mm sieve was used first to separate the coarse sand, and the leftover sand was used in the finer sieve to collect fine sediment. The flow from the wet lab was used to run water through the sand on the sieve and the sand that falls out is collected in a separate bucket underneath. Anything larger than 2 mm, so what was left on top for the 2 mm sieve, was kept for the coarse Gump feed. Everything that falls under the 500 µm sieve was the sediment needed for the fine Gump feed.

*Dirt lab raw data collection*

Each sediment sample was placed in the drying oven for 5 hours under 100° Celsius. Aluminum foil was shaped into boats to place the wet sand into, and the foil was labeled with sharpie. Afterwards, the dry sediment was placed in a weigh boat and measured with a scale. The dry sediment was kept in plastic cups and covered with Parafilm to keep moisture out.

A digital caliper was used to measure the length and width of each sea cucumber just at the moment of picking them out of the tubs to have the longest measurement they were capable of being, as they shrunk when being picked up too long. The sea cucumbers were placed in waterless tubs for about thirty seconds to allow for them to dispose of excess internal water. They were then placed individually on a large weigh boat and on a scale to measure their mass.

The final step was to place the known weights of every sediment sample through a set of sieves and figure out the percentage of sediment grains belonging to different sizes. The sieves were the following sizes: 2 mm, 1 mm, 500 µm, 250 µm, and 125 µm. The weight of each category is measured and divided by the total weight of the sample, to see later on if there was selection based on what they ate versus the available sediment sizes.

*Analyses*

Various tests and visualizations were run to see if there would be a significant difference in ingestion rates for each size class, where the control station would have the highest mass of pellets, and the finer sieved station would have the next highest mass of pellets. In addition, these tests were run to show if, in fact, the larger namakos would have less of a difference in pellet masses as they may tolerate the coarse and unsieved Gump sand better than the smaller namakos, who should have the lowest pellet masses in these two stations.

The averages of the sea cucumber measurements and masses was taken to distinguish between the two size classes. To see if there was a difference in bioturbation/ingestion rate of different sediment stations, a Kruskal-Wallis test was used on Past3 to test for a difference in the means for the small class bin and the large class bin. An alpha value of less than .05 will be needed to show significance in the difference of feeding and bioturbation preferences on the different sediment stations.

A Dunn's post hoc was used if there was significance in the Kruskal-Wallis test to see which sediment feeding and purging stations differed in ingestion rates significantly.

Percentage composition was calculated to see if there was a significant difference in the pellet composition versus the sediment station composition for each station and size class.

## RESULTS

The average mass, length and width of the specimens in the small and large size classes were taken (see Table 3). The small size class averaged masses of 22.208 g, 29.967 g, 24.794 g, and 22.274g with average length and widths of 5.71 x 1.93 cm, 6,71 x 2.86 cm, 6.75 x 2.10 cm, and 6.97 x 3.71 cm.. The large size class averaged masses of 82.103 g, 74.661 g, 78.973 g and 96.214g with average length and width of 13.70 x 3.63 cm, 14.25 x 3.14 cm, 13.95 x 3.23 cm, and 14.6 x 4.39.

A box plot was created to show the means of the data for feeding and purging with the various sediment treatments for small sea cucumbers and large sea cucumbers (see Fig. 2 and 3).

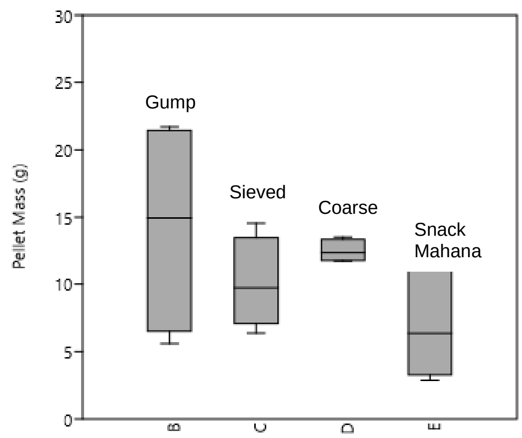


FIG.2. Box plot of the small groups for Gump, Sieved, Coarse, and Snack Mahana purging collections.

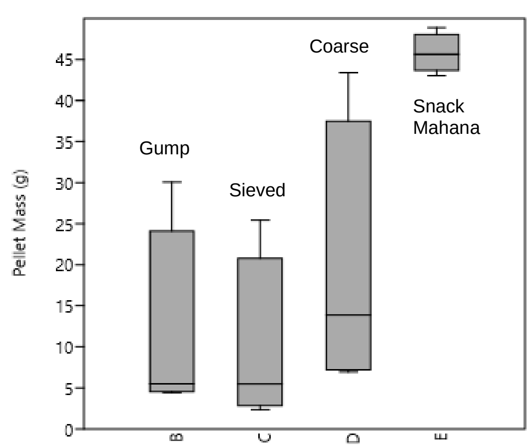


FIG.3. Box plot of the large groups for Gump, Sieved, Coarse, and Snack Mahana purging collections.

The average pellet masses for the small size namakos are the following: 14.29g, 10.09g, 12.49g, and 6.99g. The average pellet masses for the large size namakos are the following: 11.38g, 9.70g, 19.51g, and 45.79g.

An ANOVA test on Past3 was created to see if there was a significant result in the difference in the means of the sediment feeding and purging, and specifically, a Kruskal-Wallis test was used (see Table 1). A p value of .2438 was found for the small size class and a p value of .02082 was found for the large size class.

TABLE 1. Kruskal-Wallis test for the small and large classe of *H. atra* with a p value of .2438 for small and a p value of .02082 for large.

| | Small Size Class | Large Size Class |
|---|---|---|
| H (chi2) | 4.169 | 9.75 |
| Hc (tie corrected) | 4.169 | 2.39 |
| p (same) | .2438 | .02082 |

A Dunn's post hoc for the large size class shows a p value of .01157 between unsieved Gump Station sand and Snack Mahana sand, and a p value of .004774 between sieved Gump sand and Snack Mahana sand (see Table 2).

TABLE 2. Dunn's post hoc showing which categories significantly differed in means.

| | Gump Unsieved | Fine Gump | Coarse Gump | Snack Mahana |
|---|---|---|---|---|
| Gump Unsieved | | .7664 | .2985 | .01157 |
| Fine Gump | .7664 | | .1813 | .004774 |
| Coarse Gump | .2985 | .1813 | | .1375 |
| Snack Mahana | .01157 | .004774 | .1375 | |

Sieving all the Snack Mahana pellet samples was done in order to compare *H. atra's* Gump pellet composition compared to a natural sediment sample from Gump Station. The Gump Station sand is composed of the following: 51.9% >2 mm, 11.3% 1-2 mm, 14.1% 500 µm-1 mm, 15.7% 250-500 µm, 6.4% 125-250 µm, and .6% <125 µm. The average composition of the small namako pellets on Gump sand is the following: 20.7% >2 mm, 15.9% 1-2 mm, 23.1% 500 µm-1 mm, 27.2% 250-500 µm, 10.9% 125-250 µm, 2.2% <125 µm. The average composition of the large namako pellets on Gump sand is the following: 23.4% >2 mm, 16.2% 1-2 mm, 24.8% 500 µm-1 mm, 25.2% 250-500 µm, 7.9% 125-250 µm, 2.5% <125 µm.

## DISCUSSION

An ANOVA was used to analyze the data from the sea cucumber purges. The feeding data was thrown out as human error due to unconfident collection methods. The purge data would supply the best and most accurate results as each purge lasted the same amount of time, and the confidence of the mass of excrement collected was higher than the feeding collection since there was no other sand in the tubs for the purge.

A Kruskal-Wallis test was ran with a p(same) value of .2438 for the small group, and a p(same) value of .02082 for the large group. There is no significant difference between the means in sediment excrement for small groups since the p value is greater than .05, meaning that for the small sea cucumbers, neither the Gump sand, sieved Gump sand, coarse Gump sand, or Snack Mahana control sand was preferred or eaten more efficiently. Thus, there was no significant difference in the bioturbation of sediment for the small sea cucumbers and the initial hypothesis has been rejected for this size category.

The p=.02082 for the large group of sea cucumbers means that there is a significant difference in the average sediment ingestion among the four purge cycles. With this, a Dunn's post hoc revealed that there is a significant difference in the large groups between the Gump Sand pellets compared to Snack Mahana pellets (raw p value=.01157) and between the sieved Gump pellets and Snack Mahana pellets (raw p value=. 004774).

The results of the large sea cucumbers agrees with part of the initial hypothesis that the control sediment station, Snack Mahana sand, would have significantly higher pellet masses collected. This was true for Snack Mahana sand compared to Gump sand and compared to sieved Gump sand, meaning that the large namakos could possibly feed reasonably efficiently on coarse Gump sand alone, but will probably have less success in surviving and bioturbating the sediment layer at Gump Station since the natural sand is not sieved to only provide coarse sand. The fine sieved sand in Gump Station was not preferred by the large sea cucumbers compared to Snack Mahana sand, as their averages in pellets was significantly different in mass.

The findings from the small sea cucumbers had no significance which disagrees with the initial hypothesis. There was not a significant result that the small group of *H. atra* could rework and recycle the benthic layer of Snack Mahana or sieved Gump sand better than the coarser sediment stations. The box plot suggests that all average pellet masses in Gump Station were higher for the small bin, meaning that this species can actually rework the sediment better than at Snack Mahana. The wider range in deviation for 100% Gump Sediment, however, suggests that there is less predictability in how much greater can be excreted for feeding and purge cycles. For coarse Gump sand, however, the small sea cucumbers had a narrow range in deviation and this suggests that there is a better predicted rate of ingestion and bioturbation that can be studied and observed.

The large bin only had a narrow deviation range for Snack Mahana, whereas in all Gump station sediment cycles, the data varied and all were nonetheless lower than the control. This shows better predictability and reliance on the black sea cucumber's bioturbation abilities with their natural habitat's sediment than with newly introduced sediment compositions.

Lastly, Gump sand is composed of 51.9% grain sizes of >2 mm but only 20.7% and 23.4% of small and large class pellets, respectively, are composed of >2 mm grains. This shows how although half of what is offered to *H. atra* is not selected in a similar proportion in terms of feeding and excretion. In fact, 50.3% of small *H. atra* pellets and 50% of large *H. atra* pellets were composed of grains from 250 µm-1 mm, which shows that these species prefer medium size grains when feeding. Only 29.8% of Gump sediment was between this range, so the increase abundance in the pellet composition of this size range suggests that there is a preference in size grains for both size classes of namakos.

Future studies based on this experiment can include the utilization of binders in the different sediment stations, such as guar gum, xanthan gum, and carrageenan to aid in weight gain for the sea cucumbers. It has been shown that adding 10% of these binders to the sediment increases growth rate, and perhaps the significantly lower bioturbation rate of the large group can increase if binders were added to the Gump station sand (Won 2018). Adding these various binders to the sand could perhaps make the pellet masses resemble each other more despite the difference in composition, if the Gump sand which had significantly lower pellet collections for the large sea cucumbers were given the advantage of guar gum, xanthan gum, and/or carrageenan to aid in growth.

Similarly, a study performed on *Apostichopus japonicus* tested if various seaweed diets were preferred among this species, and the results found that fresh and boiled *Laminaria japonica* made up 54.73% of these holothurians' intake (Xia *et al.* 2012). Initially, however, the algae was rejected for 30 days then preferred after the sea cucumbers were accommodated with it. So, having more time to study *H. atra* could be useful to observe if the same samples will adjust better over time on the sand stations where there was a significant difference in pellet mass averages, to observe if the difference would be less such after accommodation.

Another study that could be performed on *H. atra* or other holothurians could be a long term project, similar to the two year survey on *Holothuria whitmaei* done in a Western Australian reef and lagoon (Shiell 2010). The findings were that growth rate for this species was better in the mornings but there was no significant findings for seasonal patterns in the year. A long term experiment and survey could be done on *H. atra* to see if the two experiments agree with one another. From a study done on *Apostichopus japonicus,* various categories were studied on the sea cucumber including ingestion rate, digestibility ratio, food conversion efficiency, and specific growth rate (Song *et al.* 2018). A stable isotope analysis was performed to evaluate the pellet components, so in more complex lab settings, perhaps these categories can be selected to be studied for *H. atra*.

With the important process of bioturbation that these holothurians perform in nature, and with the use of holothurians in aquaculture, the further understanding of their feeding and ingestion processes will aid in recognizing and utilizing these creatures' abilities to aid in biodiversity and ecosystem health. Their key importance to the health of coral systems also leads to focus on their feeding and living preferences that can influence the cycling of nutrients that many primary producers depend on.

## ACKNOWLEDGMENTS

I thank professors Cindy Looy, Ivo Duijnstee, Vincent Resh, Noah Whiteman, Seth Finnigan, Stephanie Carlson, Julia Sigward, as well as GSIs Renske Kirchholtes, Clay Noss, and James LaChance for their guidance, expertise, and advice throughout the course of the project. I thank Kylie Tuitavuki for help in Gump sediment collections, Kimberly Kulkovit and Emma Reich for aid in initial field site transect, Eye Sirison for aid in Snack Mahana transect study, Gump Station Facility for the lab equipment and maintenance of the station and wet labs.

## LITERATURE CITED

Chen J.X., 2004. Present status and prospects of sea cucumber industry in China. Advances in sea cucumber aquaculture and management. Fish Tech Pap **463**: 25−38.

Hou, Y.R., Y.J. Son, Q.F. Gao, S.L. Dong, B. Wen, Z. Pan, and C. Liu. 2018. Bioturbation by sea cucumbers Apostichopus Japonicus affects sediment phosphorous forms and sorption characteristics. Aquaculture Environment Interactions **10**: 201-211.

Isgoren-Emiroglu, D. and D. Gunay. 2007. The effect of sea cucumber Holothuria Tubulosa (G., 1788) on nutrient and sediment of Aegean sea shores. Pak J Biol Sci **10**: 586−589.

Mangion, P. 2004. Feeding rate and impact of sediment reworking by two deposit feeders Holothuria Leucospilota and Holothuria Atra on a fringing reef. Echinoderms: Munchen **52**:311-317.

Martinez-Garcia E, Carlsson M.S., Sanchez-Jerez P., Sánchez-Lizaso J.L., Sanz-Lazaro C., and Holmer M. 2015. Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture. Biogeochemistry **125**: 133−148.

Shiell, G.R. and B. Knott. 2010. Aggregations and temporal changes in the activity and bioturbation contribution of the sea cucumber Holothuria Whitmaei (Echinodermata: Holothuroidea). Marine Ecology Progress Series **415**: 127-139.

Song, X., C. Lin, Y. Zhou, X. Wang, R. Gu, S. Xu, Q. Xu, K. Chen, and H. Yang. 2018. Feeding preference of Apostichopus Japonicus: comparing carbon stable isotope analysis and carbon budget approach. Aquaculture Environment Interactions **10**: 243-253.

Talbout, C. and R. Hole. 1994. Fish diets and the control of eutrophication resulting from aquaculture. J Appl Ichthyol **10**: 258−270.

Uthicke, S., M. Byrne, and C. Conand. 2010. Genetic barcoding of commercial Bêchede-mer species (Echinodermata: Holothuroidea). Molecular ecology resources **10**: 634–46.

Wolfe, K., F. Vidal-Ramirez, S. Dove, D. Deaker, and M. Byrne. 2017. Altered sediment biota and lagoon habitat carbonate dynamics due to sea cucumber bioturbation in a high-pCO2 environment. Wiley Global Change Biology **24**: 465-480.

Won, S., A. Hamidoghli, J.H. Lee, J. Bae, and S.C. Bai. 2018. Effects of three different dietary binders on juvenile sea cucumber, Apostichopus japonicus. Turkish Journal of Fisheries and Aquatic Sciences. **18**: 913-920.

Xia, S., P. Zhao, K. Chen, Y. Li, S. Liu, L. Zhang, and H. Yang. 2012. Feeding preferences of the sea cucumber Apostichopus Japonicus (Selenka) on various seaweed diets. Aquaculture Volumes **344-349**: 205-209.

Yamanouti, T. 1939. Ecological and physiological studies on the holothurians in the coral reef of Palao Island. Palao Trop Bio Stud **4**: 603-636.

Yingst, J. Y. 1976. The utilization of organicmatter in shallow marine sediments by an epibenthic deposit-feeding holothurian. Journal of Experimental Marine Biology and Ecology **23(1)**: 55-69.

Yu, S. A comparative study of thermal tolerance and performance between two morphotypes of the black sea cucumber, Holothuria Atra. "Biology and Geomorphology of Tropical Islands" class, University of California, Berkeley: Student Papers. [http://ib.berkeley.edu/moorea/uploads/6/6/8/3/6683664/2001_final_papers.pdf - accessed Nov 14 2017]

**APPENDIX A**

TABLE 3. The average sizes of each group of sea cucumbers was taken to find standardized grouping measurements for each bin. Note the clear difference in mass and the average length.

| Group | Average Small Mass (g) | Avg. Small Length x Width (cm) | Avg. Large Mass (g) | Avg. Large Length x Width (cm) |
|---|---|---|---|---|
| 1 | 22.208 | 5.71x1.93 | 82.103 | 13.70x3.63 |
| 2 | 29.967 | 6.71x2.86 | 74.661 | 14.25x3.42 |
| 3 | 24.794 | 6.75x2.10 | 78.975 | 13.95x3.23 |
| 4 | 22.274 | 6.97x3.71 | 96.214 | 14.6x4.39 |