

Planktonic coral larvae have crucial environmental requirements for settling into a sessile stage. How deposit-feeding ecosystem engineers alter settlement success, and their potential effects on coral reef regeneration, has not been heavily investigated. Holothurians, echinoderms commonly called sea cucumbers, are the dominant deposit feeders on the Caribbean seafloor.¹ They play a vital role in coastal ecosystem health through nutrient cycling via bioturbation, accounting for up to 50% of dissolution of calcium carbonate, a mineral necessary for coral growth, from sediment.² Very few studies have investigated how their bioturbation affect settlement of coral larvae and survivorship of recently settled polyps. **I propose to determine the bottom-up and top-down influence of holothurians on coral larvae recruitment and survivorship after settlement, comparing the strength of the two processes to determine overall effects on coral community growth.**

Intellectual Merit and Approach: The extent to which holothurians modify sediment microbial communities is unclear, but there is evidence bacterial cultivation within the gut aids digestion. As consumed sediment is digested and excreted, excess cultivated bacteria from the gut may be transferred into the surrounding sediment.³ Through this, holothurian digestion likely alters community composition of biofilms along benthic sediment. This is important to coral recruitment as microbial composition within biofilms, including *Pseudoalteromonas luteoviolacea* (*PL*) abundance, play a fundamental role in promoting larval settlement.⁴ *PL* is also found within the guts of holothurians and are potentially a strong signal for larvae settlement.^{5,6} While the role of *PL* as a settlement cue has been studied, only one or two species of coral larvae have been used. This prevents an accurate description of the role of *PL* in larval settlement within natural coral communities. Furthermore, holothurians may directly impact survivorship of settled larvae (polyps) through sediment stress via fecal deposits.⁷ Excessive sediment on polyps hinders growth and causes tissue loss; excretion of sediment by holothurians can significantly contribute to this stress, potentially inducing coral mortality. *Isostichopus badionotus* (*IB*) is a common sea cucumber widely distributed on shallow sand beds across the Caribbean.⁸ I will collect field samples of *IB* and analyze their impacts on coral larvae settlement and polyp survivorship in St. Thomas, USVI while a PhD student in Dr. Brian Silliman's lab at Duke University. There will be on-site collaboration with Dr. Marylin Brandt's lab at the University of the Virgin Islands, St. Thomas.

Hypotheses: I hypothesize *IB* feeding increases the concentration of *PL* in the surrounding sediment (aim 1), indirectly promoting coral larval recruitment (aim 2). I also hypothesize that *IB* will have a strong negative direct effect on survivorship of coral polyps by increasing sediment stress (aim 3). I will experimentally test (1) **how *IB* influences the concentration of *PL* in the sediment through deposit feeding**, (2) **if *PL* is a settlement cue for 20 common Caribbean coral larvae**, and (3) **how *IB* abundance influences mortality of coral polyps**.

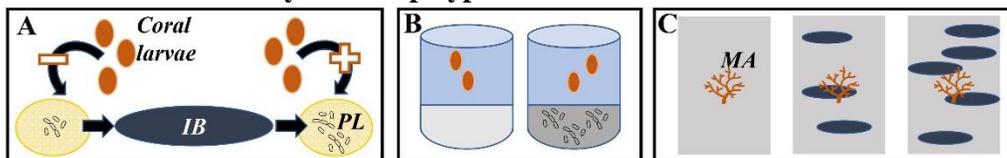


Figure 1. Illustration of mechanisms behind specific aims. A. (Aim 1) bottom-up effect of *IB* on coral settlement. B. (Aim 2) *PL* as a coral settlement cue. C. (Aim 3) top-down effect of *IB* on *MA* polyps.

Aim 1. Bottom-up effect: *IB* role in *PL* sediment concentration. In St. Thomas, 30 *IB* will be collected in the field and transported to the lab for microcosm experimentation. *PL* will be obtained from the Scripps Institution for Oceanography, incubated for 30 days to allow for sufficient growth, then incorporated into microcosm sediment. 30 total microcosms (10 control with no *IB*, 10 with 1 *IB* per microcosm, 10 with 2 *IB* per microcosm) will be used over 10 days. Bacterial abundance in sediment before *IB* introduction, 10 samples of recently excreted fecal matter (>1hr) per individual, and sediment after 10 days will be determined using 4,6-diamidino-2-phenyl-indole staining to count bacteria individuals with epifluorescence microscopy. I expect the abundance of *PL* in fecal matter and final sediment to be significantly greater compared to initial concentrations. This would suggest holothurian cultivation of bacteria within the gut enhances microbial abundance in the sediment via defecation. If abundance of *PL* is significantly lower in fecal matter and final sediment, it is possible *IB* selectively

feeds on members of this bacterial genus rather than cultivating it within the digestive system. This would subsequently affect recruitment differently by consuming *PL* and reducing its abundance in biofilms.

Aim 2. *PL* as a settlement cue for common coral larvae. 40 limestone settlement tiles will be distributed across 10 sites in St. Thomas and left for 30 days to allow natural biofilms to accumulate. During this, larvae of 20 common coral species in St. Thomas will be obtained using nighttime SCUBA during synchronous spawning events. Cultures of *PL* from Aim 1 will be grown into bacterial mats and transferred to 40 new tiles. Tiles of natural biofilms (with undesired larvae from field deployment removed) and *PL* will then be placed into one of 80 glass containers with filtered sea water. 3 larvae per coral species will be introduced to each microcosm. After 5 days to allow for settlement and metamorphosis, I will record the number of larvae settled, metamorphosed but not settled, and total surviving. Based on previous research finding *PL* as a settlement cue for the coral larvae species included in the studies, I expect the percentage of settlement as well as diversity of settled larvae to be greater in *PL* microcosms than natural biofilms.^{4,5,6} Assuming Aim 1 concludes increased *PL* in sediment with *IB* interaction, this result would indicate *IB* indirectly influences coral larval settlement by altering *PL* abundance in microbial biofilms. If the percentage of settlement is not significantly different between *PL* and natural biofilms, additional studies will be done to conclude whether *IB* alters coral larvae settlement through alternate bacteria. The application of this emerging concept on a larger diversity of Caribbean coral species will provide valuable insight into the ecological influence of bacteria on coral reef growth.

Aim 3. Top-down effect: mortality of coral polyps with *IB* presence. I will collect larvae of the common reef building coral *Montastraea annularis* (*MA*) in St. Thomas for laboratory rearing using the same methods as larval collection in Aim 2. Collections will be transported to the lab and fertilization promoted in individual petri dishes. Once the larvae settle into polyps (~1 month), individuals will be transferred to aquaria and adhered with cement to one of 9 constructed platform complexes. 20 polyps will be adhered to each platform with a 30mm plastic mesh fencing placed around the entire platform to prevent undesired transient predation. I will collect 64 *IB* in the field and placed into a respective platform treatment- *IB* absent, low intensity (3 *IB*), and high intensity (5 *IB*), with 8 replicates of each treatment. These platforms will be transplanted into the field and monitored daily for 2 weeks to record sediment accumulation (% of sediment coverage) and *MA* mortality (>80% tissue loss per individual). I expect *MA* mortality to be greater in high intensity conditions than low and control due to the accumulation of sediment and fecal matter that places sediment stress on the polyps. If there is no significant change of polyp mortality respective to *IB* intensity, then it is likely that holothurians do not exert a profound top-down influence on coral polyp survivorship through sediment stress.

Broader impacts: By expanding our understanding of coral larval settlement and survivorship to include holothurian influences, coral restoration efforts can improve management practices accordingly with minimal additional costs. Considering the rapid decline of coral reefs and surge of federal, state, and private funding into coral restoration, it is vital to investigate the relative importance of top-down vs bottom-up effects of holothurians on larval settlement. I will communicate the importance of holothurians to the USVI community through narrative blog writing on my website and organize public painting events for creative artistic outreach. Furthermore, I will mentor two Duke University undergraduate students each year of my graduate career, coined the **Sea Slugs Scholars**. Working with the Duke Undergraduate Research Society (URS), I will encourage women and LGBTQ+ individuals within URS to become a Sea Slug Scholar. This opportunity will build bridges for underrepresented groups in STEM to develop collaborative and independent research skills. Each student will contribute to my research for a semester, then develop an independent project parallel to my work the following semester. Sea Slug Scholars will be encouraged to apply for the URS Conference Grant and present their project at a national conference. If this grant does not cover all conference expenses, I will organize fundraisers to cover the difference. Providing additional funding alongside the URS Conference Grant will promote students from all financial backgrounds to develop a career within STEM beyond their undergraduate experiences.

References: ¹Roberts et al., *Oceanogr. Mar. Biol.* 2000 ²Schneider et al., *J. Geophys Res.* 2011 ³Gao et al., *J. Oceanol. Limnol.* 2021 ⁴Negri et al., *Mar. Ecol. Prog.* 2001 ⁵Bosch et al., *Curr. Top. Dev. Biol.* 2021 ⁶Zhang et al., *Coral Reefs* 2018 ⁷Sloan, *Mar. Ecol. Prog. Ser.* 1980 ⁸Purcell et al., *FAO* 2012