

HOLOTHURIAN HOST SIZE AND ITS EFFECTS ON SYMBIONT COLONIZATION

DANA LIN

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. The first organisms that come to mind at the thought of “ocean biodiversity” are usually fish and other vertebrates, often causing the more concealed forms of biodiversity to be overlooked and underestimated. A complex network of relationships exists in the marine ecosystem, with host and endo- or ectosymbiotic relationships making up just a subset of those. Several studies in the past have noticed trends with more endo- and ectosymbionts of a single species occurring on larger holothurians. This study investigates this phenomenon on a broader scale to see if biodiversity itself increases with sea cucumber size. Three categories of sea cucumbers were used: *Bohadschia argus*, sexual *Holothuria atra*, and asexual *Holothuria atra*. Organisms were collected in a clear plastic bag around the island of Moorea, French Polynesia. The length of the sea cucumber was measured. Then the number and mass of endo- and ecto-symbionts on each individual were recorded. Results yielded a general trend with larger sea cucumbers displaying higher levels of biodiversity. This knowledge would be beneficial to more accurately assess the amount of biodiversity loss as a consequence of overfishing.

Key words: sea cucumber; *Bohadschia argus*; *Holothuria atra*; holothurian; biodiversity; size; ectosymbiont; endosymbiont; parasites; commensalism; asexual reproduction; sexual reproduction

INTRODUCTION

Biodiversity is generally agreed upon to be an important element in maintaining a healthy ecological system. A higher amount of biodiversity is correlated with greater resiliency against the effects of climate change in both agricultural crops and wildlife habitats (Reiss et al 2009).

When the idea of “biodiversity” is brought up, however, the first organisms that come to mind are relatively large vertebrates. In terms of the marine habitat, fish and whales are the first animals pictured. However, an ample amount of hidden biodiversity resides in the harder-to-see crevices. Ecto- and endosymbionts that live on the inside and outside of free-living organisms, respectively, contribute a significant amount to this hidden biodiversity (Paracer and Ahmadjian 2000, Moran 2006). These symbiotic organisms can be parasitic and harm the host, commensal and not affect the host at all, or mutualistic to benefit the host and itself (Bronstein 1994, Douglas 2010, Getz 2011). Removal of the larger, free-living organisms – e.g. via fishing – also means habitat loss for these smaller organisms that rely on the host for resources (Scheffer et al 2005). Marine endo- and ectosymbionts are especially vulnerable to

such habitat loss because so few types of seafoods are farm-raised, and instead just plucked out from the sea (Coll et al 2008).

A number of studies have been conducted on the symbiotic organisms associated with holothurians, or sea cucumbers, usually examining its relationship with just one species of symbiont (Kato 1998, Lyskin and Britaev 2005). In many of these studies, there were trends showing that larger species of sea cucumbers had overall more symbionts colonizing them. For example, the scaleworm *Gastrolepidia clavigera* had higher infestation rates on larger holothurians (*Stichopus chloronotus* and *Stichopus variegatus*) at 60-80 percent, compared to the smaller species (*Holothuria atra* and *Actinopyga echinites*) at 30-50 percent infestation (Britaev and Lyskin 2002). In addition, a threshold size was found for the sea cucumber *Holothuria scabra* (80 mm) before the parasitic pea crab, *Pinnotheres halingi*, was found in the respiratory tree (Hamel et al 2011).

This study seeks to examine these trends on a broader scale, to see how size affects the amount of biodiversity in general on an organism. Two of the most prevalent species of sea cucumber in Moorea, French Polynesia include *Holothuria atra* and *Bohadschia argus*. *B. argus* occurs only in the sexual morph, while

H. atra reproduces in two ways, sexually and asexually. Preliminary data suggests that the asexual population of *H. atra* may be smaller than its sexual counterparts, so the two populations will be categorized separately. (Conand 1995, Laxminarayana 2006).

I hypothesize that larger species of holothurians will have on average more biodiversity than smaller species, because the larger ones could potentially provide more, or better resources for the symbionts. There are four associated questions I seek to answer with my hypothesis. Is there a difference in size between the asexually reproducing *H. atra*, the sexually reproducing *H. atra*, and *B. argus*? Do the distributions of macrosymbionts occurring on the three groups differ? Does the amount of biomass accumulated on the three groups differ? And finally, do the larger-sized holothurians have a greater calculated Shannon Diversity Index and evenness values?

METHODS

Study site

All organisms were collected between October 14 and November 5, 2015, with each group of holothurian taken from different sites on Moorea, French Polynesia (FIG. 1). All three groups of holothurians occurred at Temae Beach and the Haapiti reef flat, while only *B. argus* occurred inside Cook's Bay. The reason for different collection sites was because each group of holothurian occurred at the highest frequency there, ensuring the lowest chance of depopulating each group.

B. argus individuals were collected from Cook's Bay, either from within a 50 m range of Gump Station (17.490° S, 149.826° W) or at the algal ridge at the mouth of the bay (17.483° S, 149.826° W). The sexual morph of *H. atra* was acquired from the back reef of Temae Public Beach (17.498° W, 149.761832° S), and the asexual morph of *H. atra* from the Ha'apiti reef flat (17.562° W, 149.871° S) on the south side of the island.

Data Collection

Specimens were obtained by snorkeling. A clear plastic bag was used to collect the holothurian and some seawater. The bag was then sealed to ensure that no ectosymbionts were lost during the transportation back to Gump Station. Once at the station, all contents of the bag were poured into a bucket and left

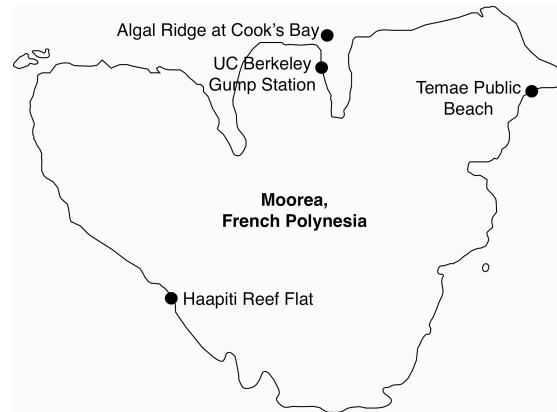


FIG. 1. Sites sampled in this study on the island of Moorea, French Polynesia. *B. argus* was collected from Cook's Bay. Sexual *H. atra* was collected from Temae Public Beach. Asexual *H. atra* was collected from the Haapiti reef flat.

to sit in stagnant seawater for approximately one to three hours. As oxygen levels decrease, endosymbiotic pearlfish emerge from the anus to breathe (Luciano et al 2002). Any pearlfish present were rapidly dabbed dry, weighed, and inserted tail-first back into the holothurian anus to ensure survival. All fish were handled in accordance with the Animal Care and Use Protocol # AUP-2015-05-7549.

The length of each holothurian was taken in their relaxed state to the nearest quarter centimeter. The surface and tentacles of the holothurian were then examined closely for known ectosymbionts (Britayev and Zamishliak 1996, Hoeksema and Gittenberger 2008, Caulier et al 2013). Those found include eulimid snails, polychaete worms (*Gastrolepidia clavigera*), and harlequin crabs (*Lisocarcinus orbicularis*). These ectosymbionts were also dabbed dry and mass recorded. All specimens were returned to their original locations.

Statistical Analysis

A one-way ANOVA was used to assess the difference in size (length) amongst the three holothurian groups (*B. argus*, sexual *H. atra*, and asexual *H. atra*). A PERMANOVA test was used to assess the differences in symbiont distribution between the three groups. A Kruskal-Wallis one-way analysis of variance was used to test for differences in symbiont biomass amongst the three groups. Finally, the Shannon Diversity Index and evenness was calculated for each group of

holothurians to quantify if diversity increases in larger species or morphs of holothurian.

RESULTS

The average length each sea cucumber group decreased in the following order: *B. argus*, sexual *H. atra*, asexual *H. atra* (FIG. 2). Upon doing a one-way ANOVA for differences in length, I found that at least one group was significantly different from the rest ($F_{2,117} = 116.5$, $p < 0.001$). Further analysis using a post-hoc test doing pair-wise comparisons between each group indicated that all size groups were significantly different from each other ($p < 0.001$ for all pair-wise comparisons).

Community composition among the three groups of sea cucumbers were different as well ($p < 0.01$). I found all four species of symbionts on the largest group of sea cucumber, *B. argus*. For both morphs of *H. atra*, I found only Eulimid snails and harlequin crabs on them. However, a greater proportion of individuals of the sexual morph had snails and crabs on them than the asexual morph (FIG. 3)

Analysis of the amount of biomass accumulated on each group of holothurian reflected the results in community composition. *B. argus* had the most accumulated biomass, with an average of 2.027 g per individual, followed by sexual *H. atra* with 0.0827 g, and asexual *H. atra* with 0.0168 g (FIG. 4). A Kruskal-Wallis test indicated that there was at least one group that had accumulated a significantly different amount of symbiont biomass from the rest (chi-squared = 28.491, $df = 2$, $p < 0.001$). A Dunn's test was then used as a post-hoc analysis, and results indicated differences

between all three pair-wise comparisons (all $p < 0.05$).

Like the trend in accumulated biomass, both the mean Shannon Diversity Indices and mean evenness values decreased as the average size of holothurian decreased (FIG. 5). A Kruskal-Wallis test indicated that at least one holothurian group had a diversity index that was different from the rest (chi-squared = 18.122, $df = 2$, $p < 0.001$). A Dunn's post-hoc test did three pair-wise comparisons identifying the differences to be between all three groups. Similarly, the Kruskal-Wallis test indicated at least one holothurian group had an evenness value that was different from the rest (chi-squared = 18.055, $df = 2$, $p < 0.001$). Dunn's test yielded a result that indicated the evenness between *B. argus* and both *H. atra* morphs were significantly different ($p < 0.01$ for both), but not between the two morphs of *H. atra* ($p = 0.0505$).

DISCUSSION

I discovered three distinct size categories among the sea cucumbers collected, and treated these three groups as separate categories for further analysis even though two of the groups were of the same species (*H. atra*). The reason the asexual group is significantly smaller could be due to the way they divide. The asexual morph divides by transverse fission, when the two ends of the holothurian essentially split in half, and the anterior and posterior portions of the new buds regenerate to form two separate individuals. After the split, a negligible amount of growth occurs, yielding the significant size difference between the sexual and asexual morphs of *H. atra*.

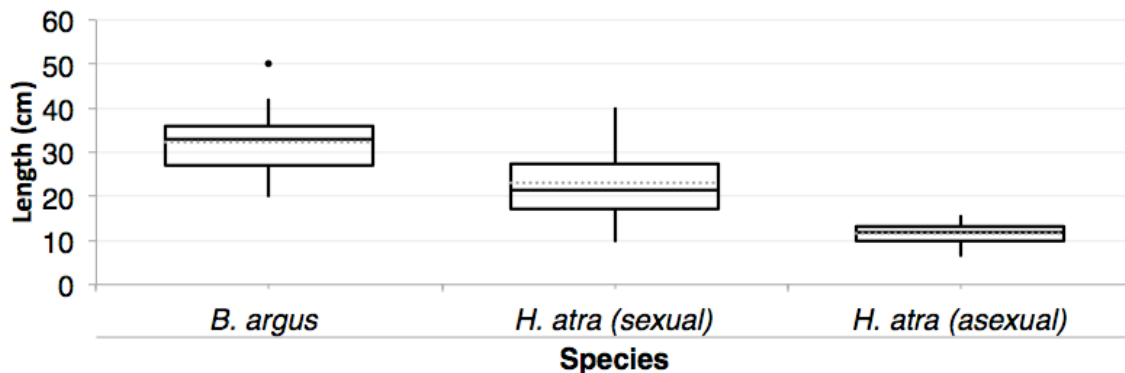


FIG. 2. Size distribution of the three groups of sea cucumbers. The dotted line represents the mean, and within the box is the interquartile range. The ends of the whiskers represent 95 percent of the data, and the horizontal line in the box represents the median value. Individual dots represent outliers.

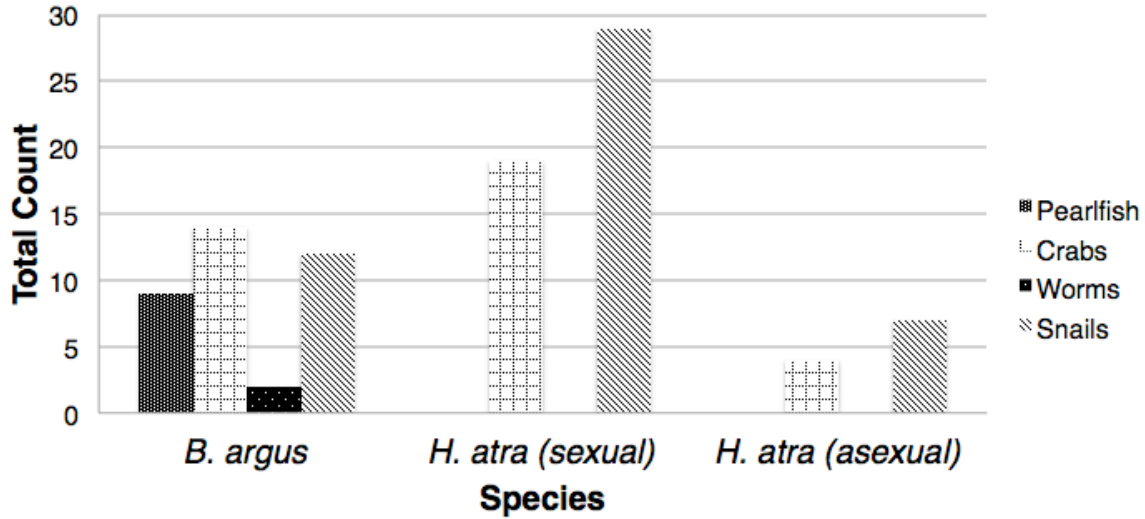


FIG. 3. Symbiont distribution among the three holothurian groups (n = 25 individuals per group).

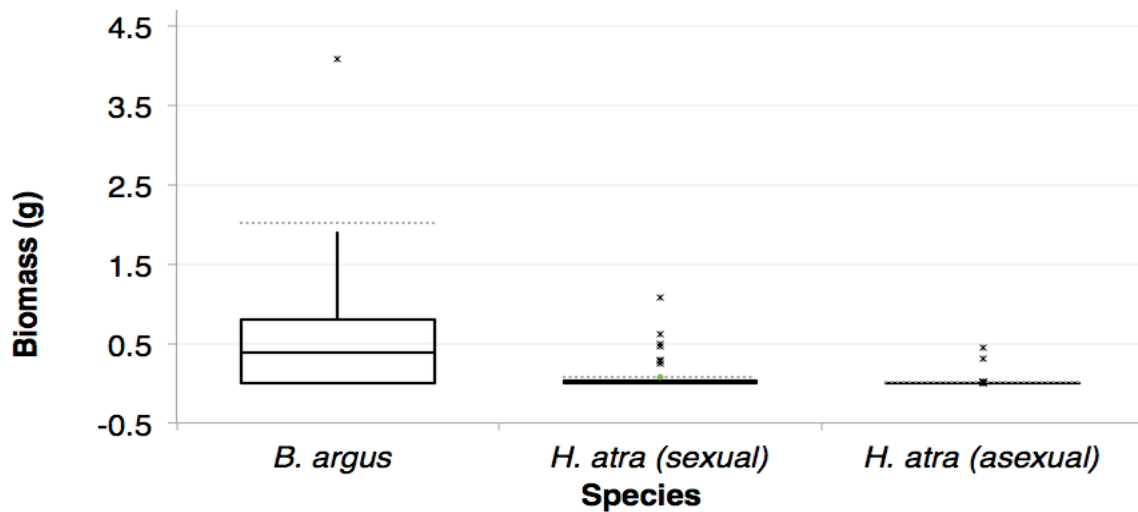


FIG. 4. Biomass distribution among the three holothurian groups. The dotted line represents the mean, and within the box is the interquartile range. The ends of the whiskers represent 95 percent of the data, and the horizontal in the box represents the median value. Individual x's represent outliers. There are two additional outliers in the data for *B. argus* not shown in the plot, one at $y = 17.6$, and another at $y = 19.8$.

The difference in community composition could possibly be explained by the same reason there was a difference in accumulated biomass of symbionts: size. By a visual assessment, *B. argus* had a wider circumference than both morphs of *H. atra*, and therefore a larger volume as well. In response to this larger volume is a larger anus and respiratory tree to accommodate a higher sediment turnover rate and a respiration rate, respectively, to sustain a larger organism. These enlarged anatomical parts allow more

physical space for the pearlfish to develop inside of the holothurian, since the pearlfish uses the anus as an entrance to swim up into the respiratory tree where it lodges itself for most of its life. The reduced size of both morphs of *H. atra* are likely too small to house the pearlfish.

It is unsure why the commensal scaleworm *Gastrolepidia clavigera* was only found on the largest species, *B. argus*. A previous study did find them on *H. atra*. However, it was more common for the

	<i>B. argus</i>	<i>H. atra</i> (sexual)	<i>H. atra</i> (asexual)
Diversity Index	0.235	0.0716	0
Standard Dev.	0.359	0.207	0
Evenness	0.316	0.103	0
Standard Dev.	0.471	0.299	0

FIG. 5. Calculated Shannon Diversity Indices and evenness values for the three groups of holothurians.

researchers to find *G. clavigera* on larger species of holothurians than smaller (12). Unlike the eulimid snails and Harlequin crabs that are still frequently found on *H. atra*, *G. clavigera*'s scales may not offer as solid of a protection as the calcified shells of the snails and crabs. I can only speculate that larger holothurians offer more ventral surface area for the worms to hide under, helping to protect them from predators.

The potential reason a lower frequency of eulimid snails was found on *B. argus* compared to sexual *H. atra* could be due to another factor that is influenced by holothurian size. *B. argus* has a thicker skin, or integument, than that of *H. atra*. This may be to hold in the greater amount of volume without tearing. Because the snails feed on the body fluid, or coelom, of the holothurian with their mouth-piece, it is likely harder for the snails to penetrate a thicker integument, therefore making it harder to sustain themselves on *B. argus* (Queiroz et al 2013).

The discrepancy in frequency of the eulimid snail on the sexual and asexual *H. atra* can likely be explained by differences in surface area. Even though the thickness of the integument on sexual and asexual *H. atra* are likely the same, a greater amount of snails were found on the sexual morph. This is likely due to the fact that the sexual morph simply has a greater surface area to hold more snails. A similar phenomenon is seen with the harlequin crab on the two morphs of *H. atra*, and could be explained with the same reasoning.

The Shannon Diversity Indices and evenness calculations support the idea that that diversity and evenness decrease as the average size of holothurian goes down. The zero values for asexual *H. atra* are not due to

the fact that no organisms were found at all on asexual individuals, but because when there were symbionts found on them, they only included organisms from a single species. A single organism dominating a habitat equates to zero diversity in this calculation index.

The general trend of increasing biodiversity in increasing sizes of holothurian has massive implications in terms of the amount of hidden biodiversity in larger organisms. Removing a larger sea cucumber from the ocean would not be the same as removing a smaller one, because taking away a larger individual would also inadvertently remove more symbionts using it as a host.

With the human population multiplying exponentially, the demand for seafood can only increase, along with the risk of overfishing (Trostle 2008). The Asian markets find a number of holothurian species to be a delicacy. A variety of factors contribute to a sea cucumber's value, including how large it is (Purcell et al 2011). Larger individuals have a higher market value, especially because processing of the holothurian shrinks the final product to about 10 percent the size of the original – making a larger one look more impressive (Conand and Byrne 1993).

A range of taxonomic groups we collectively call "fish" are overexploited for food as well—and many of them have symbionts tightly associated with them, such as copepods, worms, and even other fish (Kirtisinghe 1964, Losey Jr. 1979, Williams et al 1994). With this new awareness, we can more accurately estimate the biodiversity loss when we catch marine organisms *en masse*.

ACKNOWLEDGMENTS

I am grateful for the entire Moorea class of 2015 who have helped make my study run more smoothly and enjoyably. I want to give extra thanks to Professor Stephanie Carlson who spent extensive amounts of time to help me formulate my research questions, as well a fellow undergraduate Numfah Vanitchanant, who not only taught me everything I know about R, but was also ready to help me collect sea cucumbers at all times of day. I am also eternally grateful the graduate students who held office hours every single day of the week for three months in a row.

LITERATURE CITED

Britaev, T. A., and S. A. Lyskin. 2002. Feeding of the symbiotic polychaete *Gastrolepidia*

- clavigera* (Polynoidae) and its interactions with its hosts. *Doklady Biological Sciences* **385**:352-356.
- Britayev, T. A., and E. A. Zamishliak 1996. Association of the commensal sclaeworm *Gastrolepidia clavigera* (Polychaeta: Polynoidae) with holothurians near the coast of South Vietnam. *Ophelia* **45(3)**:175-190.
- Bronstein, J. L. 1994. Our current understanding of mutualism. *Quarterly Review of Biology* **69**: 31-51.
- Caulier, G., P. Flammang, P. Gerbaux, and I. Eeckhaut. 2013. When a repellent becomes an attractant: Harmful saponins are kairomones attracting the symbiotic harlequin crab. *Scientific Reports* **3**:2639.
- Coll, M., S. Libralato, S. Tudela, I. Palomera, and F. Pranovi. 2008. Ecosystem overfishing in the ocean. *PLoS ONE* **3**: e3881.
- Conand, C. 1995. Asexual reproduction by fission in *Holothuria atra*: variability of some parameters in populations from the tropical Indo-Pacific **19**:209-216.
- Conand, C., and M. Byrne. 1993. A review of recent developments in the world sea cucumber fisheries. *Marine Fisheries Review* **55**: 1-13.
- Douglas, A. 2010. *The Symbiotic Habit*. Princeton University Press, New Jersey.
- Hamel, J., P. K. L. Ng, A. Mercier. 1999. Life cycle of the pea crab *Pinnotheres halingi*, sp. nov., an obligate symbiont of the sea cucumber *Holothuria scabra* jaeger. *Ophelia* **50**: 149-175.
- Hoeksema, B. W., and A. Gittenberger. 2008. Records of some marine parasitic molluscs from Nha Trang, Vietnam. *Basteria* **72**:129-133.
- Kato, M. 1998. Morphological and ecological adaptations in montacutid bivalves endo- and ecto-symbiotic with holothurians. *Canadian Journal of Zoology* **76**: 1403-1410.
- Kirtisinghe, P. 1964. A review of the parasitic copepods of fish reordred from Ceylon with description of additional forms. *Bulletin of the Fisheries Research Station, Ceylon* **17**:45-132.
- Laxminarayana, A. 2006. Asexual reproduction by induced transverse fission in the sea cucumbers *Bohadschia marmorata* and *Holothuria atra*. *Beche-de-mer Information Bulletin* **23**:35-37.
- Losey Jr., G. S. 1979. Fish cleaning symbiosis: proximate causes of host behavior. *Animal Behaviour* **27**: 669-685.
- Luciano, B., A. Lyman, S. McMillan, and A. Nickels. 2002. The symbiotic relationship between sea cucumbers (Holothuriidae) and pearlfish (Carapidae). UCSC BioE 159 Moorea class papers.
- Lyskin, S. A., and T. A. Britaev. 2005. Symbionts of holothurians from South Vietnam: intra- and interspecific interactions. *Doklady Biological Sciences* **401**: 116-119.
- Moran, N. A. 2006. Symbiosis. *Current Biology* **16**: 866-871.
- Paracer, S., and V. Ahmadjian. 2000. *Symbiosis: An Introduction to Biological Associations*. Oxford University Press, Oxford.
- Purcell, S. W., A. Mercier, C. Conand, J. Hamel, M. V. Toral-Granda, A. Lovatelli, and S. Uthicke. 2011. Sea cucumber fisheries: global analysis of stocks, management measures, and drivers of overfishing. *Fish and Fisheries* **14**: 34-59.
- Queiroz, V., L. S. de Souza, A. D. Pimenta, and C. M. Cunha. 2013. New host records to *Melanella* (Caenogastropoda: Eulimidae) from the Bazilian coast. *Marine Biodiversity Records* **6**: e67.
- Reiss, J., J. R. Bridle, J. M. Montoya, and G. Woodward. 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends in Ecology and Evolution* **24**:505-514.
- Scheffer, M., S. Carpenter, and B. de Young. 2005. *Cell Press* **20**:579-581.
- Trostle, R. 2008. Global agricultural supply and demand: factors contributing to the recent increase in food commodity prices. United States Department of Agriculture, USA.
- Williams, H. H., A. Jones, and D. W. T. Crompton. 1994. *Parasitic worms of fish*. Taylor & Francis, London.