# UNDERSTANDING THE IMPORTANCE OF HABITAT COMPLEXITY FOR JUVENILE FISH AND THE APPLICATION OF 3D PRINTED CORALS FOR REEF RESTORATION

by

Emily Jean Ruhl

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Marine Studies

Summer 2018

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Approved: _____
Danielle L. Dixson, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Mark A. Moline, Ph.D.
Director of the School of Marine Science and Policy

Approved: _____
Estella A. Atekwana, Ph.D.
Dean of the College of Earth, Ocean, and Environment

Approved: _____
Ann L. Ardis, Ph.D.
Senior Vice Provost for Graduate and Professional Education

## ACKNOWLEDGMENTS

There are many people who deserve my thanks for helping me to complete this work that I am so proud of. First, thank you to Dr. Danielle Dixson for welcoming me into your lab and guiding me through this incredible experience. Conducting research in Fiji presented obstacles that I wasn't always confident I would overcome, but in the end served to make me a strong scientist. Thank you for trusting in my abilities and pushing me to finish this work. Thanks must also be given to my committee members, Dr. Mark Warner and Dr. Art Trembanis, for their valuable input with my experimental design and lots of technical support. I don't know how to thank Janis Lopez, my fellow Gettysburgian, enough for everything she has done for me these past two years. Your guidance, emotional support, and friendship have meant so much to me; thank you for everything. Luci Coumatos, administrative assistant to the stars, you seriously rock. Thank you for your incredible patience with the mess of Fiji receipts and mishaps, and doing it all with a beautiful smile. Thank you to Jason Button for teaching me your master printing skills, and to Sydney Sapp and Nicole Detorres for printing some of the corals used in these experiments. A huge thank you to all members of the Dixson Lab, past and present. I would not have accomplished everything I did without your love and support. Rohan, thank you for always having an open door, and helping me to grow as a scientist. Paul, the Dwight to my Michael, you are an incredible coworker and friend, and I feel very lucky to have you in my life. Finally, thank you to my family, Chris, and Joe for your never-ending love and support.

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## ABSTRACT

Coral reef systems have been experiencing ongoing regional declines in important topographic and biotic complexity over the last 40 years. While studies have documented the importance of live coral cover in the habitat selection process of reef fishes, the role that habitat complexity plays in these choices is poorly understood. Here, we isolated the physical structure of a common Indo-Pacific reef building coral, *Pocillopora damicornis,* using 3D printed models to investigate complexity preferences and resulting behavior of the coral-associated lemon damselfish, *Pomacentrus moluccensis*. A secondary objective of this work was to determine if 3D printed objects could be used in reef restoration by providing critical habitat for fishes and acting as viable settlement sites for recruiting reef-building corals. During a cafeteria-style choice experiment, *P. moluccensis* was highly selective of the high and medium complexity corals over the low complexity coral. During behavioral observations, fish occupying 3D printed low complexity corals had the highest rate of abandonment and most deviant behavior from live and dead control *P. damicornis* coral heads. Conversely, fish placed on 3D printed medium and high complexity corals spent more time utilizing the shelter than fish on control corals and behaved similarly to the controls when not utilizing the shelter. These results show that coral physical structure is an important factor driving habitat preferences of *P. moluccensis* and plays a critical role in influencing their behavior. Further, coral settlement was observed using 3D printed settlement tiles made of different filaments placed on a Fijian reef. Coral settlement was highest on nGen material. Thus, artificial corals made using 3D printing technology designed to mimic the structural complexity of healthy reefs have the potential to be used as reef habitat restoration structures in the future.

# Chapter 1

# UNDERSTANDING THE ROLE STRUCTURAL COMPLEXITY PLAYS IN SETTLEMENT SITE SELECTION OF JUVENILE REEF FISH

## 1.1 Introduction

Globally, coral reef systems are declining due to a combination of anthropogenic stressors (Jackson et al. 2001; Hughes et al. 2003; Anthony 2016). The susceptibility of coral reefs to these stressors has led to a significant loss of live coral cover and an increase in algae cover (Côté et al. 2005; Bruno and Selig 2007; Chong-Seng et al. 2012; Hughes et al. 2017). While recovery from short-term disturbances is possible, the compounding effects of repeated coral loss often renders a disturbed reef unable to return to a coral-dominated system (Mumby and Steneck 2008).

Phase shifts from coral-dominated to algal-dominated systems have bottom-up effects on the macrofaunal community composition (Cheal et al. 2008). Many larval reef fishes rely on positive chemical cues, such as the presence of conspecifics and live coral cover, for successful identification of reef habitat during the recruitment process (Jones et al. 2004; Dixson et al. 2014; Coppock et al. 2016). When these cues are removed or suppressed, recruitment processes are stifled. A meta-analysis of studies aimed to address the effects of disturbance-induced coral loss on reef fishes found that 62% of fishes had significant declines in abundance after a >10% loss of coral cover (Wilson et al. 2006). Further, because coral loss directly impacts specialized coral-dependent fishes, the community structure that remains after a

disturbance is less diverse and dominated by habitat generalists (Pratchett et al. 2014). This shift in biodiversity can limit reef recovery through the loss of natural recruitment and has additional socio-economic implications for coastal communities that depend on the reef (McClanahan 2008).

Coinciding with the loss of live coral, topographic complexity has also declined (Alvarez-Filip 2009; Graham and Nash 2013). The loss of structural complexity, primarily provided by scleractinian corals, results in flattened reefs that are dominated by weedy algae and causes a "death-spiral" where reefs continue to degrade (Coker et al. 2009; Darling et al. 2017). The impact of coral reef structural complexity on the macro-faunal community has been well documented on a community-level scale (Noonan et al. 2012; Pereira and Munday 2016). For example, fringing reefs around the inner Seychelles islands with high coral cover (up to 58%) and structural complexity were positively correlated with species richness of fishes, fish functional groups, and corallivorous fishes compared to reefs with high macroalgal cover (up to 95%) and low structural complexity (Chong-Seng et al. 2012). Similarly, artificial reefs on the Great Barrier Reef indicated that while differences in complexity did not have a direct effect on fish recruitment, fish abundance was increased on reefs with higher complexity (Caley and St. John 1996).

The loss of important habitat complexity limits space and refuge sites, which impacts intraspecific and interspecific competition that shapes the community structure of a reef (Hutchings and Reynolds 2004; Graham and Nash 2013). Patch reef studies have shown that survival of new recruits on reefs with medium to high topographic complexity is higher compared to reefs with low topographic complexity (Almany 2004; Noonan et al. 2012). While all recruits are subjected to strong selective

pressure during the settlement period, the availability of suitably complex space can facilitate post-settlement survival (Doherty et al. 2004; Lecchini et al. 2010). After the settlement period, significant interactions exist between the presence of predators and the availability of microhabitat refuge spaces, a defining quality of complex habitats, for survival of common prey fish (Beukers and Jones 1998).

However, juvenile reef fish respond to their habitats differently, and may also experience limitations in overly complex systems. Studies have indicated that coral-dependent prey fish exhibit higher feeding rates and bold behavior in low complexity corals that allow for a large visual field (McCormick and Lönnstedt 2013). Limited visual cues in high complexity habitats may have the greatest implications for feeding rates and survival on territorial, benthic dwelling prey fishes that can be ambushed by predators when attempting to leave their shelter. Further, a common Caribbean damselfish, *Stegastes partitus*, had reduced displays of courtship and movement in complex habitats, which may have long term impacts on fecundity (Rilov et al. 2007).

It is clear that habitat quality and structure can have significant bottom-up effects on shaping the fish community. While studies have documented the role of live coral cover (Pratchett et al. 2011; Coker et al. 2012b) and of visual and chemical cues in habitat selection for recruiting reef fish (Ben-Tzvi et al. 2010; Vail and McCormick 2011; Dixson et al. 2014), the specific effects of habitat complexity alone are not well understood. Because many small reef fish select their habitat during the recruitment stage and rarely emigrate from those initial choices (Booth and Beretta 1994), understanding the role of structural complexity should be recognized.

Studies that have aimed to examine habitat complexity in the past have used live or degraded corals to assess habitat preferences and behavioral traits of reef fishes

(Coker et al. 2009; Graham and Nash 2013). However, using natural coral cannot isolate the physical structure of the habitat from differences in the species or health status of the corals used in the study. To investigate juvenile fish preference for a coral's morphologic complexity in habitat selection, fish must be exposed to the same physical structure of a coral without other variables impacting the choice. One way to accomplish this is through the use of 3D printing technology. 3D printing technology has become commonly used as a tool for scientific applications in many fields, including oceanography (Kruszyński and Liere 2009). However, its use as a research tool in coral reef ecology has not yet been explored.

The goals of this study were to investigate the role that coral structural complexity has on habitat selection, habitat use, and resulting behavior of common Indo-Pacific damselfish using 3D printed corals. Before the question of complexity preference could be explored, the viability of 3D printed models in this context had to be addressed. The primary research objectives were:

1. To determine if 3D printed corals negatively affect the health, behavior, and survival of two Indo-Pacific damselfish species.

2. To investigate what structural characteristics of a coral are preferable in habitat selection for the lemon damsel, *Pomacentrus moluccensis*.

3. To analyze how the behavior of *P. moluccensis* is impacted by coral structural complexity.

The capability of 3D printing technology and digital sculpting software to replicate coral complexity was the primary motivation for its use in this work. The research presented in this chapter was conducted using novel methods and has added to the understanding about the relationship between coral structural complexity and

reef fish ecology. This research has also presented the framework for many other questions to be examined within this context, namely the potential applications of this technology to be successful in reef-restoration efforts.

## 1.2 Methods

### 1.2.1 Study site

The funding for this thesis research was provided through the National Institute of Health International Cooperative Biodiversity Group. The specific grant awarded aimed to explore potential drug discovery of reef organisms and subsequent protection of reefs in Fiji and the Solomon Islands, both countries being in a region that is particularly vulnerable to the synergistic effects of climate change (Mimura and Nunn 1998; Nunn 2009).

Thus, field experiments described in this thesis were conducted on the reefs surrounding Tavewa Island, Fiji (16.9231° S, 177.3652° E) (Figure 1). Experimental sites were easily accessible from the shore, making this location ideal.

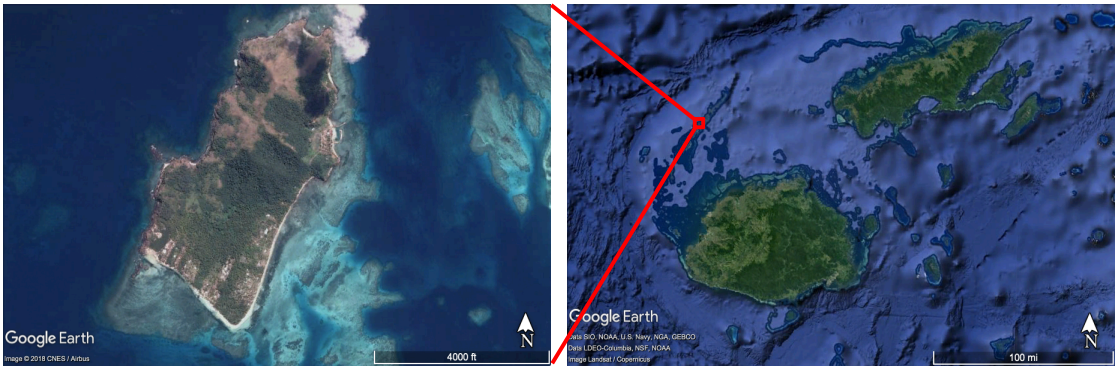


Figure 1 Field experiments were conducted on reefs surrounding Tavewa Island, Yasawas, Fiji.

### 1.2.2 Study species

#### 1.2.2.1 *Chromis viridis*

*Chromis viridis* is a coastal reef damselfish in the family Pomacentridae (Allen et al. 2003, p. 81) (Figure 2). *C. viridis* is a coral-obligate fish and is easily attainable through the aquarium trade, making this fish an ideal focal species for habitat association experiments. This species was used for laboratory-based experiments only. Individuals (n=60, SL 41.09 ± 0.74 mm) were obtained from The Fish Bowl (Dover, DE) and housed at the University of Delaware, Lewes campus. Approval from the University of Delaware IACUC committee was obtained (AUP # 1305) (Appendix A).

Fish were held in a 40 L glass aquarium connected to a 590 L sump on a re-circulating system. Tank water was maintained at 32 ppt salinity, 8.1 pH, and 26 °C. Overhead timed lighting was set at 14:10 light to dark ratio. Fish were fed daily to satiation using NRD pellets.

Figure 2 The blue-green chromis, *Chromis viridis*, was used in initial laboratory-based experiments. Image sourced from Dianne J. Bray, *Chromis viridis* in Fishes of Australia, accessed 17 Mar 2018, http://fishesofaustralia.net.au/home/species/329.

#### 1.2.2.2 *Pomacentrus moluccensis*

*Pomacentrus moluccensis* is a coral-associated damselfish in the family Pomacentridae (Figure 3). *P. moluccensis* is common and found near-shore throughout the Indo-Pacific, making it an ideal study subject for habitat selection studies in the field (Drew and Barber 2009).

Figure 3 The lemon damsel, *Pomacentrus moluccensis*, was used in field experiments. Image sourced from Fishes of Australia, accessed 16 Mar 2018, http://fishesofaustralia.net.au/home/species/2347

### 1.2.3 3D printing material suitability

#### 1.2.3.1 3D model rendering and printing

3D printing is a form of additive manufacturing where objects are created through fused deposition modeling. This process builds a model from the base up through layer-by-layer adhesion of thermoplastic filament. Using the language Gcode,

a slicing program relays a series of XYZ coordinates to a printer instructing the machine where to travel and how much filament to extrude at those points. 3D printing technology has already proved a useful tool for scientific visualization and instructional applications in many fields (Kruszyński and Liere 2009).

3D models were created using the Lulzbot Taz 5, Lulzbot Taz 6, and Makerbot Replicator 2 machines. Printing some of the models used took upwards of 18 hours, therefore multiple machines were needed to achieve the number of prints required for research to be conducted.

Two common species of scleractinian corals, *Acropora formosa* and *Pocillopora damicornis,* were used as models to create 3D printed corals for laboratory-based experiments. These species are inherently structurally different: *P. damicornis* is classified as a bushy, high complexity coral, whereas *A. formosa* has long branches and is less structurally complex. Both species of coral are significant contributors to Pacific reef matrices and play important roles in shallow benthic communities (Veron 2000). Using two species of coral with different morphologic structures as models for 3D printed corals, in addition to manipulating structural complexity within these species, increased the understanding of the coral's physical components that are preferred by *C. viridis* when selecting a suitable habitat.

Photogrammetry software was used to create 3D models of an *A. formosa* and *P. damicornis* skeleton (Figure 4). Skeletons were obtained from the University of Delaware's School of Marine Science and Policy. Individually, skeletons were placed in a well-lit area, and photographed from all angles using an iPhone 6s. Photographs were uploaded into Autodesk ReMake and stitched into a 3D model. The program was used to check for and fix model defects. The models were exported as STL files to be

printed using the slicing program Cura 21.08 (Appendix B). Corals were printed with the same dimensions as the coral skeletons to provide a controlled habitat size (10×7.2×7.8 cm).

Four filament treatments per coral species were used to test the impact of 3D printed corals on *C. viridis* habitat preference and behavior. Specific filaments were chosen that represented a range of physical properties (Table 1). All filaments were printed in neutral tones to control for any color preference (Figure 5). The natural *A. formosa* and *P. damicornis* skeletons used as models for the 3D printed treatments were used as control treatments throughout laboratory experiments. All coral treatments were pre-soaked in seawater for one week prior to use to dissipate negative cues that may be initially leached from the filaments.

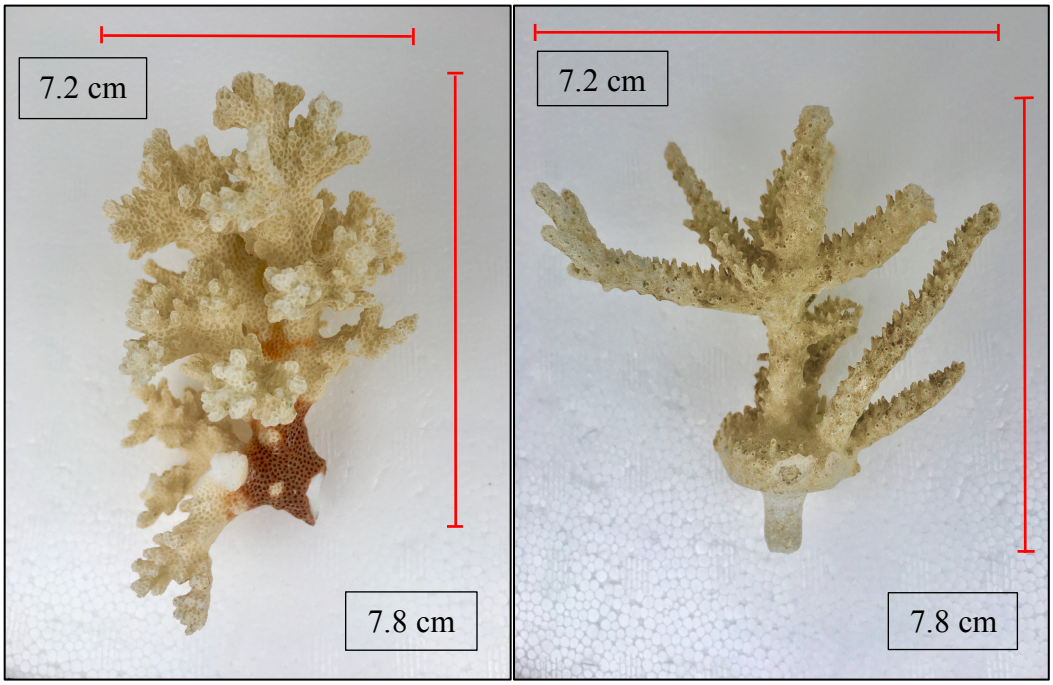


Figure 4 *P. damicornis* and *A. formosa* skeletons were used as models for 3D printed replicates.

Table 1 3D filaments used to create habitat treatments used in laboratory-based experiments with *C. viridis.*

| ***Treatment*** | ***Key Feature*** | ***Abbreviation*** |
|---|---|---|
| *Calcium carbonate skeleton* | Natural | Control |
| *nGen Co-polyester* | Hardy plastic | nGen |
| *XT Co-polyester* | Hardy plastic | XT |
| *PLA/PHA* | Biodegradable | PLA |
| *Stainless steel PLA* | Metallic | SS |

Figure 5 (From left to right) nGen, XT, PLA, and SS replicates of *P. damicornis* (top) and *A. formosa* (bottom) corals were 3D printed in neutral tones for use in laboratory-based trials (10×7.2×7.8 cm).

#### 1.2.3.2 *C. viridis* filament preference

Using 3D printed corals for ecological experiments has not yet been documented. As such, before the effect of complexity on habitat preference could be explored, it was necessary to first determine if the filaments used in 3D printing would affect the choices, behavior, or survival of fish. The objective of this experiment was to determine negative effects of 3D filaments on the survival and behavior of *C. viridis* compared to a natural coral skeleton. By exposing *C. viridis* to corals of the same size, shape, and species simultaneously, this experiment determined if 3D printed habitats would be avoided.

Experimental trials were conducted in a 1800L circular tank. In a single trial, only one species of coral was used. All five coral treatments were tested in a cafeteria style choice experiment with corals arranged in a circular pattern spaced 50cm apart from the corals directly adjacent (Figure 7). Coral species and treatment order in the tank were randomized.

Individual fish were placed into an 18cm diameter mesh cylinder with $1cm^2$ mesh size at the center of the experimental tank and left to habituate for a 15-minute period. The cylinder's large mesh construction allowed the fish to observe all habitat treatments during this period. After the habituation period, the cylinder was slowly lifted upward to begin the observation period.

Habitat associations were recorded continuously for 15 minutes. A fish was considered to be associated with a habitat if it was within 5cm of the habitat and in either a stationary or slow swimming position. A fish was considered to not be associated with any habitat if it was beyond 5cm from any treatment. A second consecutive association at the same habitat was recorded if the fish swam more than 5cm from a coral and then returned. A timer was used to record the amount of time

that each fish spent associating with a habitat. Fish were unable to see the observer during trials.

Each fish was used only once (*A. formosa* n = 29, *P. damicornis* n = 15). The length of the habituation period, experimental period, and number of trials in this experiment has been used in other similar laboratory experiments that aimed to analyze reef fish habitat associations (Coker et al. 2009; Brooker et al. 2013).

Due to the non-independence of corals, association times at each coral treatment were transformed into a proportion of the total time associated spent associating with any coral during a given trial. The proportion of association time was first compared between the coral species using Welch's t-test. These proportions of association time were then analyzed using a nonparametric Kruskal-Wallis test (Bonferroni correction), with post-hoc comparisons performed using Dunn's test.

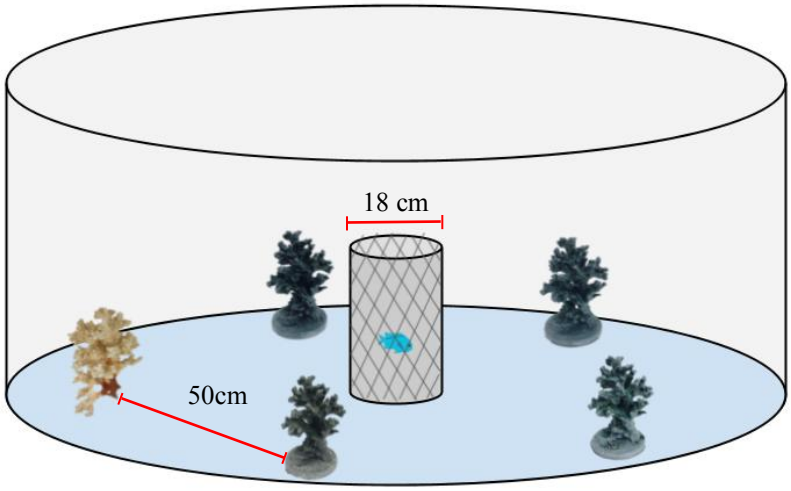


Figure 6 Experimental design of the *C. viridis* cafeteria-style choice of 3D printed *P. damicornis* (n=15) or *A. formosa* (n=29) and respective control habitats.

#### 1.2.3.3 *C. viridis* behavioral effects

Many studies have evaluated how coral reef fish behave when exposed to predator cues, conspecifics, or various stressors such as ocean acidification (Manassa and McCormick 2012; Manassa et al. 2013; Chivers et al. 2016). Such behavioral studies help to explain important aspects of coral reef ecology, such as the defense mechanisms of coral reef fish. Understanding these mechanisms, and the consequences of them, is a critical first step to elucidating the impacts these behaviors have at a community level.

The objective of this experiment was to understand how the behavior of *C. viridis* would be affected when they were exposed to 3D printed coral habitats as opposed to a natural coral habitat. Because the ability of prey fish to behave defensively and seek refuge is crucial to their longevity, a primary aim of this experiment was to determine if any of the 3D printed habitats would impact the behavior of *C. viridis* in a way that would likely compromise their survival.

A single coral treatment was placed in the back-left corner of an observation tank. A total of ten observation tanks were used (Figure 8). Tanks were allowed flow-through water to ensure constant temperature (26 °C) and aerated water throughout trial periods. The tanks were covered in black plastic on three sides to block visual cues from adjacent tanks. Randomly chosen *C. viridis* were added individually to each experimental tank and allowed to habituate for 24 hours (Holmes and McCormick 2010; Manassa et al. 2014).

This experiment focused on measuring activity level, a commonly studied behavioral trait, of *C. viridis* in relation to the coral habitat treatments (Réale et al. 2007). Activity level was measured by the mean frequency and extent (horizontal and vertical) of an individual's movement throughout the tank and in relation to the

habitat. Measuring rates of activity in individuals can indicate the likelihood of food acquisition, encounter rates with predators, and courting behavior (Biro et al. 2010).

Observation tanks were divided into four equal vertical areas (4cm) and six equal horizontal areas (4cm), marked with lines on the back side of the tank. If the fish was within the coral habitat, the distance was marked as 0cm, and the length of time inside was recorded. The total number of lines crossed during the observation period was recorded. At least half the body length must have crossed a line to be considered movement into a new section. These parameters were used to determine activity level, average distance from the habitat, and average amount of time that each habitat treatment was utilized (Holmes and McCormick 2010; Manassa and McCormick 2012).

Each fish was observed for a period of ten minutes and was used only once ($n=6^{-1}$ filament treatment$^{-1}$ coral species). A full water change was conducted after the completion of each trial.

The difference in total counts of line crosses, time spent in shelter, and maximum distance ventured from shelter were individually compared between the coral species using Welch's t-test. These parameters were then compared among the four filament treatments and control coral using a one-way MANOVA. Residual analysis found that the assumptions of normality and homogeneity of variance were satisfied.

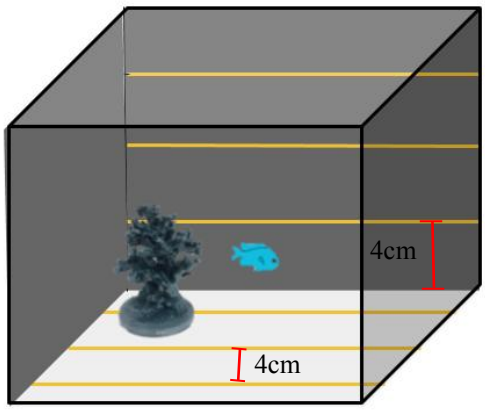


Figure 7 Experimental design of observation tanks testing the behavioral impacts of 3D printed corals on *C. viridis* ($n=6^{-1}$ filament treatment$^{-1}$ coral species).

### 1.2.4 Habitat complexity preference and influence on fish behavior

#### 1.2.4.1 3D model rendering and printing

Only *P. damicornis* coral treatments were used in field experiments. *P. damicornis* is a brooding coral, is commonly found throughout its distribution, and is classified as "least concern" by the International Union for the Conservation of Nature (Hoeksema et al. 2014; Jiang et al. 2018). *A. formosa* is listed as "near-threatened", likely due to its fragile, branching morphology and its higher susceptibility to bleaching (Bruno et al. 2001; Harithsa et al. 2005). Thus, removing live *P. damicornis* for experimental trials would have fewer detrimental impacts to the reef community. Additionally, *P. damicornis* tends to grow as distinct, and often solitary, coral colonies, making it easy to remove and manipulate.

Three *P. damicornis* models were created with varying levels of complexity, from here on classified as low, medium, and high treatments. The digital clay sculpting software Cubify® Sculpt™ was used to manipulate the complexity of the

original *P. damicornis* model created using Autodesk ReMake. Because this model was based off of a natural coral skeleton, it was presumed that it's structure was representative of a standard *P. damicornis* colony that would be found naturally, and thus was classified as the medium complexity treatment.

Branches were quantitatively removed or added to the medium complexity model to create the low and high treatments, respectively. To maintain a standard volume, branches that extended the farthest from the base of the medium complexity coral were not manipulated. So, if each coral treatment was placed inside of a box, all corals would be limited by the same size container. This program was also used to calculate the total surface area of each model. While the overall volume of the treatments was consistent, the total surface area ratios differed, with the highest complexity model having the highest surface area to volume ratio (Table 2). The parameters manipulated to achieve different complexity classes are key factors associated with coral structure and have been used as indicators of complexity in other studies (Almany 2004; Rilov et al. 2007; Noonan et al. 2012).

Low, medium, and high complexity corals were printed using the same methods as the coral treatments used in laboratory treatments (Figure 6). However, only the PLA/PHA filament was used, as explained in the 3D printing material suitability results. Corals were printed with the same dimensions to maintain constant habitat volume (14×12×13cm) and pre-soaked in seawater for one week prior to experimental use to dissipate negative cues that may be initially leached from the filament.

Table 2 3D coral habitats were created with different levels of complexity by altering the surface area to volume ratio between the treatments.

| *Complexity Treatment* | *Branch tips* | *Surface Area ($cm^2$)* | *Volume ($cm^3$)* | *SA to V ratio* |
|---|---|---|---|---|
| *Low* | 13 | 563.58 | 2,184 | 0.26 |
| *Medium* | 23 | 714.55 | 2,184 | 0.32 |
| *High* | 35 | 766.94 | 2,184 | 0.35 |

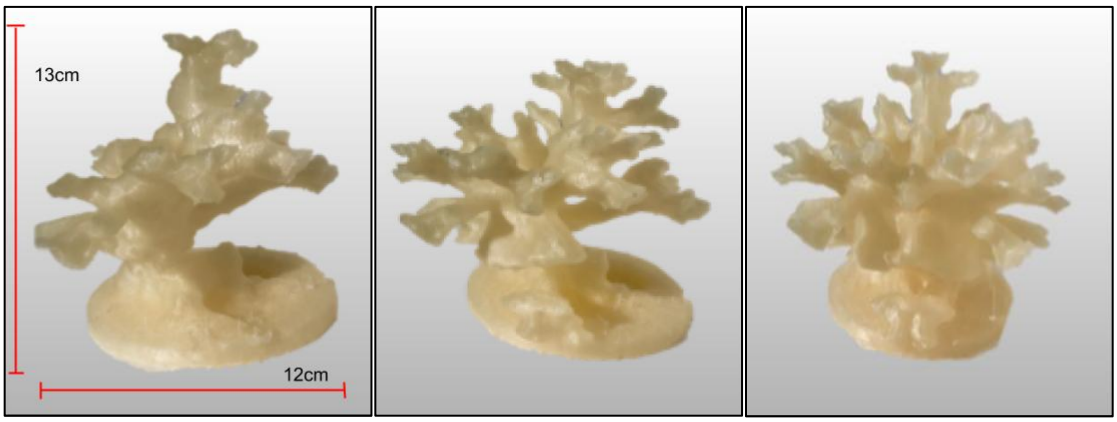


Figure 8 Low, medium, and high complexity *P. damicornis* corals were created and 3D printed to be used in habitat association experiments. Scale bar applies to all depicted corals.

#### 1.2.4.2 *P. moluccensis* complexity preference

A cafeteria-style patch reef experimental design was used to assess if *P. moluccensis* exhibited a preference for a specific level of coral complexity, independent of live coral tissue, using 3D printed corals. While other studies have already explored the role of complexity in habitat choice for associated reef fish using live or dead corals, these experiments were not able to isolate the coral complexity as the only variable affecting these choices. 3D printing technology and sculpting software can quantifiably manipulate coral structure to not only more accurately

manipulate coral complexity, but also isolate the coral morphology as the only factor having an effect on habitat choice.

Patch reefs (n=7) were constructed in an open sandy area 10m from the contiguous reef. Patch reefs each consisted of one low, medium, and high complexity 3D coral spaced 30cm away from each other in a triangular pattern, allowing equal access for the fish (Figure 9). The order of coral treatments was randomized for each patch reef. Each coral was weighted down with a rock secured to the base using clear fishing line, which also acted as a type of rubble base typically used in patch reef experiments that include live coral. Each piece of coral was cleaned of silt, algae, and other organisms before each trial to prevent any outstanding factors from affecting preference. Patch reefs were equal in size (~1$m^2$) and were spaced 2m away from any other patch reef. Cages made of chicken wire and mesh were constructed to surround each patch reef to ensure that *P. moluccensis* used in this experiment would not be eaten or abandon the patches mid-trial. Cages were 90cm in diameter by 50cm tall. The mesh wrapped around the perimeter of the cage ensured that the fish could not escape and that predators could not intrude, while still allowing for water flow-through and visibility into and out of the cage. Each coral was placed 20cm from the side of the cage.

Experimental trials were run between 0800 and 1100. Juvenile *P. moluccensis* (< 2.5cm) were collected on SCUBA the morning of trials from the nearby reef using a mixture of clove oil, ethanol and seawater (ratio 1:1:5) and hand nets. There are no long-term effects on the fish using this method and is commonly used in coral reef environments (Munday and Wilson 1997). Once captured, individual fish were placed into gallon zip-lock bags filled with seawater for a maximum of one hour.

Individual *P. moluccensis* were randomly placed onto a patch reef through a re-sealable door constructed into the wire cage. The door was placed so that the fish could be released into the middle of the patch reef. Fish were allowed to habituate for a 15-minute period. A 15-minute trial period followed, where associations with each complexity coral were continuously recorded. A fish was considered to be associated with a coral of it was within 5cm of the coral and in either a stationary or slow-swimming position. A fish was considered to be not associated with any coral habitat if the fish was beyond 5cm away from any of the treatments. A stopwatch was used to record the amount of time that each fish spent associating with each coral habitat. Each *P. moluccensis* was used only once and was placed back onto the contiguous reef after the 15-minute trial period.

A total of 36 trials were run in this experiment. Due to the non-independence of corals, association times at each coral treatment were transformed into a proportion of the total time associated spent associating with any coral during a given trial. These proportions of association time were then analyzed using a nonparametric Kruskal-Wallis test (Bonferroni correction), with post-hoc comparisons performed using Dunn's test.

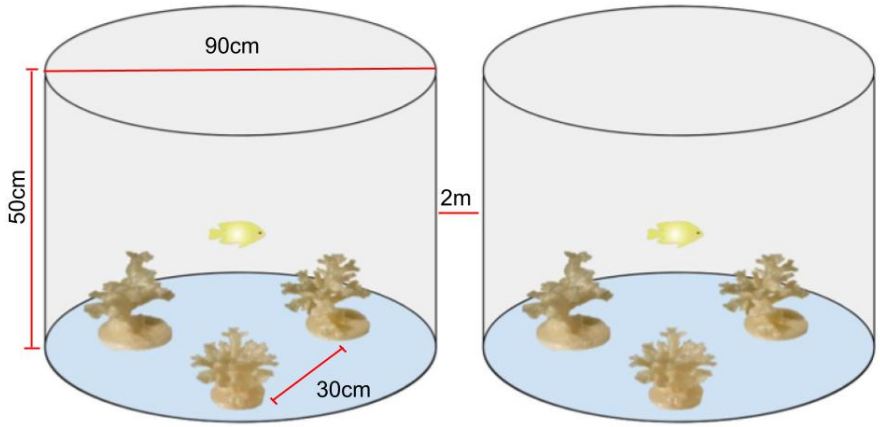


Figure 9 Experimental design of caged patch reef field experiments testing the habitat complexity preferences of *P. moluccensis* using 3D printed *P. damicornis* corals (n=36).

#### 1.2.4.3 *P. moluccensis* habitat use and behavior

Research conducted on the behavior of reef fishes has elucidated their defense mechanisms employed against various stressors (Manassa and McCormick 2012; Manassa et al. 2013; Chivers et al. 2016). Such defense mechanisms include seeking refuge when startled, remaining concealed in the shelter, and not venturing far from the shelter. Because the ability of prey fish to behave defensively is crucial to their longevity, this experiment was conducted to determine if the behavior of *P. moluccensis* would change in a way that may compromise their survival when placed onto a 3D coral compared to a live coral. Further, this experiment aimed to identify differences in behavioral metrics measured between the different 3D coral complexities in relation to a complex live coral.

A live *P. damicornis* coral was used as an overall control treatment in this experiment. Dead *P. damicornis* coral was also used as procedural control to provide a

structurally complex, naturally occurring habitat option without the associated live coral tissue. Corals were searched for on the reef adjacent to where trials would occur. Because the medium complexity 3D coral was a replicate of a natural *P. damicornis* coral skeleton, it's structural complexity was decided to be the standard level of coral complexity that would likely be seen in a natural reef environment. Thus, the live and dead control coral treatments were aimed to match this complexity level.

A 100cm$^2$ quadrat was used to ensure that the number of branch tips in the selected live and dead corals was similar to the number of branches on the medium complexity 3D printed coral (Table 3). Additionally, length, width, and height measurements indicated that overall volume of the selected corals was similar to the medium complexity 3D coral.

Table 3 Complexity measurements of live and dead *P. damicornis* corals used as controls in a behavioral experiment.

| ***Coral Treatment*** | ***Branch Tips*** | ***Volume (cm²)*** |
|---|---|---|
| *3D medium* | 23 | 2184 |
| *Live coral 1* | 20 | 1960 |
| *Live coral 2* | 22 | 2002 |
| *Dead coral 1* | 25 | 2700 |
| *Dead coral 2* | 26 | 2508 |

The dead coral treatments were transported on shore to be experimentally bleached. This process removed any cues that could impact fish behavior. Live corals used for the dead treatment were placed into 7.5L of freshwater and left for a 24-hour period. The corals were inspected for any remaining live coral tissue prior to use.

Patch reefs (n=10) were constructed in an open sandy area 10m from the contiguous reef. Patch reefs consisted of one coral: either a live *P. damicornis*, a dead

*P. damicornis*, a low complexity 3D printed coral, a medium complexity 3D printed coral, or a high complexity 3D printed coral. Each 3D printed coral was weighted down with a rock secured to the bottom using clear fishing line, which also acted as a type of rubble base typically used in patch reef experiments that include live coral. For consistency, the live and dead coral pieces were also placed on a rubble base.

Each piece of coral was cleaned of silt, algae, and other organisms before each trial to prevent any outstanding factors from affecting preference. Patch reefs were equal in size (~10cm$^2$) and were spaced 5m away from any other patch reef. Cages made of chicken wire and mesh were constructed to surround each patch reef to ensure that *P. moluccensis* used in this experiment would not be eaten or abandon the patches during the habituation period. Cages were 15cm in diameter by 30cm tall. The mesh wrapped around the perimeter of the cage ensured that the fish could not escape and that predators could not intrude, while still allowing for water flow-through and visibility into and out of the cage.

Experimental trials were run between 0800 and 1100. Juvenile *P. moluccensis* (< 2.5cm) were collected on SCUBA the morning of trials from the nearby reef using a mixture of clove oil, ethanol and seawater (ratio 1:1:5) and hand nets. Once captured, individual fish were placed into gallon zip-lock bags filled with fresh seawater for a maximum of one hour.

Individual *P. moluccensis* were randomly placed inside of the cage onto each patch reef. Fish were allowed to habituate for 30 minutes. After the habituation period, the cage was slowly lifted upward, and behavioral observations were continually made for 5-minutes (Figure 10). Observations were made 1.5m away from each patch reef. Five factors were used to assess behavior: 1) maximum distance traveled from the

coral shelter, 2) relative distance traveled from the coral shelter, 3) total time spent in coral shelter, 4) position within the coral habitat, and 5) boldness. To assess boldness, the fish was approached with a wooden dowel, at the end of the observation period. The fish's reaction and/or latency to emerge from the shelter was defined on a discrete scale from 0-3, where: 0) fish hid in coral shelter and did not emerge; 1) fish retreated to shelter when approached and took >5 seconds to emerge; 2) fish retreated to shelter when startled and took <5 seconds to emerge; 3) fish did not retreat when startled. These metrics for assessing behavior have been used in other studies and have been related to survival (McCormick 2009; McCormick and Meekan 2010; Holmes et al. 2017).

A stopwatch was used to record the amount of time that each fish spent exhibiting the behavioral metrics. Each *P. moluccensis* was used only once and was placed back onto the contiguous reef after the trial.

A total of 50 trials were run in this experiment (3D Low n=5; 3D Medium n=9, 3D High n=10; Live n=13; Dead n=13). Fish that abandoned the patch reef before the end of the observation period were not used in statistical analyses, as the behaviors these fish had exhibited were likely not a true representation of their normal behavior. Abandonment was defined as any fish that swam more than 10cm away from the coral before the five-minute observation period concluded. The likelihood of abandonment within habitat treatments was examined using the binomial exact test. Rates of abandonment across treatments were compared to the live control treatment using Fisher's exact test. Four contingency tests were run, calling for an adjusted significance threshold of $p<0.0125$ (Bonferroni correction).

Differences in the proportion of time spent utilizing the habitat treatments were analyzed using a one-way ANOVA, with post-hoc comparisons performed using Dunnett's test, where all treatments were compared to the live control coral only.

The time *P. moluccensis* spent at the bottom, middle, or top of each coral habitat was transformed into a proportion of the total time spent within the habitat. These proportions of association time were then analyzed using the nonparametric Kruskal-Wallis test, with post-hoc comparisons performed using Dunn's test.

Distance ventured from the coral and boldness were compared across habitat treatments using a one-way MANOVA. One-way ANOVAs were used to explore significant differences found.

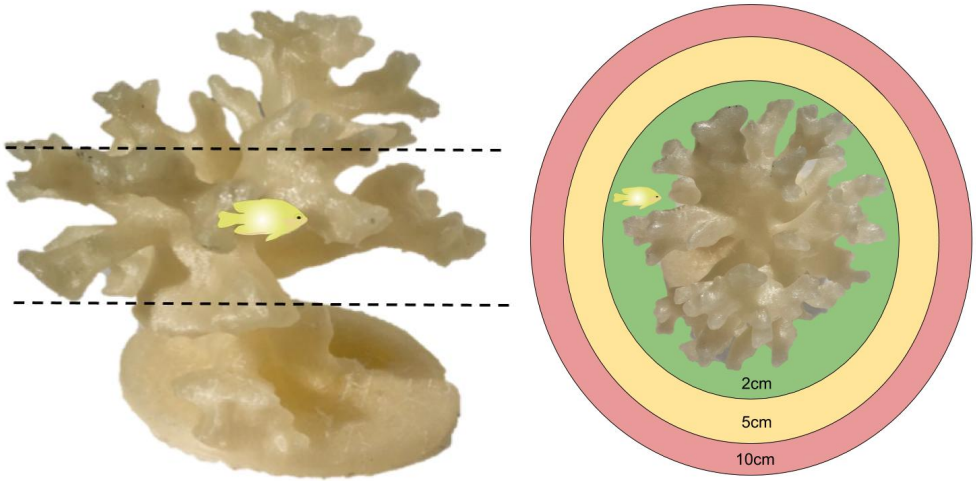


Figure 10 Experimental design of a behavioral study of juvenile *P. moluccensis* on 3D printed and natural *P. damicornis* corals (3D Low n=5; 3D Medium n=9, 3D High n=10, Live n=13; Dead n=13).

### 1.2.5 Habitat association surveys

To better understand the habitat preferences of *P. moluccensis* in a natural reef setting, transect surveys were conducted around Tavewa Island to search for *P. moluccensis* associated with *P. damicornis* and other species of coral colonies. Coral colonies containing *P. moluccensis* were searched for along 25×1m transects (n=78). Transects were conducted at three depths in each area surveyed: along the reef crest, 10 meters toward the shore from the reef crest, and 10 meters beyond the reef crest (Figure 11).

When *P. moluccensis* was observed associating with a coral colony, the length, width, and height of the colony were recorded. The total number and size estimates (total length) of each *P. moluccensis* were also recorded. Other fish within surveyed coral colonies were disregarded. Finally, the coral species and number of branch tips within a 10×10 $cm^2$ quadrat were recorded. The density of branch tips was used as a measure of habitat complexity (Noonan et al. 2012). Corals were categorized into low, medium, and high complexity after identifying the median value of branch tips of all corals surveyed. Corals with fewer or more branch tips than one standard deviation away from the median were classified as low and high complexities, respectively; corals with branch tips within one standard deviation from the median were classified as medium complexity.

Regression analyses were used to explore patterns of habitat choices of *P. moluccensis*. Volume of colonies measured was log transformed to meet assumptions of normality. Linear regression was used to assess the relationship between coral complexity and the number of *P. moluccensis* found in the colonies. Non-parametric quantile regression was used to assess the relationships between the number of *P.*

*moluccensis* found in colonies surveyed and the log volume of those colonies. The non-parametric Kruskal-Wallis test was used to test for pair-wise differences in number of *P. moluccensis* found in low, medium, and high complexity corals surveyed. This test was repeated for *P. damicornis* corals alone.

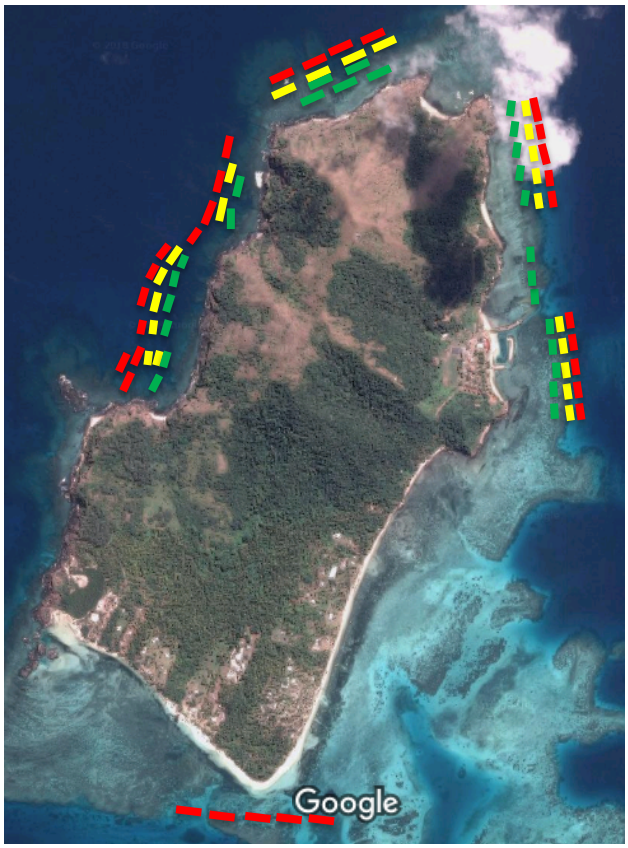


Figure 11 Location of transects conducted beyond the crest (red), along the crest (yellow), and inshore from the crest (green) around Tavewa Island to analyze natural habitat complexity associations of *P. moluccensis* (n=78).

## 1.3 Results

### 1.3.1 3D printing material suitability

#### 1.3.1.1 *C. viridis* filament preference

Differences in associations were first analyzed between the coral species treatments. No significant difference was found between the total proportion of time that *C. viridis* associated with any of the filament treatments between the *A. formosa* and *P. damicornis* treatments (Welch's t-test: $t_{(27.247)}$= 1.018, p=0.32). The lack of difference in association times between the coral species indicated that *C. viridis* was not making habitat choices based on the morphology of the corals (Figure 12). Thus, the data for the two coral species treatments were combined to increase the power of the statistical testing for differences in associations with the filament treatments.

*C. viridis* associated with any of the filament treatments for 48.48% (± 6.65) of the time, which was not significantly different than the average amount of time spent not associating (Welch's t-test: $t_{(43)}$= -0.228, p = 0.82) (Figure 13).

*C. viridis* was not significantly selective of any of the coral habitats present during the experiment (Kruskal-Wallis: $\chi^2_{(4)}$ = 7.896, p=0.1) (Figure 14).

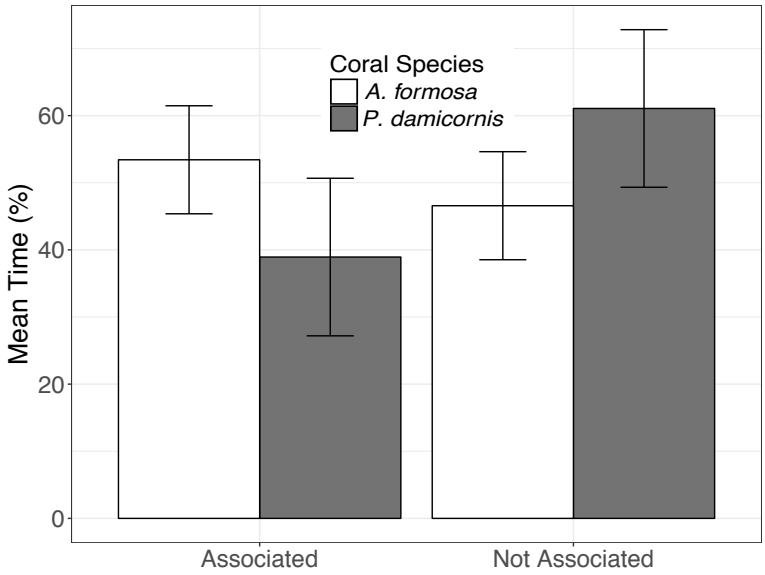


Figure 12 No significant difference was found for the mean percent time (± SE) *C. viridis* spent associating with any of the habitat treatments between the coral species treatments (*A. formosa* n=29; *P. damicornis* n=15).

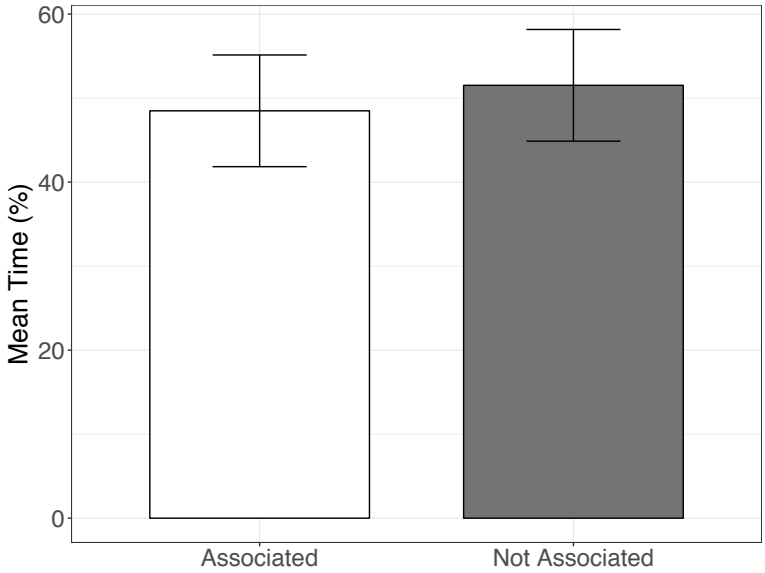


Figure 13 No significant difference was found for the mean percent time (± SE) *C. viridis* spent associating and not associating with any of the habitat treatments (n=44).

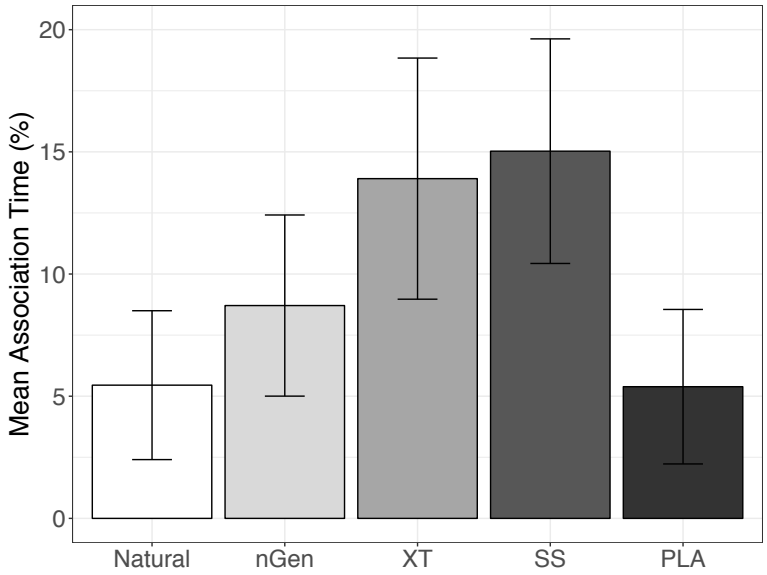


Figure 14 No significant difference was found for the mean percent time (± SE) *C. viridis* spent in association with any of the habitat treatments (n=44).

#### 1.3.1.2 *C. viridis* behavioral effects

Differences in behavioral responses were first analyzed between *C. viridis* exposed to *A. formosa* or *P. damicornis* corals. No significant differences were found for time spent in the habitat provided (Welch's t-test: $t_{(58)}$= -0.81, p=0.42), maximum distance ventured from the habitat (Welch's t-test: $t_{(58)}$= 0.44, p=0.66), modal distance from the habitat (Welch's t-test: $t_{(58)}$= 1.08, p=0.28) or the number of lines crossed (Welch's t-test: $t_{(44.16)}$= 0.59, p=0.55), regardless of filament treatment (Figures 15-17). Because the behavior of *C. viridis* was not significantly affected by coral species, the data for the species treatments were combined to increase the power of the statistical testing for differences in associations with the filament treatments.

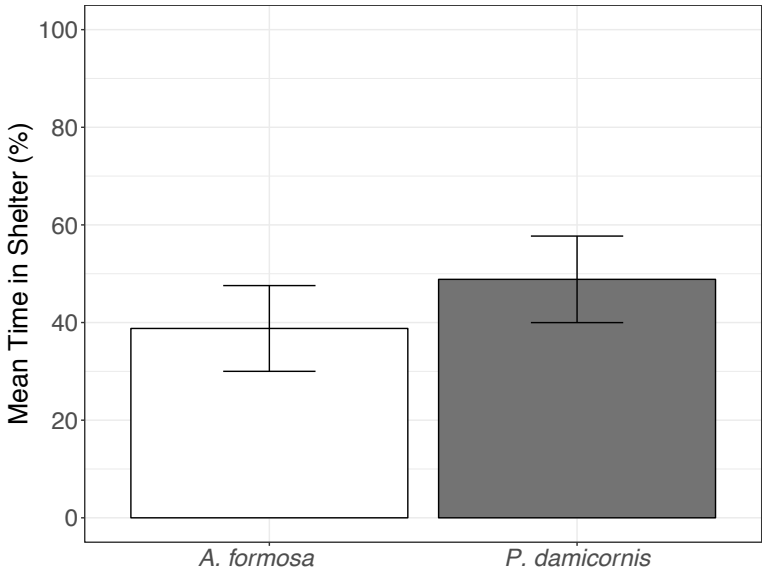


Figure 15 No significant difference was found for the mean percent time (± SE) *C. viridis* spent associating with any of the habitat treatments between the coral species (*A. formosa* n=30; *P. damicornis* n=30).

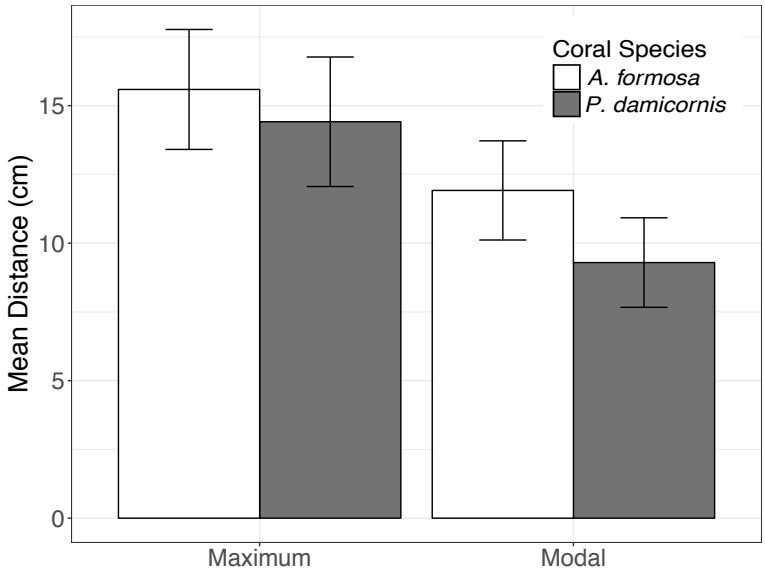


Figure 16 No significant difference was found for the mean distances (cm) (± SE) *C. viridis* ventured from any of the habitat treatments between the coral species (*A. formosa* n=30; *P. damicornis* n=30).

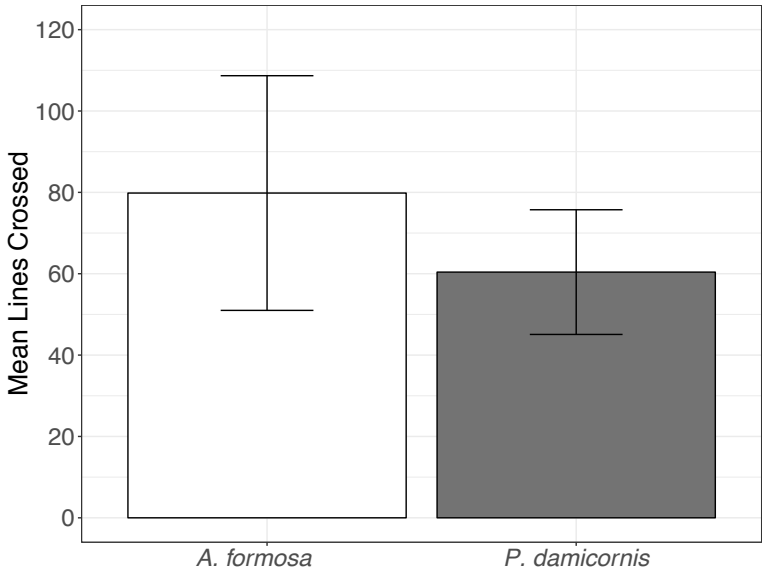


Figure 17 No significant difference was found for the mean number of lines (± SE) *C. viridis* crossed when exposed to any of the habitat treatments between the coral species (*A. formosa* n=30; *P. damicornis* n=30).

No significant differences were found between the 5 treatments for any of the behavioral traits analyzed (MANOVA: Pillai's trace = 0.19, $F_{(4)} = 0.70$, p = 0.8) (Figure 18).

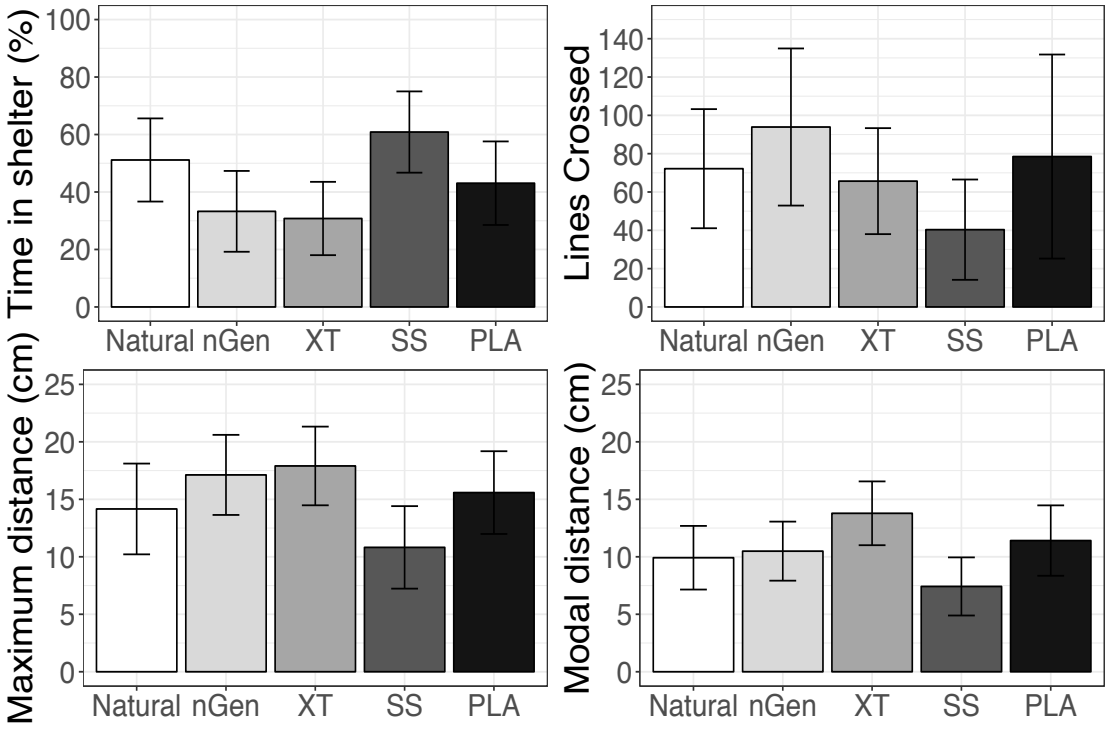


Figure 18 No significant differences were found in mean behavioral responses (± SE) by *C. viridis* when exposed to 3D printed or coral skeleton habitats ($n=12^{-1}$ treatment).

### 1.3.2 *P. moluccensis* complexity preference

During a cafeteria-style experiment, the total time that *P. moluccensis* associated with any of the 3D printed treatments was significantly greater than the total time spent not associating, indicating that 3D printed corals could be utilized as habitat (Welch's t-test: $t_{(35)}=7.98$, $p<0.0001$) (Figure 19).

*P. moluccensis* was highly selective between coral complexity treatments (Kruskal-Wallis: $\chi^2_{(3)} = 29.86$, $p<0.0001$) (Figure 20). The association with the high complexity 3D printed coral was stronger than any other complexity (mean % time = 45 ± 0.06 SE).

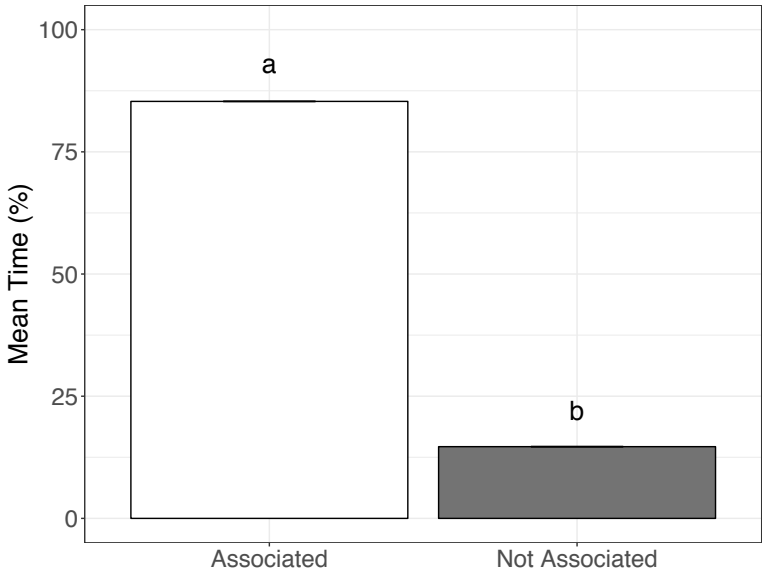


Figure 19 *P. moluccensis* spent significantly more time associating compared to not associating (± SE) with any of the 3D printed complexity treatments during a cafeteria-style experiment.

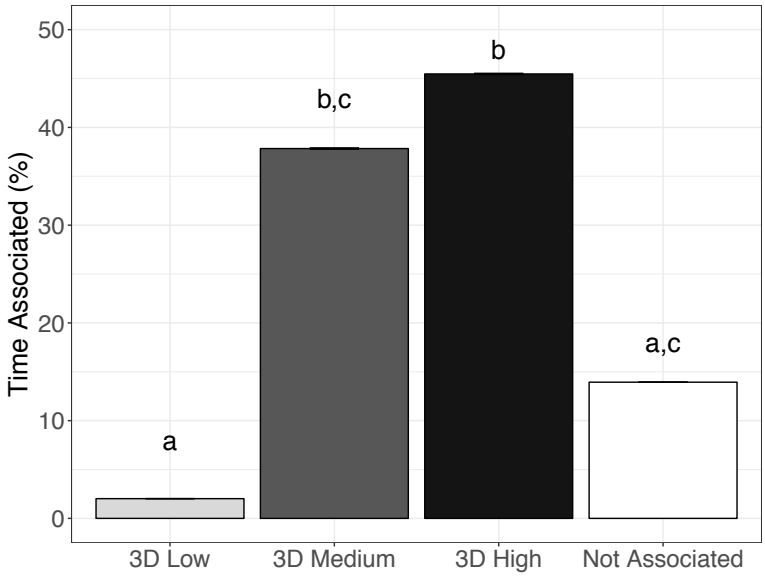


Figure 20 Mean percent time (± SE) *P. moluccensis* spent in association with each 3D printed complexity treatments during a cafeteria-style experiment. Not Associated represents the mean percent time fish were not associated with any of the habitat treatments present. Letters indicate significant differences (n=36).

### 1.3.3 *P. moluccensis* habitat use and behavior

All habitat treatments had some rate of abandonment by *P. moluccensis* during experimental trials. While no habitat treatment saw significant abandonment, fish placed on the live and dead *P. damicornis* habitats were more likely to remain than to abandon their respective habitat treatments (Table 4). The low complexity 3D printed habitat had the highest probability of abandonment at 0.61.

No significant differences in abandonment were found between the live control and all other habitat treatments with the appropriately adjusted p-value (Fisher's exact test: p=0.02) (Figure 21). Fish that abandoned were removed from subsequent analyses.

Table 4 The probability of abandonment by *P. moluccensis* differed across *P. damicornis* habitat treatments.

| ***Habitat*** | ***Frequency Abandoned*** | ***Frequency Not Abandoned*** | ***Probability of Abandonment*** | ***p-value*** |
|---|---|---|---|---|
| *Live* | 2 | 12 | 0.14 | 0.001** |
| *3D Low* | 8 | 5 | 0.61 | 0.58 |
| *3D Medium* | 4 | 9 | 0.31 | 0.27 |
| *3D High* | 5 | 10 | 0.33 | 0.30 |
| *Dead* | 1 | 13 | 0.07 | 0.002** |

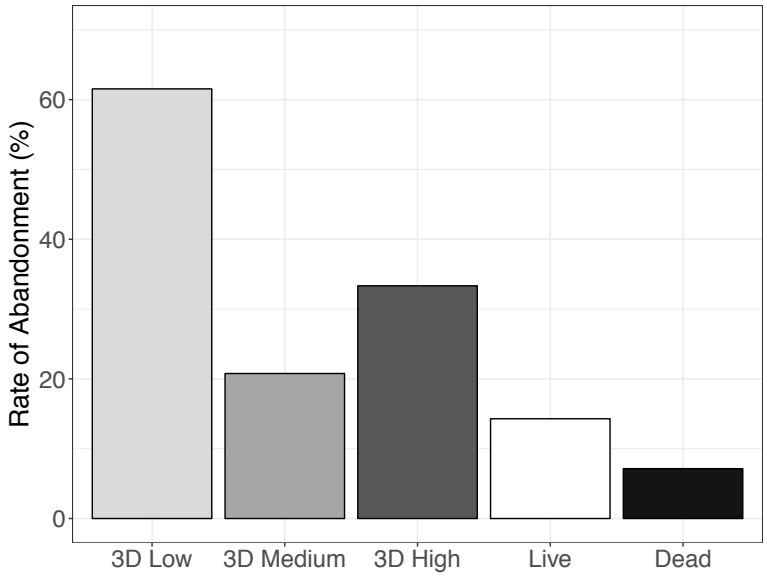


Figure 21 *P. moluccensis* did not show significantly different rates of abandonment when placed on different coral habitat treatments (3D Low n=13; 3D Medium n=13, 3D High: n=15; Live n=14; Dead n=14).

Total time spent utilizing the experimental habitat treatments did not differ compared to the live control coral (one-way ANOVA, Dunnett's test: 3D low, t=-2.128, p=0.13; 3D medium, t=1.307, p=0.53; 3D high, t=1.265, p=0.56; dead**,** t=0.444,

p=0.98). However, *P. moluccensis* showed higher average association times with the 3D printed medium and high complexity habitat treatments compared to the live control (Figure 22).

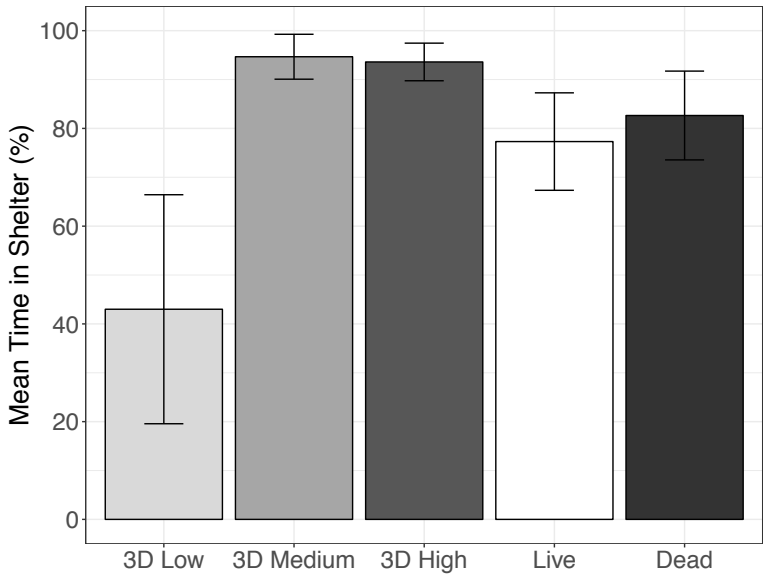


Figure 22 No differences were found between the mean time (± SE) spent utilizing each experimental habitat treatment compared to the live coral control (3D Low n=5; 3D Medium n=9, 3D High n=10; Live n=13; Dead n=13).

Habitat treatment had a significant effect on the proportion of time that *P. moluccensis* spent at either the bottom, middle, or top of the habitat (Table 5). Fish placed on the medium or high complexity 3D printed corals spent significantly more time in the middle of the habitat when utilizing it (Figure 23). In contrast, fish placed on the live or dead *P. damicornis* habitats spent more time at the bottom of the coral.

Table 5 Habitat treatment had a significant effect on the proportion of time that *P. moluccensis* spent at either the bottom (B), middle (M), or top (T) of the habitat (* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001).

| *Habitat* | *X²* | *df* | *p-value* | *Interactions* |
|---|---|---|---|---|
| *3D Low* | 2.22 | 2 | 0.33 | NA |
| *3D Medium* | 6.823 | 2 | 0.03* | M–B: 0.0401*<br>M–T: 0.0315* |
| *3D High* | 14.81 | 2 | 0*** | M–B: 0.0008***<br>M–T: 0.0023** |
| *Live* | 8.56 | 2 | 0.01* | B-M: 0.0325*<br>B-T: 0.0098* |
| *Dead* | 10.58 | 2 | 0.01* | B-T: 0.0019** |

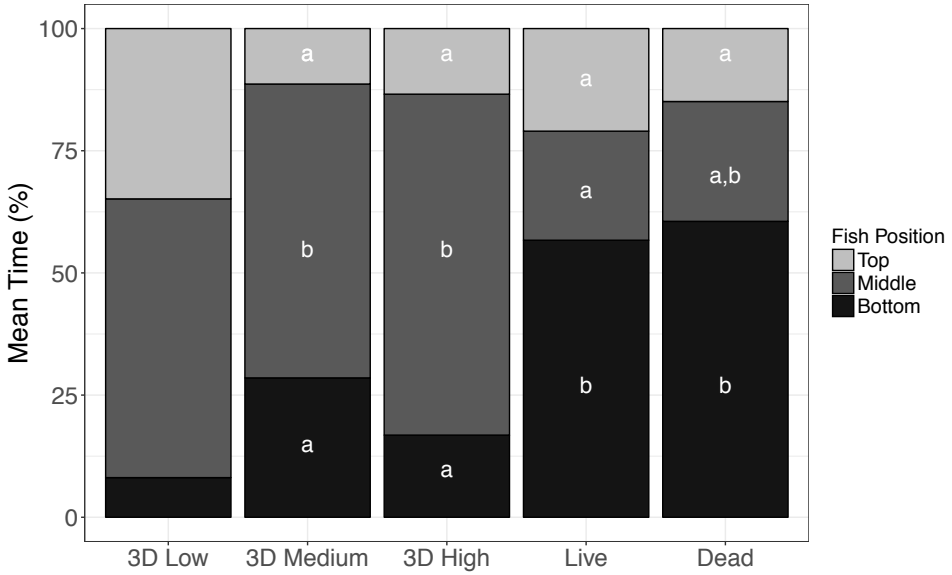


Figure 23 Mean percent time *P. moluccensis* spent at each position when occupying each coral habitat during a behavioral assessment. Letters within each treatment represent significant differences between the positions (3D Low n=5; 3D Medium n=9, 3D High n=10; Live n=13; Dead n=13).

Without looking at the effect of habitat treatment, *P. moluccensis* exhibited a strong preference for remaining close to their coral habitat (Figure 24). The vast majority spent almost the entire trial period at 0cm away from the coral habitat. Conversely, very little time was spent venturing to 2, 5, or 10cm away from the habitat, regardless of treatment.

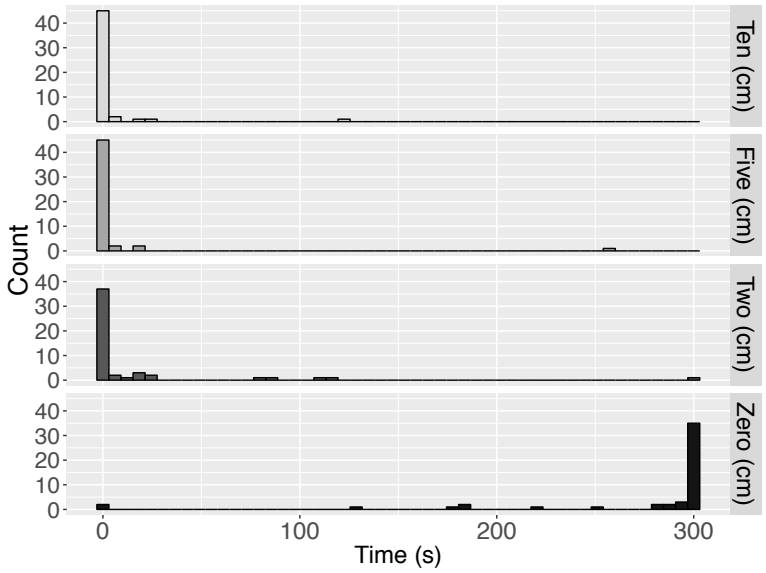


Figure 24 *P. moluccensis* spent the majority of trial time (s) within 0cm of their coral habitat, regardless of treatment.

When looking at how *P. moluccensis* behaved within coral habitat treatments, variability can be seen particularly with fish placed on the low complexity 3D printed treatment. A multi-dimensional scaling plot showed how distance ventured by *P. moluccensis* from this habitat treatment vary greatly (Figure 25). Conversely, the other treatments showed relative uniformity, indicating similar distances ventured within each treatment.

An analysis of the distance *P. moluccensis* spent the majority of its time in an individual trial across treatments was conducted using Fisher's exact test. The results were highly significant ($p<0.01$), indicating that *P. moluccensis* was spending the majority of the time at different distances across habitat treatments. When the data for the low complexity 3D printed coral treatment was removed, Fisher's exact test was not significant between the other treatments ($p=1$). Thus, this habitat treatment was removed for the interactive analysis of variance between distance ventured from each coral habitat and boldness of *P. moluccensis*. Subsequently, no significant interactions were found between the mean time spent at different distances ventured from each coral habitat (MANOVA: $F_{(3)}= 0.84$, $p=0.63$).

The boldness of *P. moluccensis* differed significantly between treatments (one-way ANOVA: $F_{(3)}=3.17$, $p=0.03$). *P. moluccensis* occupying high complexity 3D printed corals had the highest average boldness rating, which was significantly higher than *P. moluccensis* occupying live corals (Figure 26).

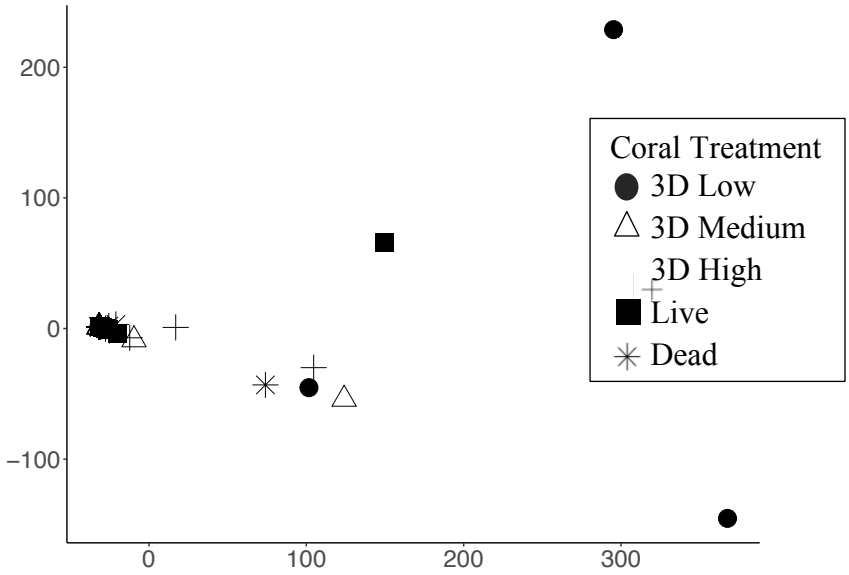


Figure 25 A multi-dimensional scaling (MDS) plot indicated how similar the distances ventured by *P. moluccensis* were within habitat treatments (3D Low n=5; 3D Medium n=9, 3D High n=10; Live n=13; Dead n=13).

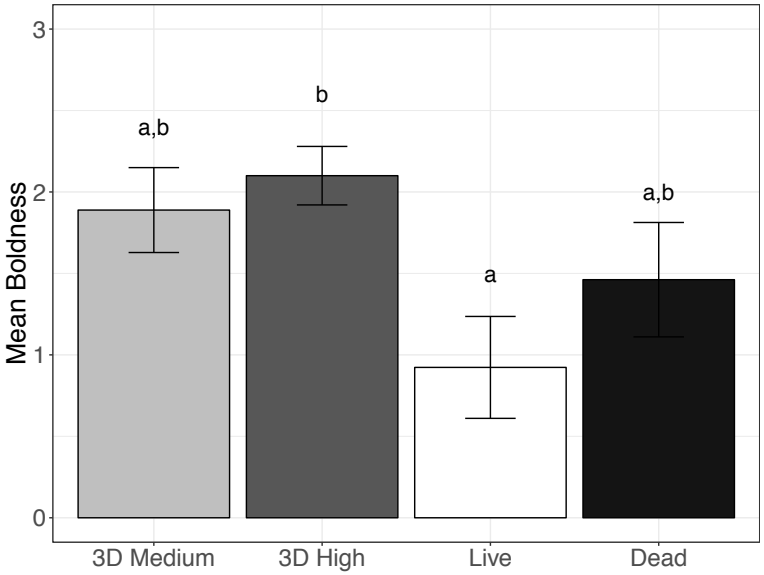


Figure 26 The mean boldness metric of *P. moluccensis* placed on high complexity 3D printed corals was significantly higher than fish occupying live *P. damicornis* coral treatments (3D Medium n=9, 3D High n=10; Live n=13; Dead n=13).

### 1.3.4 Habitat association surveys

A total of 50 corals containing *P. moluccensis* were surveyed along transects around Tavewa Island. These corals were representative of ten different scleractinian species (Figure 27).

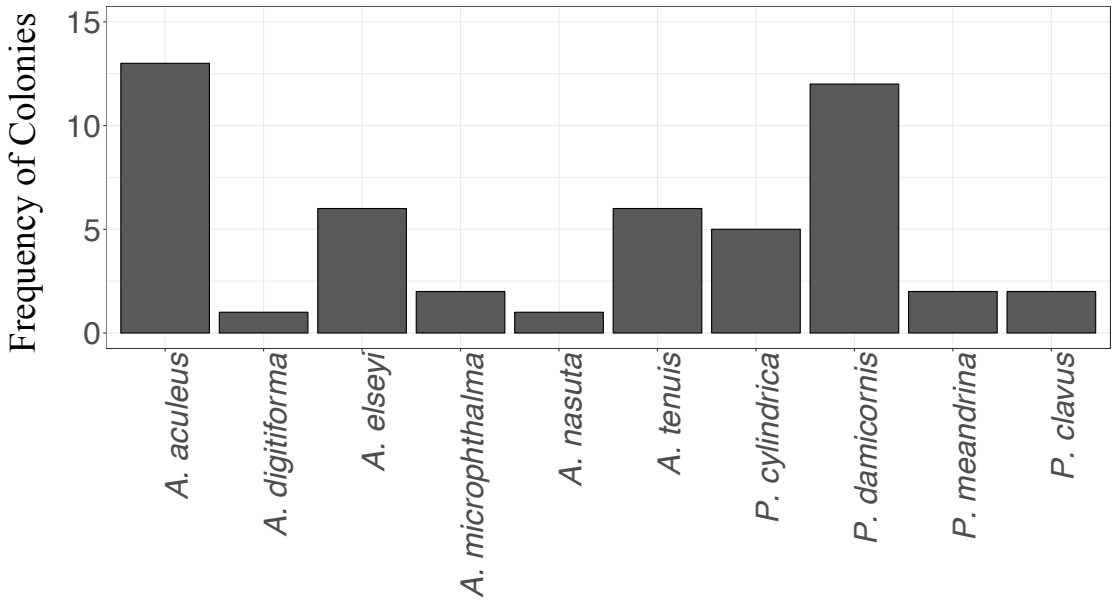


Figure 27 *P. moluccensis* was found in ten species of coral along transects around Tavewa Island.

The total number of *P. moluccensis* in each colony surveyed had a weak positive relationship with the volume of the colony. Conversely, no relationship was found between the total number of *P. moluccensis* and the branch tip density of the colonies (Figure 28).

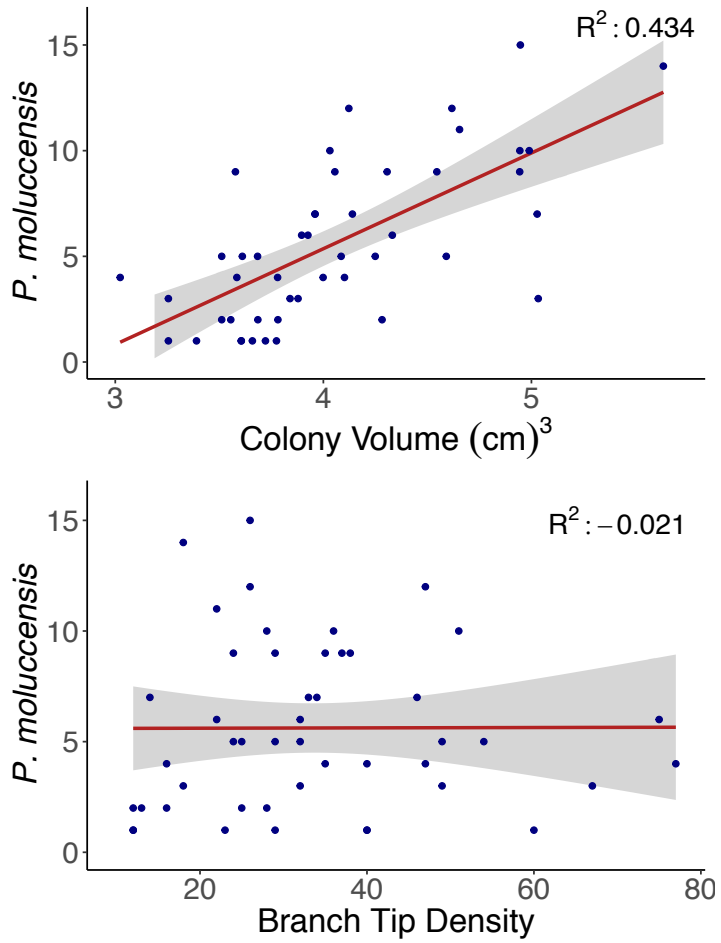


Figure 28 Linear and quantile regression analyses were used to analyze the relationships between *P. moluccensis* and the coral colonies they were found in.

Corals surveyed were categorized into low, medium, and high complexity categories to make comparisons with results found for the behavior of *P. moluccensis* when occupying 3D printed corals of varying complexities.

The median branch tip value of all corals surveyed was 29 branches (± 14.78 SD). Corals with fewer than 15 branches were considered low complexity; corals with 15-43 were considered medium complexity; corals with more than 44 branches were

considered high complexity. Of all corals surveyed, the medium complexity was most frequently occupied by *P. moluccensis* (Figure 29). However, no difference was found in the total number of *P. moluccensis* found in each complexity ($\chi^2_{(2)} = 2.04$, p-value = 0.36) (Figure 30).

The same conclusion was found when only considering *P. moluccensis* found in *P. damicornis* corals of different complexities ($\chi^2_{(1)} = 0.105$, p-value = 0.75).

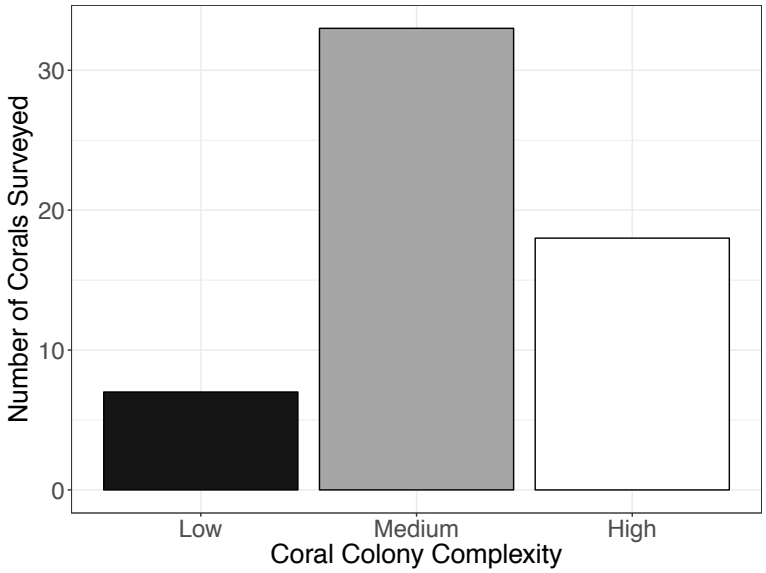


Figure 29 Frequency of occurrences of *P. moluccensis* in colonies of different complexities.

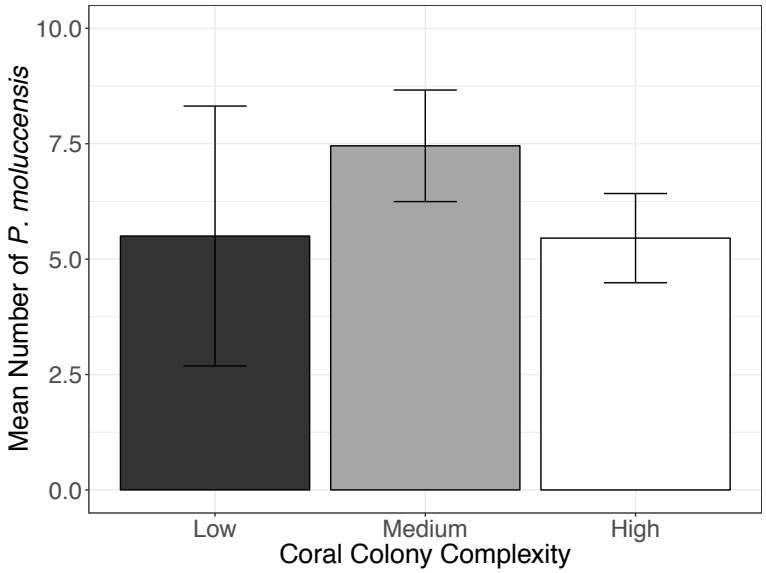


Figure 30 No difference was found in the mean number of *P. moluccensis* found in colonies of different complexities (n=50).

## 1.4 Discussion

Understanding specific structural habitat preferences of coral reef fish has been a key focus of coral reef ecology in the recent past (Almany 2004; Kerry and Bellwood 2012; Darling et al. 2017). To the best of our knowledge, this is the first study to use 3D printed habitats to explore habitat preferences and resulting behavior of coral reef fish. As hypothesized, results of the initial laboratory experiments indicated that *C. viridis* had no preference for different types of filament used to 3D print artificial habitats. Further, none of the filament treatments presented to *C. viridis* caused the fish to behave in a deviant manner compared to their behavior when exposed to a natural coral skeleton. Many studies have evaluated how coral reef fish behavior is changed when exposed to various stressors, such as poor habitat quality (Manassa and McCormick 2012; Manassa et al. 2013; Chivers et al. 2016). These studies are important because they elucidate the defense mechanisms of coral reef fish, and how those mechanisms may be impacted when in degraded environments. Because the exploratory experiments conducted in this study indicated that 3D printed habitats would not act as a stressor to a common coral reef fish, the use of these habitats to analyze habitat complexity preferences *in situ* was justified.

*P. moluccensis* was highly selective of higher complexity habitats than a low complexity habitat in a three-way choice experiment, which aligned with the results of other similar studies. For example, the coral-obligate goby, *Gobiodon histrio*, exhibited a strong preference for coral colonies with narrow interbranchial widths over wide interbranchial widths when tested in a binary choice experiment, which is another common measurement of habitat complexity (Pereira and Munday 2016). The

preference for higher complexity habitats has also been observed across other reef species including coral and non-sessile invertebrates (Graham and Nash 2013).

The risk of predation likely plays a role in influencing habitat complexity preferences of prey fish. In a laboratory setting, survivorship of *P. moluccensis* was significantly higher in the presence of predators on *P. damicornis* compared to *Acropora nobilis,* a low complexity coral (Beukers and Jones 1998). Further, the number of predatory strikes was lower for certain species when *P. moluccensis* was occupying a high complexity coral compared to *A. nobilis*. While successful predation effort is partially dependent upon the traits of the predator, the greater degree of protection offered by comparatively high complexity corals clearly influences these interspecific interactions.

In the current study, *P. moluccensis* associated equally with medium or high complexity habitats in choice experiments and spent almost identical amounts of time inside the respective shelters during behavior experiments. This is contradictory to the findings of Noonan et al. (2012), who concluded that too much structural complexity on a micro-habitat level was not a positive attribute for a resident damselfish, *Chromis retrofasciata,* in Papua New Guinea. In both field observations and patch reef experiments, *C. retrofasciata* had the highest rate of abundance and survival on medium complexity compared to high complexity *Seriatopora hystrix* corals. As other studies have also observed this trend, it has been proposed that small prey fish may choose to occupy corals with intermediate branch spacing and thickness that provide refuge while also not limiting visual predatory cues (Rilov et al. 2007). It is possible that while the overall surface area to volume ratio of the medium and high complexity

3D printed corals used in this experiment were different, the specific features of these habitats (i.e. branch spacing) were too similar for a preference to be shown.

The behavior of *P. moluccensis* was dependent upon coral habitat treatment, with the most deviant behaviors observed from fish placed on the lowest complexity 3D printed coral. The rate of abandonment from low complexity corals during trials was 61%, almost double the next highest rate from the high complexity 3D printed corals. Fish on the low complexity corals that did not abandon spent the least amount of time utilizing the shelter and showed the most inconsistent behavior in terms of distance they ventured from the habitat. As these behaviors are crucial factors that impact rates of predation, these results further confirm that microhabitat features can have significant bottom-up influences on reef fish community structures.

The behaviors of fish placed on 3D printed medium or high complexity corals compared to natural *P. damicornis* corals were not very aberrant. No significant differences were found for the rate of abandonment, amount of time spent utilizing the shelter, or distance ventured from the shelter between any of these treatments. These results indicate that 3D printed habitats, if created with a favorable level of complexity, can act similarly to live corals, despite lacking positive chemical cues associated with live coral tissue.

However, some behavioral differences were identified between fish placed on a 3D printed habitat or a natural habitat. While *P. moluccensis* occupying 3D printed medium or high complexity corals did not behave significantly different from each other, both treatments utilized their respective shelters differently from *P. moluccensis* occupying live *P. damicornis* corals. When inside of the 3D printed corals *P. moluccensis* spent significantly more time in the middle of the shelter, a presumably a

riskier position in terms of potential predation. When inside live coral, significantly more time was spent at the bottom of the shelter. Measures of boldness between these treatments also varied. Fish on the 3D printed corals on average were bolder and emerged from their habitats more quickly when approached than fish on the live corals. While occupying a more exposed area of their habitat and emerging more quickly or not retreating at all could be interpreted as risky behaviors, increased boldness by fish on 3D printed corals may not directly correlate with compromised survival. Studies have shown that pre-settlement stage damselfish that exhibited bold behaviors had higher survival rates after settlement than their shyer conspecifics (McCormick and Meekan 2010). During observational experiments on patch reefs, newly settled *Pomacentrus wardi* that were classified as survivors scored a median boldness metric of two, the same as *P. moluccensis* on 3D medium and high complexity corals in the current study, whereas non-survivors scored a median boldness metric of one, the same as *P. moluccensis* on live corals in the current study (Fuiman et al. 2010). Boldness could be interpreted as an indicator of alertness and suggests that bold fish may minimize the risk of attack. Thus, these differences in behavior of *P. moluccensis* on 3D printed corals versus live corals may not inherently be negative.

While it is important to investigate the habitat preferences and behavior of individuals in a controlled environment, it is well understood that foraging (Gil and Hein 2017), migratory (Haulsee et al. 2016), and defensive behaviors (Manassa et al. 2013) of fishes are influenced by the actions of social groups. As such, transects were conducted to analyze if the choices made by *P. moluccensis* in patch reef experiments would be reflected in natural habitat associations on the reef when many other factors

would be influencing their decision-making. Although it was predicted that a positive correlation would be found between *P. moluccensis* density and habitat complexity, this was not the case. Instead, a relationship was found between the volume of the colonies surveyed and *P. moluccensis* density, indicating that the size of the habitat space was a more important factor than complexity in influencing habitat choice.

The apparent lack of influence complexity had on natural *P. moluccensis* communities surveyed in this study may be misconstrued. When corals surveyed were binned into complexity classes, *P. moluccensis* was identified in medium complexity corals over three times as much as low complexity corals, and almost twice as often as high complexity corals. The lack of correlation found between complexity and presence of *P. moluccensis* can be explained by the frequency with which *P. moluccensis* was found inside the same type of coral complexity. The strong association of *P. moluccensis* with medium, and to a lesser extent, high, complexity corals supports the results of the habitat choice experiment. Complexity appears to be a playing a role in habitat choice for *P. moluccensis,* with higher complexity habitats likely offering important protection from predators.

*P. moluccensis* were still found in low complexity habitats, however. Further, when the average number of *P. moluccensis* found in each complexity class was compared, no significant differences were found. Because *P. moluccensis* prefer higher complexity corals, the presence of *P. moluccensis* in relatively dense numbers in low complexity corals may indicate a lack of suitable habitat space on the reefs surveyed. As previously mentioned, it has been well illustrated that behavioral mechanisms play a strong role in shaping the distribution of coral reef fishes, and a propensity for aggression can force weaker individuals into compromised habitats

with a greater risk of predation (Holbrook and Schmitt 2002; Coker et al. 2012a). As coral reef degradation continues, environmental traits and changes can exacerbate these exclusionary behaviors (Kok et al. 2016) and can ultimately lead to phylogenetic clustering within species localities and habitat associations (Head et al. 2018). Thus, the availability of suitable habitat space is crucial for maintaining species population stability.

# Chapter 2

# SETTLEMENT, SURVIVAL, AND GROWTH OF CORAL ON 3D PRINTED SUBSTRATES: SUPPLEMENTARY ECOLOGICAL APPLICATIONS OF 3D PRINTED OBJECTS

## 2.1 Introduction

The successful recruitment of reef-building corals is a complicated, yet crucial process for ensuring the longevity of coral reef ecosystems (Mumby and Steneck 2008). To maximize chances of survival, recruiting corals need suitable habitat space, and are known to probe available surfaces to actively select desirable substrate (Harrison and Wallace 1990). Crustose coralline algae (CCA) is known to be a favored substrate for many coral species and has been shown to induce settlement (Heyward and Negri 1999). The exact mechanisms of this process are not completely understood as certain species of CCA are strongly preferred by corals, while others emit strong anti-settlement defense strategies (Harrington et al. 2004). The chemical cues released from bacterial films associated with CCA have also been explored as the primary influence on settlement (Sharp et al. 2015). The Caribbean coral species *Porites astreoides*, *Orbicella franksi*, and *Acropora palmata* larvae selectively settled in response to biofilms of a particular strain that produced the compound tetrabromopyrrole (Sneed et al. 2014). Again, this compound does not have universally positive effects, and was found to induce metamorphosis prior to settlement for several Pacific coral species (Tebben et al. 2015).

Additional habitat features are important in determining successful settlement for corals. In controlled settings, studies have shown that *P. astreoides* planulae larvae

chose to settle significantly more on textured surfaces that offered protective microhabitats (Davies et al. 2013). In situ, crevices and cryptic microhabitats were effective at reducing predation rates by corallivores both on newly settled recruits (Nozawa 2008) and juveniles (Gallagher and Doropoulos 2017).

Abiotic factors such as sedimentation (Babcock and Smith 2000) and poor water quality (Albright et al. 2008) can have compounding effects on the survival and growth rate of coral spat. Most notably, recruiting corals are suffering from a loss of critical habitat space as reef systems shift to a macro-algal dominated state. Coral planulae larvae are deterred by some allelopathic compounds produced by macroalgae (Paul et al. 2011, Dixson et al. 2014). *P. astreoides* recruitment was either deterred or compromised by the presence of three widespread macroalgal genera, indicating lower recruitment rates and survival in algal-dominated areas (Edmunds and Carpenter 2001; Kuffner et al. 2006). As reef systems continue to degrade and become flattened, intraspecific competition for available, yet viable, microhabitats is intensifying and can force some recruits to settle in less desirable habitats (Harrington et al. 2004).

To re-establish coral cover on degraded reefs, the process of coral colonization and how settlement choices affect survival must be better understood. Because habitat loss is a major factor in impacting recruitment rates, the addition of favorable substrate should facilitate invertebrate colonization and growth. Many different materials have been used, with varying levels of success, as substrate options for settling corals including terracotta (Peterson et al. 2005), cement (Babcock and Mundy 1996), and petri dishes (Harriott and Fisk 1987). To the best of my knowledge, no research has been conducted on the use of 3D printed settlement tiles as sites for invertebrate colonization. Because the sustainability of coral reefs is critically dependent on the

colonization and growth of reef-building species, investigating the efficacy of new methods and materials to facilitate this process has become vital.

The goals of this study were to examine the settlement and growth of Caribbean and Indo-Pacific species of invertebrates on 3D printed settlement tiles created using different materials. The primary research objectives were:

1. To determine if a common Caribbean coral, *P. astreoides*, will successfully settle on 3D printed tiles.

2. To analyze and compare the growth rates of *P. astreoides* settled on different 3D printed materials.

3. To investigate the natural colonization patterns by invertebrates on 3D printed settlement tiles in the Indo-Pacific.

The capability of 3D printing technology and digital sculpting software to quantifiably manipulate model features was the primary motivation for its use in this work. The tiles created in this study were designed with a textured surface to provide cryptic microhabitats that should facilitate successful settlement and survival. Because the use of 3D printing technology as a tool for reef restoration has yet to be explored, this study included both laboratory and field experiments to elucidate coral habitat preferences under experimental conditions, and to determine if these tiles would be utilized by planulae larvae *in situ*. Further, understanding if CCA and other algal species grow on 3D printed materials in a natural reef setting could reveal future patterns of coral settlement, survival, and growth on these surfaces.

## 2.2 Methods

### 2.2.1 Study site

The funding for this thesis research was provided through the National Institute of Health International Cooperative Biodiversity Group. The specific grant awarded aimed to explore potential drug discovery of reef organisms and subsequent protection of reefs in Fiji and the Solomon Islands, both countries in a region that is particularly vulnerable to the synergistic effects of climate change (Mimura and Nunn 1998; Nunn 2009).

The field settlement experiment was conducted within the marine protected area outside of Coralview Island Resort on Tavewa Island, Fiji (16.9231° S, 177.3652° E) (Figure 31). This reef was easily accessible from the shore and undisturbed by fishing activities, making this location an ideal location. Additionally, a protected reef was chosen for this experiment to investigate coral settlement on 3D printed tiles in an ecosystem with high coral coverage compared to other locations around the island. Tiles were deployed at the site before a coral recruitment pulse to increase the likelihood that settlement on the tiles would occur.


Figure 31 Coral settlement on 3D printed tiles was analyzed on a protected reef, highlighted in red, around Tavewa Island, Fiji.

### 2.2.2 Study species

P. astreoides, known commonly as mustard hill coral, was used in laboratory-based experiments (Figure 32). This species is common throughout the Caribbean and Atlantic basin, reproducing by brooding planulae larvae around the lunar cycle (Green et al. 2008). P. astreoides larvae are hardy and commonly used in laboratory experiments (Sharp et al. 2015).

During the May 2017 lunar spawning event, 42 adult colonies were collected from Wonderland Reef, FL Keys (24° 34.130' N, 81° 22.868' W) using the spill-over method (Kuffner et al. 2006; Paul et al. 2011). Each adult colony was placed in an individual flow-through aquarium with the outflow spilling into a 180mm mesh bottom container resting in a flowing seawater bath. This method separates the positively buoyant ~1mm planulae larvae from their parent colony. The larvae were packaged in falcon tubes in filtered seawater and shipped the day after the release to the University of Delaware where experiments were conducted.

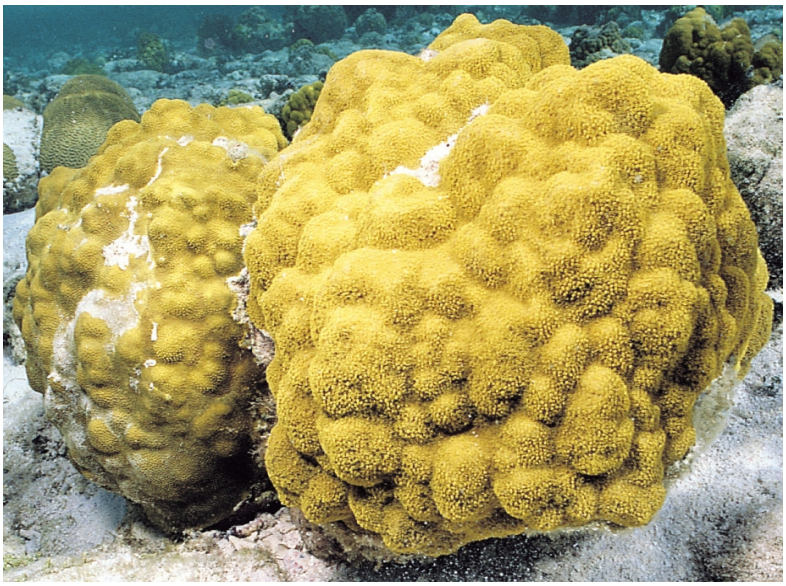

Figure 32 Mustard hill coral, *Porites astreoides*, was used to compare settlement, survival, and growth on 3D printed settlement tiles. Image sourced from Charlie Veron, The Australian Institute of Marine Science, accessed 12 April, 2018, http://coral.aims.gov.au/factsheet.jsp?speciesCode=0603.

### 2.2.3 3D model rendering and printing

For the work done in this chapter, 3D models were created using the Lulzbot Taz 5, Lulzbot Taz 6, and Makerbot Replicator 2 machines.

#### 2.2.3.1 *P. astreoides* experiment

A terracotta tile was used as a model to create 3D printed settlement tiles for a laboratory-based settlement experiment. A terracotta tile was used to mimic a textured substrate with microhabitats commonly used in other settlement experiments (Sato 1985; Albright et al. 2008). Photogrammetry software created the 3D tile model. The tile was placed in a well-lit area and photographed from all angles using an iPhone 6s. Photographs were uploaded into the ReMake program and were stitched together into

a 3D model. The program was also used to check for and fix model defects when encountered. The model was exported as an STL file to be printed using the slicing program Cura 21.08. Tiles were printed with the same dimensions to provide a controlled habitat size (4×4×0.2cm).

Four filament treatments were used to test the settlement and growth rates of *P. astreoides* on 3D tiles (Table 6). Specific filaments were chosen that represented a range of chemical compositions. Tiles were pre-conditioned in artificial seawater (34 ppt) for one week to dissipate negative cues that may initially be leached. While pre-soaking the tiles in artificial seawater did not produce a beneficial biofilm that could positively influence settlement, soaking the tiles in Delaware Bay water would also not have created the same biofilm used by settling corals (Decho 1990; Huang and Hadfield 2003).

Table 6 Filaments used to create 3D printed settlement tiles used in laboratory-based experiments.

| ***Treatment*** | ***Key Feature*** | ***Abbreviation*** |
|---|---|---|
| *Control (no tile)* | - | Control |
| *nGen Co-polyester* | Hardy plastic | nGen |
| *XT Co-polyester* | Hardy plastic | XT |
| *PLA/PHA* | Biodegradable | PLA |
| *Stainless steel PLA* | Metallic | SS |

#### 2.2.3.2 Field Experiment

The design software Autodesk was used to create a textured settlement tile to be used in field experiments. The model was created as a square face with parallel rows of small, indented squares (0.5×0.5×0.1cm). A small hole was inserted in the

center of the model so tiles could be easily nailed to the reef substrate. The model was exported as an STL file to be printed using the slicing program Cura 21.08. Tiles were printed with the same dimensions (40×40×2cm).

Four filament treatments were used to assess natural settlement patterns (Table 7). Different colors of the same filament were incorporated into this experiment to determine any potential impacts color had on natural colonization.

Table 7 Filaments used to create 3D printed settlement tiles used in a field-based experiment in Fiji.

| ***Treatment*** | ***Key Feature*** | ***Abbreviation*** |
|---|---|---|
| *nGen Co-polyester, Red + Blue* | Hardy plastic, color variation | nGen |
| *XT Co-polyester* | Hardy plastic | XT |
| *PLA/PHA, Red + Blue* | Biodegradable, color variation | PLA |
| *Stainless steel PLA* | Metallic | SS |

### 2.2.4 *P. astreoides* settlement and growth

Settlement of *P. astreoides* was tracked under controlled conditions for 14 days. The experiment was conducted in 500 ml glass aquaria, filled with 200 mL of artificial seawater that was changed every 48 hours. In each aquarium, 1 tile treatment was placed texture-side up on the smooth glass bottom. Control treatment aquaria contained no tile. Aquaria were randomly arranged in a 380 L water bath to ensure constant temperature (26.2°C) throughout the experiment. Twelve replicates for each treatment were used.

Seven *P. astreoides* planulae larvae were pipetted into each aquarium. Assessments of settlement were made daily. Settlement was scored as the number of corals that had attached and metamorphosed on any surface (Figure 33). Spat that had

metamorphosed but were still free-floating in the water column were scored as dead. Settled spat were considered attached to a surface if they could not be dislodged by light agitation of the water (Sharp et al. 2015).

After the 14-day settlement period, only the tiles with metamorphosed *P. astreoides* settled on them were tracked for growth and survivorship. Tiles were placed on elevated racks directly in the 380 L water bath to allow provide a continual supply of aerated water. A pipe cleaner was used weekly to keep tiles clean of algae to prevent factors from impacting growth and survivorship. Each settled juvenile was monitored for growth and survivorship for 12 weeks. The surface area of *P. astreoides* was measured every 3rd week using an imaging microscope and the program ImageJ.

To determine if 3D printed settlement tiles would inhibit settlement of *P. astreoides,* individual chi-square analyses were conducted between the total spat that settled within each experimental treatment aquaria to the total spat that settled within the control aquaria. Here, it was assumed that the absence of a provided settlement surface within the control treatment did not deter planulae larvae from settling on the glass. In this analysis, settlement included *P. astreoides* that settled anywhere in the aquaria for all treatments (i.e. glass or tile) at any point during the settlement period, including individuals that later died. Corals were considered not settled if they were still free swimming at the end of the 14-day period. Coral larvae that died before settling were not included in this analysis.

Due to the correlation of individual *P. astreoides* settled within each replicate aquaria, a repeated measure ANOVA was used to analyze total settlement between the 3D printed tile treatments. In this analysis, settlement only included *P. astreoides* that

settled on the tile at any point during the settlement period, including individuals that later died.

Percent mortality of *P. astreoides* between treatment tile conditions was analyzed using a one-way ANOVA. Percent mortality was calculated from the total number of *P. astreoides* that settled at any point and the final count that survived on each tile treatment at the end of the 12-week growth period.

Growth rates were determined by measuring the total surface area and rate of change in surface area over time (Albright et al. 2008). A repeated measure ANOVA was used to determine if the growth rate of *P. astreoides* individuals settled on 3D printed tiles was significantly affected by tile filament type. Growth rate was used as the dependent variable instead of total surface area to account for initial size differences of the individual corals.

Figure 33 Example of a *P. astreoides* juvenile, highlighted in red, metamorphosed on a 3D printed nGen tile.

### 2.2.5 Natural coral settlement

Tiles were placed in clusters at three depths at the time of low tide systematically throughout the protected reef on Tavewa Island, Fiji (Figure 34). For ease of relocating at the end of the experiment, clusters were placed in parallel lines along and within depth categories. Clusters at the same depth were spaced 30m away from adjacent clusters, and 10m away from adjacent clusters at different depths. Tiles were secured as horizontally as possible with 2-inch galvanized roofing nails to the reef substrate. Tiles were identified by individual numbered tags nailed into the substrate.

A cluster ideally consisted of one replicate of each treatment tile, including both color variations for the nGen and PLA treatments (Figure 35). Over the course of transport and deployment, several tiles were broken or lost (Table 8). As such, not every cluster consisted of all tile treatments. However, the replication for this experiment was sufficient to account for these losses.

All tiles were photographed at the time of placement on July 25, 2017. Tiles were removed from the reef and photographed on both sides on January 27, 2018. Natural coral settlement was quantified by counting the total number of individual colonies that settled on the top and bottom of each tile treatment. Colonies were not quantified by the number of polyps and were not identified to the species level. Coral colonies were identified using a 30x magnification microscope attachment (Figure 36).

These data could not be transformed to meet parametric assumptions and were evaluated with the nonparametric Kruskal-Wallis test, with post-hoc comparisons performed using Dunn's test.

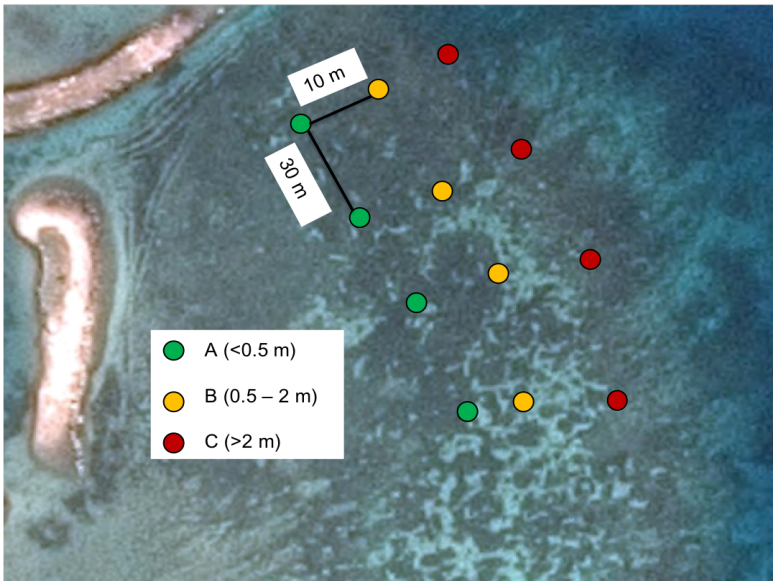


Figure 34 Settlement tiles were placed in clusters of one replicate per treatment at three depths within the protected reef outside of Tavewa Island, Fiji (n=66).

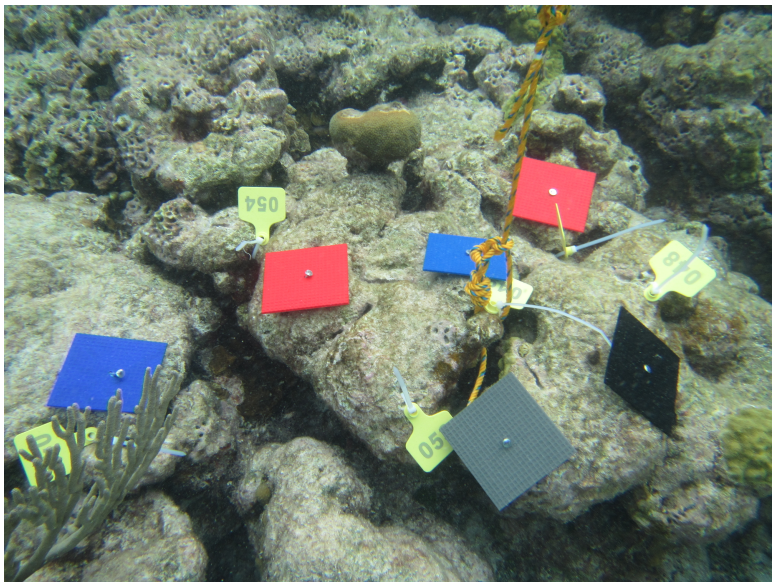

Figure 35 A cluster of 3D printed tiles secured horizontally onto the reef substrate.

Table 8 Missing or broken tile replicates from each depth in the protected reef outside of Tavewa Island, Fiji.

| ***Depth*** | ***Treatment*** | ***# of Replicates*** |
|---|---|---|
| *Shallow (A)* | Red nGen | 3 |
| | Blue nGen | 2 |
| | Nylon | 3 |
| | Red PLA | 1 |
| *Reef Crest (B)* | Red nGen | 1 |
| | Blue nGen | 1 |
| | Blue PLA | 1 |
| *Fore Reef (C)* | Red nGen | 1 |
| | Blue nGen | 2 |
| | Red PLA | 2 |
| | Blue PLA | 1 |

Figure 36 An example of a coral colony, highlighted in red, identified on the bottom of a blue nGen tile (top). Tiles were inspected for coral colonies on the top and bottom of all tiles using a 30x magnification microscope attachment (bottom).

## 2.3 Results

### 2.3.1 *P. astreoides* settlement and growth

All treatments were found to have significantly higher settlement compared to the control aquaria (Table 9).

Table 9 Chi-square analyses of total *P. astreoides* settlement anywhere in each treatment aquaria compared to settlement when no tile was present.

| ***Filament*** | ***Total Settled*** | ***$X^2$*** | ***df*** | ***p-value*** |
|---|---|---|---|---|
| *Control* | 15 | - | - | - |
| *nGen* | 32 | 27.43 | 1 | P<0.001 |
| *XT* | 52 | 88.04 | 1 | P<0.001 |
| *SS* | 36 | 71.11 | 1 | P<0.001 |
| *PLA* | 41 | 46.57 | 1 | P<0.001 |

No significant differences were found in total settlement on tiles between the treatments (repeated measures ANOVA: $F_{(3)}$=1.355, p=0.2557) (Figure 37). No significant differences were found in percent mortality of settled *P. astreoides* between the experimental treatments (one-way ANOVA: $F_{(3)}$=0.5943, p=0.6235) (Figure 38).

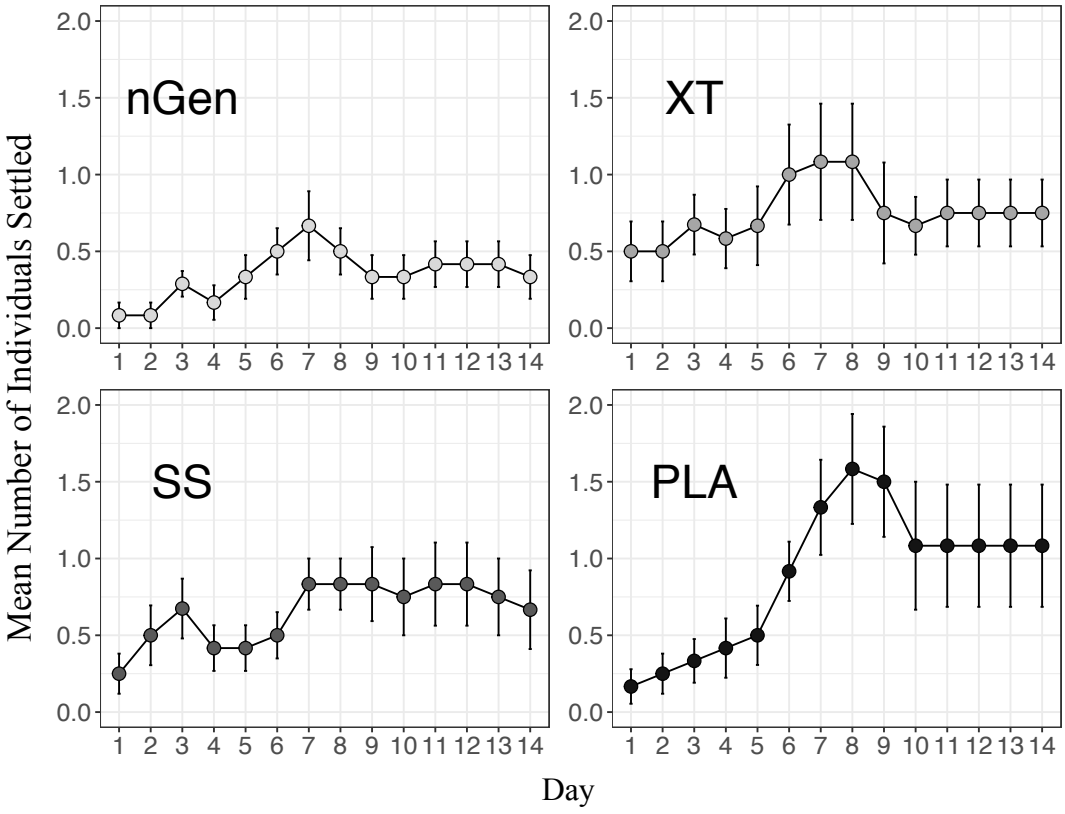


Figure 37 No differences were found for the mean number (± SE) of *P. astreoides* settled on each 3D printed treatment tile during a 14-day settlement period.

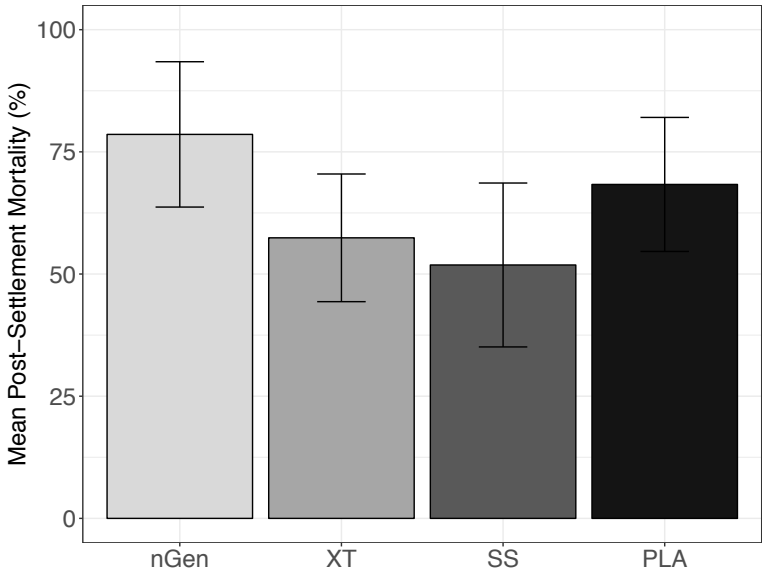


Figure 38 No differences were found in mean post-settlement mortality (± SE) of *P. astreoides* settled on different 3D printed tile treatments.

No significant differences in average growth rate of *P. astreoides* on different tile treatments or growth rates of individuals within treatments were found ($F_{(3)}$=1.099, p=0.357) (Table 10).

Table 10 Average weekly growth rates of *P. astreoides* on each settlement tile treatment.

| ***Filament*** | ***Average Growth Rate (mm²/week)*** | ***SE*** |
|---|---|---|
| *nGen* | 0.078 | 0.01 |
| *XT* | 0.201 | 0.01 |
| *SS* | 0.211 | 0.02 |
| *PLA* | 0.162 | 0.05 |

### 2.3.2 Natural coral settlement

Every tile treatment had some settlement of coral over the course of the six months (Table 11). There were no significant differences in the total number of colonies settled between the six tile treatments (Kruskal-Wallis: $\chi^2_{(5)}$=1.7743, p-value= 0.88) (Figure 39).

Table 11 Total number of colonies found on each 3D printed settlement tile treatment placed on a protected Fijian reef.

| ***Treatment*** | ***Treatment Replicates*** | ***Number of Coral Colonies*** |
|---|---|---|
| *nGenB* | 12 | 14 |
| *nGenR* | 16 | 16 |
| *Nylon* | 22 | 7 |
| *PLAB* | 20 | 19 |
| *PLAR* | 18 | 6 |
| *SS* | 26 | 13 |

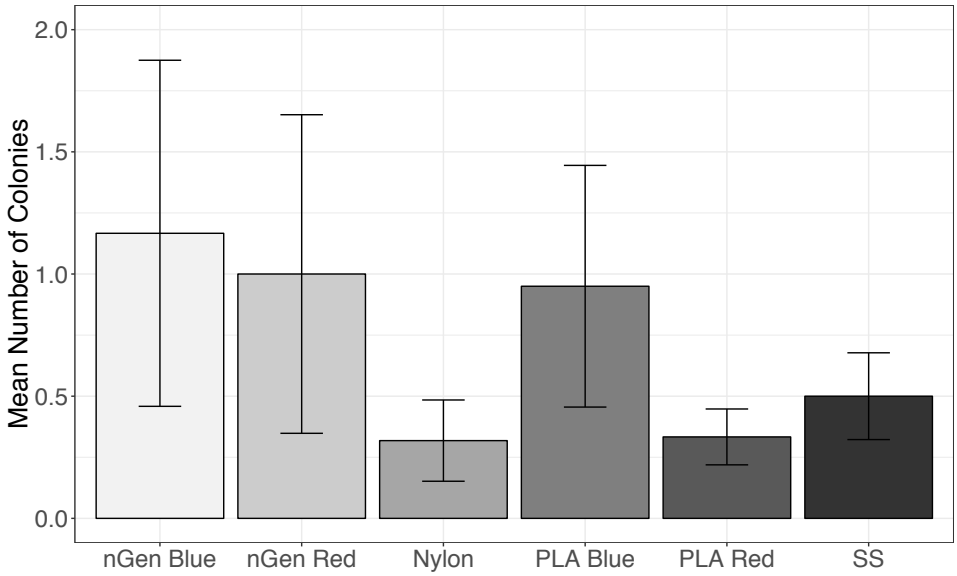


Figure 39 No significant differences were found in mean coral colonization (± SE) on 3D printed settlement tile treatments.

Settlement was also evaluated within depths across 3D filament treatments. Blue nGen, red nGen, and blue PLA, which had the highest average settlements, had the most settlement occur on tiles placed deepest on the fore reef (Figure 40). Contrastingly, nylon, red PLA, and SS had similar average coral settlement across all depths. However, no significant differences were found between average coral settlement on tiles placed shallow, on the reef crest, or on the fore reef across the filament treatments.

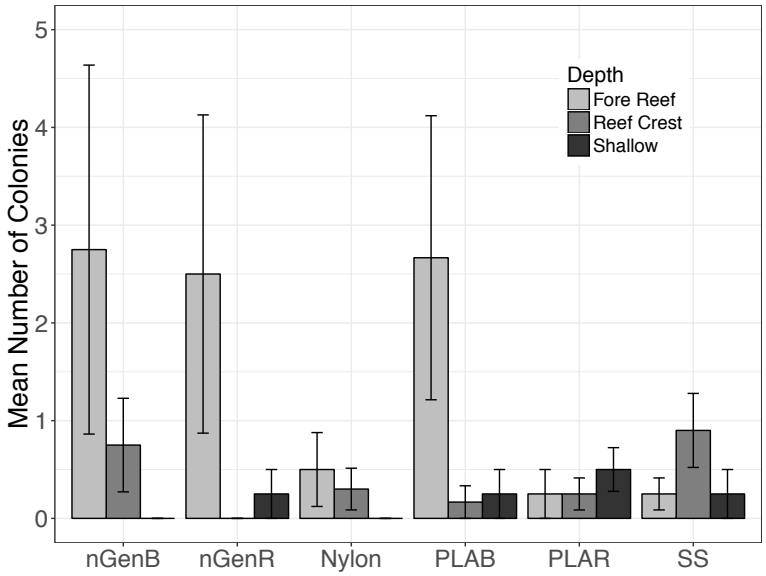


Figure 40 No significant differences were found in mean coral colonization (± SE) on 3D printed settlement tile treatments placed at different depths.

## 2.4 Discussion

Under controlled conditions, *P. astreoides* chose to settle significantly more in treatment jars containing a 3D printed tile compared to control jars with no tile. This result was expected, as the 3D tiles were designed to provide a textured surface that recruiting corals have been shown to prefer (Thomason et al. 2002). Not considering other environmental factors, textured surfaces provide a more protective habitat than smooth surfaces and can reduce coral mortality from predation (Keough and Downes 1982).

The higher settlement observed in treatment conditions also indicated that the materials in the 3D printed tiles did not inhibit *P. astreoides* from successfully settling. Further, time to settlement for most treatments used was comparable to settlement rates observed in the same time period on more commonly used materials. Albright et al. (2008) analyzed the settlement of *P. astreoides* on limestone tiles, pre-conditioned *in situ*, in two separate trials. After one week, the first trial had 34.72% (±7.29) settlement, while the second trial had 12.5% (±4.95). After one week in the current study, all treatments had higher percent settlement on the tiles compared to Albright et al.'s second trial, and all but the XT treatment had a settlement rate within ±6.5% of the first trial. The settlement rates observed in the current study, where treatment tiles were not pre-conditioned to produce a biofilm to facilitate settlement, indicated that 3D printed surfaces can act as suitable substrate for recruiting corals.

Similarly, percent mortality of corals settled on treatment tiles was comparable to rates observed for *P. astreoides* in other studies. When analyzing the latent effects of oxidative stress on settling *P. astreoides*, Ross et al. (2013) saw only 10% survival of settled spat in the control treatment (individuals were not exposed to oxidative

stress) after 24 hours on a patch reef in the Florida Keys. After 3-months in the current study, the lowest rate of survival (nGen treatment) was approximately 21% while the highest (SS treatment) was approximately 48%. Newly settled corals are extremely vulnerable to predation and abiotic stressors, resulting in low rates of juvenile survivorship in many areas (Wilson and Harrison 2005; Penin et al. 2010). While the rate of mortality for *P. astreoides* in all treatments obtained in the current study is not higher the rates of other studies, it is likely that mortality would have been higher if it had been tracked *in situ.* It can be inferred from the obtained results, however, that 3D printed materials did not have immediate or latent post-settlement effects on *P. astreoides* survival.

Coral settlement was observed on every tile treatment when secured *in situ* for approximately six months. Depth had a non-significant but noticeable effect on settlement, with the highest average number of colonies settled on nGenB, nGenR, and PLAB tiles placed on the fore reef. This study has demonstrated that recruiting corals can successfully colonize and survive on 3D printed surfaces both in a laboratory and field setting. As results indicated that no particular filament had a substantial influence on the settlement, growth, or survivorship of *P. astreoides* and settlement of Indo-Pacific corals, it does not seem likely that the 3D printing process or common printing materials would have deleterious consequences for reef-building invertebrates.

This exploratory research has opened the door to many other relevant questions regarding the potential of 3D printed materials to act as a viable substrate alternative on degraded reefs. Mainly, how would settlement on 3D printed tile be impacted if they were associated with cues known to attract coral larvae and induce settlement? The positive influence of live coral (Lee et al. 2009), CCA, and associated biofilms on

inducing coral settlement and juvenile growth rate (Albright and Langdon 2011) has already been investigated for a variety of coral species and across many substrate types. Under controlled conditions, Dixson et al. (2014) showed that larval settlement on traditional tiles was increased by 1600% when the tiles were rubbed with a CCA cue. Thus, if 3D printed settlement tiles designed with microhabitats could be incorporated with positive cues, or positioned on degraded reefs with outplanted live corals, settlement and survival on these materials would expectedly proliferate.

CCA and other benthic invertebrates were already observed growing on all 3D filament treatments used in this field study. In addition to coral colony density, tiles will be analyzed for percent cover and composition of CCA and filamentous algae to more accurately predict how the presence of these materials may influence the reef benthos. While increasing coral cover on unhealthy reefs requires consideration of the interactions between many environmental factors, replenishing habitat space for calcifying species is a critical first step for reef restoration.

# Chapter 3

# CONCLUSIONS

The combination of successful coral and fish recruitment is essential for a stable reef environment (Hughes et al. 2003; Mumby and Steneck 2008; Bellwood et al. 2004; Anthony 2016). The global decline of coral reefs has compromised the stability of these vital ecosystems and has caused a shift to an environment with reduced biodiversity and loss of topographic complexity (McClanahan et al. 2002; Pratchett et al. 2014). Although the loss of topographic complexity has been correlated with a loss of fish abundance and biodiversity (Wilson et al. 2006), the relationship between reef organisms and habitat structure is not well understood (Noonan et al. 2012).

This thesis demonstrated that complexity plays an important role in determining habitat associations and subsequent behavior of the coral-associated damselfish, *P. moluccensis*. Low complexity corals were least favorable and resulted in very deviant behavior among individuals, implying compromised survival if occupying such a precarious habitat. By having a clearer understanding of the role that reef complexity plays, conservation efforts can be directed at protecting and outplanting specific species and sizes of corals that can offer the most beneficial substrate for coral-associated fish.

Further, this thesis successfully isolated the physical structure of a coral skeleton using 3D printing technology, a methodology that has not previously been explored in ecological research. This study has provided evidence that coral reef damselfish will utilize artificial corals designed with appropriate habitat features

similarly to how they utilize live coral colonies. This finding further emphasizes the importance of a coral's physical structure in habitat selection, as the 3D printed corals used did not emit positive chemical cues from live coral tissue. When considering that settling corals and CCA will also utilize 3D printed surfaces as habitat, the use of these synthetic coral colonies and tiles for bolstering coral reef communities and adding structure and suitable habitat warrants further investigation.

Artificial reefs and structures are already common tools that have been used to provide substrate and habitat space for recruiting invertebrates and fish (Seaman and Sprague 1991). These reefs are typically composed of large and heavy concrete materials, rock, stone, or repurposed materials such as tires, vehicle frames, and sunken ships (Baine 2001). Many artificial reefs currently deployed are placed in one focal area and are intended to increase aggregations of fish to a specific location to create a spillover effect that will benefit the system as a whole (Grossman et al. 1997). However, a review of 30 artificial reef case studies determined that only 50% of reefs met the restoration objectives set by the managing body (Baine 2001). While many of these reefs prove unsuccessful due to a combination of factors (improper management, location, etc.), the majority of studies cited poor design and insufficient provision of crevices and refuges to be significant downfalls.

For example, concrete Reef Balls™ are some of the most commonly used objects in artificial reef creation, yet their design lacks the complexity needed to support healthy fish communities. Reef balls are dome-shaped structures with holes in the side leading to a large central void space. Reef balls with added structural elements placed in this void space had significantly higher fish abundance, number of species, and fish biomass across multiple size classes compared to standard reef balls,

indicating that these structures are not appropriately designed to maximize reef restoration success (Sherman et al. 2002).

As habitat characteristics are major drivers of the structure and function of animal communities, the design of artificial reefs should mimic that of the environment that they are intending to rebuild. This thesis accomplished this through the use of 3D printing technology, successfully replicating the skeleton of an important reef-building coral species. Versions of large, concrete 3D printed coral reefs have already been deployed off the coast of Bahrain, where coastal development has significantly impacted reef health and acroporid cover (Burt et al. 2013). While no studies could be found that explored the efficacy of this reef structure since it was deployed in 2012, the nature of the printing process and materials used are not easily repeatable. If structurally complex 3D printed reefs are used for a new era of reef restoration efforts, repeatable studies are necessary to answer important questions about the impacts of these reefs. Small-scale machines, such as the LulzBot and MakerBot series, are more universal than construction-sized printers due to the significantly lower costs, ease of operation, availability of filament, and portability of the prints. Additionally, bioplastic filaments meant to naturally degrade over time are available. Thus, using this material would be more ecologically sustainable than using other materials currently utilized in artificial reef construction.

More research needs to be done to investigate the efficacy of complex, 3D printed corals as artificial habitats for reef organisms. Several dozen models (PLA/PHA) of *A. formosa* coral colonies were placed on the protected reef on Tavewa Island, Yasawas, Fiji from February 6, 2018 to October 15, 2018. These corals will be analyzed for composition of invertebrate colonization and associated fishes after this

time. The results of this exploratory study should add to our understanding of how the natural reef environment will respond to the long-term presence of complex 3D printed corals when placed alongside positive chemical cues.

Additional future studies should analyze habitat use and complexity preferences of newly settled recruits on 3D printed corals, and track growth of settled corals on these materials. Further, rate of degradation of biodegradable artificial corals needs to be examined in a reef setting to ensure that sufficient coral growth can occur before the degradations process compromises the temporary habitat.

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## Appendix A

## STATEMENT OF ETHICS

All research conducted in this thesis was within the guidelines of the Institutional Animal Care and Use Committee (IACUC). This committee is constituted according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals. As the grant under which this project was funded included multiple universities, approval was obtained from both the University of Delaware (AUP number: 1305) and the Georgia Institute of Technology (Protocol Number: A16112).

Additionally, research was conducted within the guidelines of the Fijian government. A binding memorandum of understanding (MOU) was obtained through the Ministry of Agriculture, Fisheries, and Forests stating conditions for the proposed work.

**University of Delaware**
**Institutional Animal Care and Use Committee**
**Annual Review**

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| | |
|---|---|
| **Title of Protocol:** Exploration, conservation and development of marine biodiversity in Fiji and the Solomon Islands | |
| **AUP Number:** 1305-2018-2 | ← **(4 digits only)** |
| **Principal Investigator:** Danielle Dixson | |
| **Common Name:** The study may involve but is not limited to, the following species: network pipefish, cardinalfish, candy stripped cardinalfish, green chromis, white tailed damselfish, twinspot damselfish, blue spot damselfish, domino damselfish, vagabond butterflyfish, latticed butterflyfish, convit surgeonfish, tang, rabbitfish, Forsten's parrotfish, daisy parrotfish, stripped cardinalfish, threespot wrasse, honeycombed grouper<br><br>**Genus Species:** As above: Corythoichthys flavofasciatus, Apogon fuscus, Apogon endekataenia, Chromis viridis, Dascyllus aruanus, Chrysitptera biocellata, Crysiptera cyanae, Dascyllus trimaculatus, Cheatodon vagabudnus, Chaetodon raffelsi, Acanthurus triostegus, Ctenochaetus striatus, Signaus spinus, Chlorurus sordidus, Apogon angustatus, Halichoeres trimaculatus, Epinephelus merra | |

**Pain Category:** ***(please mark one)***

**USDA PAIN CATEGORY:** ***(Note change of categories from previous form)***

| Category | Description |
|---|---|
| ☐ **B** | Breeding or holding where NO research is conducted |
| ☐ **C** | Procedure involving momentary or no pain or distress |
| ☐ **D** | Procedure where pain or distress is alleviated by appropriate means (analgesics tranquilizers, euthanasia etc.) |
| ☐ **E** | Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation |

**Official Use Only**

IACUC Approval Signature: Glenn Tatlock, DVM

Date of Approval: 6.1.18

November 11, 2016

Mark Hay, Ph.D.
Department of Biology

| | |
|---|---|
| **Protocol Number:** | **A16112 Approval** |
| **Project Title(s):** | **Exploration, Conservation and Development of Marine Biodiversity in Fiji and the Solomon Islands** |
| **Approval Period:** | **November 11, 2016 – November 10, 2019** |
| **Funding Source / OSP Number:** | **NIH / Doc ID# 127167 (#3206ANC)** |

Dear Dr. Hay:

The Georgia Tech Institutional Animal Care and Use Committee (IACUC) has reviewed the above-referenced protocol application and acknowledges the receipt of a copy of the approved IACUC protocol and approval letter for the research to be conducted by Dr. Danielle Dixson, University of Delaware.

The proposed procedures were found to comply with the Institute's Policy for Humane Care and Use of Laboratory Animals; therefore, effective this date, the project is approved.

The current approval period is shown above. **After the third year approval, if research is to continue, you will need to submit a new application at least 60 days prior to expiration.**

You are asked to provide this office with copies of annual letters of continuing approval for the vertebrate animal research conducted by Dr. Dixson, University of Delaware.

You are required to submit any change in procedures to the IACUC for review prior to implementation of the change. Approval is contingent upon your agreement to abide by the policies and procedures of the Georgia Institute of Technology regarding the use of animals in research.

The full text of Georgia Tech's Assurance (A3822-01) can be found online at http://researchintegrity.gatech.edu/about-iacuc/assurance-a3822-01/.

Problems affecting the care or use of animals should be reported immediately to the Research Veterinarian, Dr. Laura O'Farrell (404.385.6233) and to the Office of Research Integrity Assurance (404.385.7316) or (404.894.7044). Please do not hesitate to contact me if you have any questions.

Sincerely,

Anna Marie Lee, B.S., CPIA
Research Associate
Office of Research Integrity Assurance

cc: Nena Gray, Biology
Danielle Dixson, University of Delaware
David Moore, CoS
Laura Letbetter, OSP

MINISTRY OF AGRICULTURE, FISHERIES & FORESTS
DEPARTMENT OF FISHERIES

**MOU between 1) Georgia Tech Research Corporation, United States of America 2) the people of Ba Province and 3) Fisheries Department of the Ministry of Agriculture, Fisheries and Forests.**

This Memorandum of Understanding [MOU] is a binding agreement between 1) Georgia Tech Research Corporation, United States of America, 2) the people of the Ba Province – as owners of customary fishing right areas and 3) Fisheries Department of the Ministry of Agriculture, Fisheries and Forests – as representative of Fiji Government, pertaining to the taking of living marine organisms within Fiji fisheries waters for the general purpose of research work.

**Summary:**
The Intellectual Property Right Unit [IPRU] of the Ministry of Agriculture, Fisheries and Forests is in place, where its primary purpose is fully covered under the provisions in the Convention of Biological Diversity, which Fiji has ratified. The role of the IPRU is to ensure the protection of indigenous rights and resources from the exploits of outside forces and demands. It is paramount that indigenous and rural communities participation in the development of their natural resources are respected by all parties, and that sustainable resource use are the over-arching goals, and that any benefits derived should be equally enjoyed by the same.

**Conditions:**
The following conditions shall apply:

1. All developments pertaining to the taking of living marine organisms within customary fishing right areas in the Fiji Islands shall be subjected to consent by the custodian resource owners;
2. Any removal of live marine organisms [biological material] or materials derived therefrom as specimens out of the country for the purpose of scientific research work will require formal certification from the Fisheries Department;
3. Relevant copies of reports on these scientific research work will be made available to Fisheries Department and customary fishing right owners as and when required for their information, reference and record;
4. Permission must be obtained from the Permanent Secretary for Agriculture, Fisheries and Forests before the samples covered in this agreement are conveyed to any third party or any commercial use is made of the samples except for taxonomic identification purposes only;
5. Any scientific research work undertaken on these specimens for commercial purposes should have definite benefit sharing in place for the customary fishing right owners, the Fisheries Department and the country as a whole.
6. A local sample collection center shall be set up in Fiji, with the assistance of Institute of Applied Sciences, University of the South Pacific.

Acknowledged and agreed on:

this …….. day of …… 20 ……

| | | |
|---|---|---|
| ……. | …… | …… |
| **1) Georgia Tech Research Corporation United States of America** | **2) People of Ba Province** | **3) Permanent Secretary for Agriculture Fisheries and Forests** |

**Qoliqoli: _____**

**Signature: _____**

**Appendix B**

**3D CORAL MODEL CREATION**