PHOTOGRAPHS:
Front Cover: Chris Savage
Forward: (Top to bottom) Chris Savage, Kelsey Burns, Chris Savage, Christina Wickman
Profile Pictures: Anoushka van de Ven
Intern Page: (left to right) Amanda Parra, Christina Wickman, Chris Savage
Table of Contents: (Top to bottom) Chris Savage, Christina Wickman, Chris Savage (both)
Back Cover: Kelsey Burns

CIEE Research Station Bonaire
Tropical Marine Ecology & Conservation Program
Vol. IV Fall 2008
As understood by the Greeks, Physis simply meant nature. Nature in the sense that it was
dynamic and flowing. Nature in the sense of growth and in close proximity to essence. Always,
Physis represents growth free of external forces. In the eyes of Thoreau, this growth came in the
form of knowledge, of the transition from darkness to light—cognitively, cosmically,
bio-logically. Physis, is in essence, the entirety of the surrounding natural world.

Our studies over the past fifteen weeks have taken us from clear, blue waters at 60 feet to the
murky shallows at the roots of mangroves, we have tagged turtles and collected trash, we have
sat in darkness listening for bats among the limestone and scaled windows to the world looking
far beyond the horizon.

Here, we present our brush with nature. Here, we present a culmination of our adventures, our
conservation efforts, and our growth as individuals and as scientists. Here, we present to you
Volume IV of Physis.

Enjoy!
Lauren Van Thiel
CIEE Bonaire Fall 2008
FORWARD

The Council on International Educational Exchange (CIEE) is an American non-profit organization with nearly 100 study abroad programs in 35 countries around the world. Since 1947, CIEE has been guided by its mission…to help people gain understanding, acquire knowledge, and develop skills for living in a globally interdependent and culturally diverse world. As a membership organization, composed of mainly U.S. institutions of higher education, CIEE responds quickly to the changing academic needs and desires of its member institutions.

The Tropical Marine Ecology and Conservation program in Bonaire is one of the newest programs offered by CIEE and is an example of our ability to foresee the need for science-based programs abroad. Our goal is to provide a world-class learning experience in Marine Ecology and Conservation. Our program is designed to prepare students for graduate programs in Marine Science, Environmental Science, or for state and federal jobs in Natural Resource Management. Student participants enroll in five courses: Coral Reef Ecology, Scientific Diving, Human Ecology, Marine Resource Management and Independent Study. In addition to a full program of study, this program provides dive training that prepares students for certification with the American Academy of Underwater Scientists, a leader in the scientific dive industry, at their home universities.

The proceedings of this journal are the result of each student’s Independent Research project. The research was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Netherlands Antilles. Students presented their findings in a public forum 26 November 2008 at the CIEE Research Station in Bonaire.

The Independent Research Advisors for the projects published in this journal were: Rita B.J. Peachey, Ph.D, and Amanda Hollebone, Ph.D. Caren Eckrich M.S. acted as Diving Safety Officer, instructing the students on research diving techniques. Kelsey Burns and Amy Milman worked as research interns for CIEE assisting the students in field work. Brief biographies of the advisors and interns are presented on the next page.
Dr. Rita Peachey is the Resident Director in Bonaire. She received her B.S. in Biology and M.S. in Zoology from the University of South Florida and her Ph.D. in Marine Science from the University of South Alabama. Her research interests include coral biology and how UV affects the early stages of life in the ocean. In addition, she has studied how pollution can enhance the detrimental effects of sunlight on larval crabs, corals and oysters. Rita has years of experience conducting ecological research in a variety of ecosystems such as oyster reefs, seagrasses, coral reefs, and mangrove swamps. 

*Primary advisees: Kara Kozak, Amanda Parra, Christopher Savage, Christina Wickman*

Caren Eckrich is the Assistant Resident Director and, as a resident of Bonaire for the last eight years, she brings local experience and a wealth of information on diving and marine ecology in Bonaire. She is a SCUBA instructor and has taught Marine Ecology in Puerto Rico, Curacao and Bonaire. Her educational background includes a B.S. in Wildlife and Fisheries from Texas A&M University and a M.S. in Biological Oceanography at the University of Puerto Rico in Mayaguez. Caren’s research interests include fish behavior, seagrass ecology, sea turtle ecology and coral disease.

Dr. Amanda Hollebone is the Marine Conservation Biology faculty at CIEE Bonaire. She received her B.S. in Biology from the UNC Chapel Hill and Ph.D. in Marine Ecology from the Georgia Tech and has recently taught in the Biology Department at Georgia Southern University. Amanda’s research interests lie in the areas of reef community ecology and invasive species with her dissertation research focusing on the population dynamics and pre- and post-settlement ecology of a non-native porcelain crab in the oyster reefs of Georgia, USA. Amanda has had several years of experience conducting research in marine ecosystems of the southeastern US including mud flats, salt marshes, oyster reefs, and offshore reefs, as well as in the mangrove forests, seagrass beds, and coral reefs of Florida and the Bahamas. 

*Primary advisees: Chiu Cheng, Lauren Pacheco, Annemarie Rini, and Lauren Van Thiel*
INTERNS

Kelsey Burns is one of the 2008 Tropical Marine Ecology interns. She was the teaching assistant for Coral Reef Ecology, Culture and History of Bonaire and Independent Research. Kelsey graduated in July 2008 from James Cook University in Townsville, Australia with a Bachelor’s of Science, Marine Biology. In the future, she plans on attending graduate school in pursuit of a PhD in marine biology. Her main interests include larval ecology, ichthyology and food web dynamics.

Amy Milman is the Conservation Biology and Scientific Diving Teaching Assistant for the Fall 2008 semester. Amy graduated in 2007 with a masters degree in Marine Environmental Management from the University of York. Since graduating she worked as a Marine Scientist and Divemaster on a coral reef conservation project in Mexico before coming to Bonaire. Her research during her time here has been to record the rate of spread of dark spot disease at reefs with different environmental conditions.
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Can native sessile species resist the settlement of the orange cup coral, *Tubastrea coccinea*, on hard substrate communities of Bonaire, Netherlands Antilles?

Chiu Cheng*
Moravian College

Abstract
*Tubastrea coccinea* is an invasive coral species found on the reefs of Bonaire. These corals are typically seen at various densities (up to 80% m⁻²) on hard, vertical substrata suggesting that biotic resistance could be one possible biological factor preventing settlement of *T. coccinea* elsewhere (e.g., horizontal substrata). The impact potential competitors have on the successful invasion, recruitment and growth of *T. coccinea* was experimentally assessed by establishing replicated 15 x 15 cm plots of substrata already inhabited by single species or combinations of native species (0-3 and 3 seeded with adult *T. coccinea*) at the Harbor Village jetty, Kralendijk, Bonaire, which had the necessary vertical substrata. Monitoring occurred over a period of three weeks to assess percent cover change of the studied organisms. Additionally 15 vertical, 5 m transects were run to evaluate mean percent cover of all sessile species that inhabited the surveyed locations for a general representation of species diversity at the jetty. *T. coccinea* was not observed to settle in any of the experimental plots nor did the seeded adult conspecifics show any evidence of growth or recruitment. Observational data indicated that an algal turf had the highest mean percent cover, but in areas around *T. coccinea*, algal turf percent cover decreased by almost 20%, suggesting competition between the two organisms. No firm conclusions could be drawn about *T. coccinea* recruitment or growth, but results suggested that the presence of an invasive species may negatively affect the growth of native species when they are found in close proximity to it.

Introduction
The study of invasions in marine systems is still in its infancy, and the potential factors determining the susceptibility of a community to invasions still often remains unclear when compared to studies on terrestrial ecosystems (but see Stachowicz et al. 1999; Duffy and Stachowicz 2006). The concept of native biotic resistance relates to a community’s ability to resist invasion. The mechanisms affecting this resistance are still debated. However, previous studies have shown, on a small-scale, that the negative effect of diversity on invasion success is largely due to its effects on resource availability, such as space (Stachowicz et al. 1999; Stachowicz et al. 2002). Similarly, a terrestrial plant study found that invasion resistance is due to diverse plant assemblages that use resources more completely through maximum niche occupation (Pokorny et al. 2005). However, while it is commonly believed that diversity can enhance resistance to invasion, arguments can also be made that diversity may be ineffective against invasion or, in other cases, possibly enhance it (Levine and D’Antonio 1999). One study has shown that while native species richness slows initial invasion, the early invaders stimulate further settlement and thus, any potential biotic resistance is eventually overwhelmed (Hollebone and Hay 2007).

In certain locations on the reefs of Bonaire, Netherlands Antilles the invasive orange cup coral, *Tubastrea coccinea*, is found at high densities (up to 80% m⁻²) along vertical substrata. It is believed to be the only scleractinian unintentionally introduced into the western Atlantic and was found on the hull of ships between 1948 and 1950 in the Netherlands Antilles (Humann and Deloach 2003a). One possible explanation for the variation in *T. coccinea*’s development (its abundance and distribution) may be the horizontal heterogeneity of the environment; that is, the functional diversity within a trophic level that comprises of species with similar roles that require them to compete for the same resources (Duffy 2002). On the reefs of Bonaire, it is possible that there is competition for space between native species and *T. coccinea*.

The purpose of this study was to determine the potential of *T. coccinea* to settle and grow among the native, sessile organisms (e.g., coral and algae) of Bonaire that appeared to be potential competitors in hard-substrate marine habitats. Creed and De Paula (2007) found that *T. coccinea* is not very selective to substrata and could ably recruit to all materials. A challenge was to figure out the ecological mechanisms that keep some invaders (*T. coccinea*) locally rare in their introduced range (Ruesink 2007). This study has attempted to elucidate why there appeared to be “hotspots” of *T. coccinea* recruitment on Bonaire’s reefs, as well as contributing to the...
growing body of knowledge concerning successful marine invasions on hard substrata.

I hypothesized that the higher the native species diversity the greater the horizontal biotic resistance, thus reducing the invasion success of *T. coccinea*. The following questions were addressed: 1) Is the absence of *T. coccinea* the result of biotic resistance (i.e. competition) by native species? 2) Which native species, or combination of species, are most effective at limiting the recruitment and growth of *T. coccinea*? 3) Does the presence of adult *T. coccinea* conspecifics increase the successful settlement or growth of conspecific recruits?

**Materials and Methods**

This study was conducted using SCUBA at the Harbor Village Jetty (N 12º 16’ 27.6”, W 068º 28’ 54.0”), just north of the dive site “Something Special,” on the western shoreline of Kralendijk, Bonaire, Netherlands Antilles. This site was chosen because it has the appropriate hard, vertical substrata for native species and the invasive coral to grow. Along the jetty, *T. coccinea* distribution was variable, with areas of up to 80% cover m⁻² to those where the coral was completely absent.

In order to assess the ability of native species to resist the successful settlement and growth of *T. coccinea* through competitive interactions, I haphazardly chose 15 x 15 cm plots on the vertical substrata and established replicated communities (0 spp. n=3, 1 spp. n=3, 2 spp. n=3, 3 spp. n=2, 3 spp. + *T. coccinea* n=2) of 0-3 native species and all combinations thereof at 0.5-5 m depth. These treatments were naturally set-up at the southern face of the jetty where the desired single species or combination of species already existed. I identified the following organisms (using the Humann and Deloach Reef creature and Reef coral identification guides 2003a; 2003b), chosen for this study: the coral *Porites astreoides*, an algal turf and the hydroid *Halocordyle disticha*, which already existed at high densities at the jetty and appeared to be potential competitors with *T. coccinea*. Physical manipulation occurred for 14 of the 25 treatments where I had to remove certain organisms to establish the desired combination.

Furthermore, two adult conspecifics of *T. coccinea* were haphazardly removed and seeded onto the 3-species treatments (n=2) using an epoxy (z-spar) that was prepared and mixed within 30 minutes prior to application underwater. These conspecifics comprised an area about 8% of a 15 x 15 cm quadrat and were used to determine if recruitment to the adult corals would be an important factor affecting settlement and survivorship. Nails and string were used as additional support while the epoxy hardened. Concrete nails were hammered into the top-left corner of the plots and colored cable ties were used as labels for each treatment and as permanent identifiers for the replicate number and for each of the organisms.

For a period of 3 weeks (October 29 to November 12, 2008), I monitored the plots and recorded percent composition of all species present, including any recruitment or growth of *T. coccinea* where applicable. Any of the changes in community composition was acquired by placing the top-left corner of a 15 x 15 cm PVC quadrat onto the permanent nail. Percent cover for all species observed, including ones not found initially, was recorded each Wednesday. The quadrat was subdivided into 25 squares, each square representing 4% cover, which allowed for rapid assessment of each species within the defined space. Bare substrate, which had developed over time as a result of die-off of certain organisms, was also accounted for as it had possible implications for interactions among the different organisms. An analysis of variance (ANOVA) was run on 1 and 2-species plots to test for differences among the treatments single species or combinations.

In addition to the experimental study directly assessing biotic resistance, I also attempted an observational assessment on post-settlement community composition and dynamics by running transects along the jetty. I collected data on the general distribution and abundance of all sessile species present to identify native species that may have an effect on the recruitment and growth of the invasive coral. A total of 15 vertical, 5 m transects were monitored. The first transect was set up nearest to shore and all subsequent transects placed 1 m apart with a progression away from shore.

I selected 5, non-overlapping points, between 0 and 475 cm (inclusive), randomly for the length of each transect, where 0 cm was the highest point and 500 cm the lowest point. A 25 x 25 cm PVC quadrat, subdivided into 25 squares, was used to assess percent cover of all the organisms within the defined area; organisms were later identified using the Reef identification guides by Humann and Deloach (2003a; 2003b). I calculated the mean of means for each identified species’ percent cover by averaging the percent cover in each of the 5 quadrats and then averaging the mean from all 15 transects. Similarly, the mean of means was calculated only for quadrats that contained *T. coccinea* to see if there was any evidence of interactions or trends between the 2 scenarios. An unpaired t-test was run to determine whether there was a significant difference between the 2 scenarios.
Results

Over the duration of the study, *T. coccinea* was never observed to settle or grow in any of the experimental plots, including the treatments seeded with an adult *T. coccinea* conspecific. The conspecific colonies themselves did not appear to increase in size over the period of 3 weeks. However I did detect a change in percent cover in the algal turf in my experimental plots over time; an ANOVA test revealed a significant difference between the different treatments (Figures 1a-c). However I did detect a change in percent cover in the algal turf in my experimental plots over time; an ANOVA test revealed a significant difference between the different treatments (Figures 1a-c). In addition there was a notable trend in the change of algal turf cover, depending on the type of treatment. In plots containing 0-1 species, the percent cover of algal turf either increased sharply, from 0% up to 65% and 100% or had a gradual decrease from 100% to 80% (Figure 1a). The 2-species treatments showed a similar trend where percent cover remained consistent around 60% and 90% or increased from 0-40% (Figure 1b). The 3-species plots exhibited the most drastic decreases in turf cover, particularly in treatments seeded with the invasive coral, where it dropped from 60% to 10% by week 3 (Figure 1c).

The observational component of the study revealed a visible inverse relationship between *T. coccinea* and algal turf. At the jetty, algal turf was the most abundant organism with the highest average mean coverage over the substrate, exceeding 60%, while all other organisms did not have percent cover exceeding 6%. *T. coccinea* represented 2% (Figure 2). Only 11% of the surveyed quadrats (25 x 25 cm) contained *T. coccinea*. In these quadrats, the mean percent cover of *T. coccinea* comparatively increased to 12% while the algal turf decreased to 45%. Additionally, the mean percent cover of bare substrate also increased from 17-24% (Figures 2 and 3). All other organisms represented did not reveal any noticeable increases or decreases in cover. An unpaired t-test with results comparing percent cover of the algal turf in the 2 scenarios showed a significant difference (p = 0.011).

Discussion

Biotic resistance of a community determines its ability to prevent invasion success (Stachowicz et al. 1999). The influential mechanisms affecting resistance remains an on-going debate, but certain studies have identified some of the possible factors. Stachowicz et al. (1999; 2002) suggested that species diversity, at least on a small-scale, is proportional to the resistance of a particular community or simply to resource availability. Furthermore, as *T. coccinea* is typically seen in high densities (up to 80% m^-2_), it is quite possible that the presence of adult species could encourage recruitment and settlement. As a result,
competition, species diversity and the presence of adult conspecifics were all considered as possible factors involved in an environment that is limited in space and addressed in my hypothesis. The results of this study were not able to effectively answer the questions I had set out to address. Through the duration of my study, no settlement or growth of *T. coccinea* was ever observed, even in treatments seeded with the adult conspecifics.

Although I was unable to draw any definite conclusions pertaining to the invasion dynamics of *T. coccinea*, the results of the experimental work suggest that some form of post-settlement interaction, physical or chemical, may exist between native and non-native organisms, as evidenced from the experimental and observational data. In plots that contained 0 and 1-species, percent cover of the algal turf generally showed an upward trend over time or remained consistent throughout (Figure 1a). A similar pattern could be seen in 2-species treatments, but for the 3-species treatments, particularly those seeded with *T. coccinea*, the percent of algal turf decreased by 30-50 (Figures 1b and 1c). Whether or not these suggested interactions may have any effect on biotic resistance or the invisibility by *T. coccinea* in the longer term will require continued monitoring of my plots at the jetty.

The observational study showed an inverse relationship between *T. coccinea* with algal turf. On average, the mean percent cover of algal turf was 62% over the entire jetty while all other species occupied less than 6% cover. In quadrats that contained *T. coccinea*, the turf saw a decrease from 62-45% while the invasive coral increased from 2-12%. This suggests possible competitive interaction between *T. coccinea* and the algal turf (Figures 2 and 3). These interactions could involve defenses that are physical, where the invasive coral is literally overgrowing the algal turf and vice versa, or chemical, through allelopathy. It may also be possible that a higher amount of bare substrata and a low amount of competition may promote invasion. Furthermore, characterization of the defenses of the native species as well as their threshold for invasion are imperative (Hollebone and Hay 2007). In marine systems, research on the invasibility by exotic species is still in its infancy and potential factors determining susceptibility of a community are still debated (Stachowicz et al. 1999; Duffy and Stachowicz 2005). It is unclear if invasion barriers are the result of dispersal limitation, initial colonization, factors limiting growth or survival of established individuals. Even upon successful settlement additional factors may contribute to the invasion window, including the physical environment or species interactions (Dethier and Hacker 2005).

Some of these possible factors were not addressed in this study, but should not be ignored. Predation is a biotic factor that could potentially determine the success of an invasion. Sometimes predation pressures can favor introduced species, at least initially, where native predators prey only on native species, presumably eliminating them from competition for space (Byers 2002). Comparatively abiotic conditions (e.g., salinity, water chemistry, temperature, nutrient runoff, natural disasters, etc.) are less well-studied, yet they can affect the outcome of an invasion in communities by placing stress on either the native species or the invading ones (Gerhardt and Collinge 2007). A continuation of this study should consider these additional factors and...
also attempt to determine the main drivers behind invasion success.

Results from this experiment suggest possible physical interactions, among native and non-native species, such as competition for space. It remains to be determined the mechanisms driving competition and, potentially, biotic resistance in hard substrate communities of Bonaire. It also remains to be determined if T. coccinea recruits depend on the presence of adult conspecifics or whether high densities of the coral could amplify invasion success. Perhaps given enough time, successful settlement or growth by T. coccinea will occur. A continuation of this study might provide more insight about the ecology and mechanisms that affect biotic resistance and contribute to the current body of knowledge pertaining to invasions in benthic, marine communities.

Acknowledgements

This study was made possible by the CIEE Bonaire research program and could not have happened without the help of Rita Peachey for providing all the necessary equipment and advice. Thanks also to Caren Eckrich for coordinating the dives. I would like to thank my advisor, Amanda Hollebone, for her supervision and support through the duration of this study. Special thanks to Christopher Savage for the use of his camera and to him and Christina Wickman for taking my fieldwork photos. I am also grateful to Amanda Parra and Lauren Pacheco for providing transportation and needed supplies. Lastly I want to express my thanks to BNMP and Ramon de Leon for allowing me to work on and manipulate the location of the invasive coral.

References


Gross anatomical findings from the fall 2008 Bonaire moray eel mortalities

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Abstract
Unexplained moray eel mortalities have been occurring in Bonaire since July 2008. The number of mortalities increased over time, peaking in October and sharply declining in November. Eel die-offs have occurred in Bonaire in the past and there are reports of eel die-offs in the wild populations but no causative agent has been identified. Mass eel die-offs in aquaculture facilities have been recorded for decades. Only within the past three decades has a causative agent been isolated and studied. This pathogen, *Vibrio vulnificus* serovar E, is considered primarily an eel pathogen but has been reported in several cases to infect humans. At the CIEE Research Facility, necropsies have been performed on twenty-three eel specimens. The eels have had several gross and microscopic abnormalities in common. For example, all had a hemorrhagic gastrointestinal tract and no outward signs of disease. Various parasites were noted in the swim bladder and GI tract. In many of the specimens, the liver was pale in color and the swim bladder contained a thick viscous material. Based upon these observations and clinical signs of *V. vulnificus* infection in eels, it is hypothesized that this bacterium is likely the causative agent responsible for the mass die-off of moray eels. However, diagnosis is dependent on testing at a laboratory using genetic or microbial techniques.

Introduction
Mass die-offs of organisms, both terrestrial and aquatic, have been documented for centuries. In the summer of 1997, there was a mass die-off of the short-tailed shearwater bird (*Puffinus tenuirostris*) in the Bering Sea that was eventually linked to scarce food sources (Baduini et al. 2001). During the summer of 1999, there was also a mass mortality event that occurred to gorgonians in the Northwestern Mediterranean Sea. Researchers eventually found that above average water temperatures had led to a rise in opportunistic infection (Cerrano et al. 2000). One of the more recent and notable mass mortality events that occurred in the Caribbean was that of the long-spined sea urchin (*Diadema antillarum*). The event began in January 1983 near the Panama Canal. The mass die-off followed the movement of the ocean currents, with the epidemic covering the entire Caribbean and some of the tropical West Atlantic by February of 1984. It is estimated that ninety-three percent of the entire *D. antillarum* population died. No other Caribbean echinoid was affected, which suggests that the pathogen was host specific. However, the causative agent has yet to be identified and the *D. antillarum* population has yet to fully recover more than twenty years later (Lessios 1988).

Mass mortalities result in bottleneck populations. A specific population maintains roughly the same size with minor fluctuations. When a bottleneck event occurs, it results in a significant loss of a population (Lessios 1988). The population may reach a critical point at which either recovery or extinction can occur. If there are enough members of the population remaining to reproduce successfully, recovery is possible. However, genetic diversity is greatly decreased, leaving the population even more susceptible to future disease and environmental stresses. The quick die-off of a key species in the reef ecosystem also results in many changes. Algae are the main item in *D. antillarum*’s diet. The mass reduction in the population resulted in excessive algae growth, which in turn smothered some corals. The loss of just one key species affected the overall community structure through the changes in resource availability (Lessios 1988).

Viruses, parasites, and bacteria have all been linked as causative agents in eel mortalities. Mass die-offs in cultured eels have been reported since the early 1970’s. The causative agent was initially identified as a gram-negative bacterium belonging to the genus *Vibrio* (Biosca et al. 1991). In 1982, the pathogen was identified to the species level as *Vibrio vulnificus*. Nine years later, the bacterium was isolated from diseased European eels for the first time. It was found to be a slightly different variant of a bacterium that usually infects people and was classified as biotype 2 (Biosca et al. 1991). Biotype 2, today reclassified as serovar E, is a primary pathogen for eels. It has been documented in farmed populations but its incidence in wild eels is unknown (Marco-Noales et al. 2001).

Unexplained eel mortalities have been occurring in the waters surrounding Bonaire, Netherlands Antilles, since July 2008 with no significant increase or decrease in death rates for other marine organism...
(K. Kozak, personal observation). In the case of *D. antillarum*, the causative agent has never been isolated and the population has yet to recover (Lessios 1988). Determining the causative agent of the eel mortalities could potentially prevent a cascade of events that could result in the community dynamics and resource availability changing on the reef.

**Materials and Methods**

**Location and Spread**

The eel mortality event occurred along the leeward side of the island of Bonaire, located in the Netherland Antilles (Figure 1). Eels have a cryptic life style during daylight hours. The fringing reefs of Bonaire offer abundant places for eels to hide, which is why a larger number of eels are found here. Reports of dead eels were collected by J. Ligon and shared on a Bonairian website. Divers gave reports that included the species of eel, location, and date. Divers also collected specimens and brought them to the field lab at the CIEE Research Station Bonaire.

**Necropsy – External Examination**

During the height of the eel deaths, necropsies were performed on specimens provided to the CIEE Research Facility. Specimens were placed on ice until they could be necropsied. Eels were examined externally for abnormalities or pre-mortem injuries, measured, and photographed.

**Necropsy – Internal Examination**

Specimens were placed into lateral recumbency and an incision was made along the ventral midline starting at the anus and ending ventral to the gills. Once through the epidermis, surgical scissors were used to finish separating the muscle tissue, taking care not to puncture internal organs (Figure 2). An initial overview of the organs was done, making note of abnormalities. The viscera was then separated from the peritoneal lining using small surgical scissors. The final cut was made proximal to the esophagus and distal to the lower intestines (Figure 3) to remove the viscera from the body cavity (Meyers 2006).

The heart, spleen, kidney tissue, liver tissue, intestines, swim bladder, and gallbladder were all externally examined for gross abnormalities. The gastrointestinal tract was cut open to inspect the contents. Samples of the gut material were collected and examined using a light microscope. Parasites found within the gut material were photographed for record. The stomach and intestinal walls were also examined and photographed. The swim bladder was opened and the contents were studied using a light microscope.

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**Figure 1.** Map of the spread of eel mortalities on Bonaire. The green line represents the area where the eels were found during the month of August (3 eels). The red line represents the area where the eels were found during the month of September (22 eels). The blue line represents the area where the eels were found during the month of October (48 eels).

**Figure 2.** The author making the initial incision along the ventral midline of a green moray that was brought to the lab on October 4, 2008.

**Figure 3.** The author cutting open the gastrointestinal tract of a spotted moray that was brought to the lab on October 1, 2008.
Local divers made reports on the behavior of infected eels. Photographs and video recordings documenting the behavior of sick eels were donated to CIEE. Reports, photographs, and videos were used to describe the typical behavior of sick eels to aid in diagnosis.

Results

Location and Spread

The first eels collected during August were found on the northern end of the island at Ol’ Blue and Karpata. During September the eel specimens were collected as far north as 1000 Steps and as far south as Tori’s Reef. As the death toll rose, the spread of collected specimens decreased and became more localized around Kralendijk. Specifically, during the month of October, the majority of eel specimens were found near Something Special.

Reports of dead moray eels on Bonaire began in late July 2008. In the subsequent months, the death toll increased dramatically. During August, only three eel deaths were reported (Figure 4). In September, the number of dead eels reported increased to 22 eels, doubling in October to over 48 reported eel deaths (J. Ligon, unpublished data). The majority of eel species reported dead were the spotted moray eel, Gymnothorax moringa, with a total of 57 mortalities. However, reported deaths also included 9 green moray eels (Gymnothorax funebris), 5 viper moray eels (Enchelynassa formosa), 2 purplemouth moray eels (Gymnothorax vicinus), 1 chain moray eel (Echidna catenata), 1 golden tail moray eel (Gymnothorax miliaris), and a report of 2 sharptail eels (Myrichthys breviceps). The deaths markedly decreased in November with only three eels being reported. As for other marine organisms, such as cartilaginous fish and invertebrates, there was no marked increase or decrease in fatality reports, pointing to a causative agent that is strictly pathogenic to eels.

Necropsy – External Examination

Twenty-three eel specimens were dissected between September and October 2008 for this study. Externally, there were no signs of disease but several specimens did have external puncture wounds. The eels appeared to have died quickly because there were no outward signs of deterioration or starvation.
Necropsy – Internal Examination

The inner lining of the gastrointestinal tract was hemorrhaged in all specimens (Figures 5a-c) amount and degree to which the intestines were hemorrhaged varied from specimen to specimen. Several of the more severe cases of hemorrhaged gastrointestinal tracts also had petechiae (minor hemorrhaging of blood vessels) present in the tissue lining the body cavity (Figure 6). Another observation was that none of the twenty-three eels had food material present in their stomach. The intestines were either filled with liquid fecal matter or mucus (Figure 5c). The liver of many eels was also abnormally pale (Figure 7) and in some eels the presence of bloody fluid in the abdominal cavity was noted (Figure 8). Nine of the twenty-three eels dissected had the presence of a viscous material in the swim bladder (Figure 9). Lastly, a variety of parasites were found in a number of the eel specimens (Figure 10), including eggs, cysts, and nematodes.

Behavior

Several divers gave accounts of the behavior of the sick eels. There were reports of eels, which are normally cryptic during daylight hours, being out in open areas. Eels were seen biting their abdomen (Figure 11), to the point in which a few specimens had self-inflicted abdominal punctures (K. Kozak, personal observation). Seizing in the water column was also observed, while some eels were seen lying upon their back, lethargically swaying with the current. Healthy eels stay hidden under rocks and coral during the day, coming out to hunt in the evening and at night (K. Kozak, personal observation).

Discussion

Based upon the data collected on eel mortalities, it appears that the pathogen was introduced in July 2008. There were no eel deaths reported on Curacao, which is only thirty-five miles away (E. Newton, personal communication). The occurrence of Hurricane Omar during the middle of the eel mortalities caused a massive flush out of the water surrounding Bonaire. If poor water quality were the cause, then a rapid end to the eel mortalities would be expected. Soon following Hurricane Omar, there was a spike in the number of eel mortalities reported (Figure 4). The number of eel deaths reported increased ten-fold in September, then doubled during the month of October, exhibiting a high capacity for this pathogen to spread.

The number of reported mortalities sharply decreased during the month of November (J. Ligon, unpublished data). *V. vulnificus* is a bacterium that prefers high temperatures and low salinity (Biosca et al. 1991). The sharp decrease in the mortality rate could be due to decreasing temperatures as a result of
seasonal change. Another factor is that the present eel population has sharply decreased. A decrease, overall, in population size also results in a decrease in the number of susceptible individuals that can become infected.

The gross abnormalities found in the twenty-three eel specimens concur with the pathophysiology of vibriosis caused by *V. vulnificus*. Vibriosis is caused by endotoxins produced by the bacterium. One of these endotoxins has been implicated in the death of peritoneal cells (Amaro et al. 1997). The bacterium causes a relatively rapid death, with mortalities occurring within 4 to 48 hours after exposure (Biosca and Amaro 1996). The bacterium grows and divides relatively rapidly following infection (Biosca et al. 1996). All of the specimens presented for dissection had an overall healthy appearance, giving rise to the likelihood of a quick and rapid death. In a simulated “natural outbreak”, infected eels showed external hemorrhages near the head and ventral parts of the body. This could be due to less optimal conditions for eels in the lab. Internally, the main signs were hemorrhagic intestines and pale livers (Marco-Noales 2001). In addition, vibriosis results in hemorrhagic septicemia. Physical signs of hemorrhagic septicemia include hemorrhages in the peritoneum, body wall, and viscera (Kahn 2005).

One of the first studies done on *V. vulnificus* in eels, researchers found inflammation of internal tissues and the intestines, a pale hemorrhagic liver, and fluid within the abdominal cavity (Biosca et al. 1991). Predisposing factors for the bacterium are nutritional deficiencies, traumatic injuries, parasitism, and sharp seasonal temperature changes (Kahn 2005). Notably, many of the dissected eels had some form of parasite present within them.

The behavior of the eels before death is supportive evidence that the causative agent is *V. vulnificus*. Serovar E infects eels, however, biotype 1 of this same bacteria infects people. The symptoms of infection include watery diarrhea, weakness, and abdominal pain (Beers 2006a; Beers 2006b). Divers reported sick eels were attacking their own abdomen (Figure 11). One of the first clinical features observed in eels purposefully infected with *V. vulnificus* are behavioral changes.

The changes noted included loss of activity and eels found resting on the ocean bottom (Biosca et al. 1991), which was also reported by divers.

**Conclusion**

There have been several reports of farmed eel populations dying due to the bacterium *V. vulnificus*, but the incidence of the bacterium in wild populations is unknown (Marco-Noales et al. 2001). The gross abnormalities found during the necropsies of the twenty-three eels on Bonaire concur with the pathophysiology of vibriosis caused by the bacterium *V. vulnificus* reported in the literature. The observed behavior of sick eels also concurs with previous studies on infected individuals. There were no notable die-offs of other fish species during the episode. However, a few specimens of other reef species were brought to the lab but did not exhibit the same gross abnormalities. An alternate pathogenic agent, such as a virus, could be the cause of the die-off. Analyses of eel tissue or blood samples are...
necesary to reveal the pathogen, assuming it is caused by something currently described in the literature.

**Acknowledgements**

I would like to first thank CIEE Research Station for allowing me to have this opportunity and for providing me with the necessary materials to complete this study. I want to thank my advisor Rita Peachey for guiding, advising, and supporting me on this project and to my professors, Caren Eckrich and Amanda Hollebone, for also guiding me with this study. Thanks to my parents and brother for always supporting me in all my endeavors as well as my co-workers at Queen City Animal Hospital for being great teachers and supporters. I also want to thank the local divers, Albert Bianculli, Yellow Submarine Dive Shop, and my classmates for collecting all of the eel specimens. Ramon de Leon and BNMP for allowing the eel specimens to be removed from the park for necropsy and Jerry Ligon for providing data on the eel deaths. Lastly, I want to thank Dawn Miller, David Tilzer, and Jeanne Chin for providing video and photographs of the sick eels.

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Invading is not always bad: A study of positive interactions between the invasive coral *Tubastraea coccinea* and native reef species of Bonaire, NA

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Abstract

The orange cup coral, *Tubastraea coccinea*, was introduced into the Caribbean in the 1930s from the Indo-Pacific. Since then, it has spread throughout the Caribbean and into the near-shore reefs of Bonaire. In this study, I assessed the interaction of this exotic coral with the native reef community. I hypothesized that the three-dimensional structure of *T. coccinea* facilitates native species among which it successfully grows by providing habitat and food. To investigate this, colonies of *T. coccinea* were visually monitored in the field over several the morning (8:00), noon (12:00) and evening (18:00) sessions to capture how native species interact with and use the coral in a natural setting. Colonies of *T. coccinea* were also collected, defaunated, and experimentally caged-off so that consumers would not be able to graze the biofilm and/or algae growth on the colonies. Percent cover of cyanobacteria and macroalgae growing on the corals was monitored over the duration of the study. Species richness within open and closed cages was also measured to assess which native species utilized the habitat as well as biofilm and algae. Cyanobacterial percent cover changed significantly over the duration on the study (increasing to 18% and decreasing to 3% in closed and increasing to 10% and decreasing to 1% in open cages ) as well as differed between closed and open cages (18% versus 9% at highest percent cover, respectively). The percent cover of macroalgae in closed cages was significantly higher than in partially closed cages (45% versus 25%, respectively) from day ten to the completion of the study. This was likely due to the exclusion of herbivorous fishes in the closed cages. Native species richness within both cage treatments increased throughout the duration of the experiment, but showed a four-fold increase between day 5 and 10 within closed cages versus a leveling-out in open cages. Native fishes and annelids were observed in both the natural and experimental settings utilizing *T. coccinea* as both a habitat and a food source. These interactions of native species with *T. coccinea* suggest that the coral is positively interacting with the ecosystem in which it has successfully invade settled in and has become a facilitator of native species.

Introduction

Invasive species have historically been prevented from spreading to different regions by natural barriers (Carlton and Ruiz 2005). However, in the last several hundred years, human activity and an increase in global travel has contributed to the introduction of exotic species to new regions at a rate that exceeds that which has been documented in the past (Carlton and Ruiz 2005). Invasive species are often perceived as negative interactors in their new range (Bruno et al. 2005). For instance, it has been demonstrated that the non-native brown tree snake (*Boiga irregularis*) consumes and decimates populations of endemic birds on Guam (Savidge 1987), the zebra mussel (*Dreissena polymorpha*) in North American freshwater lakes and streams has virtually eliminated native unionids (Strayer 1999), and the mass mortality of Australia’s freshwater crocodiles (*Crocodylus johnstoni*) is due to the intentional introduction of toxic cane toads (*Bufo marinus*) as a biocontrol agent (Letnic et al. 2008).

Surprisingly though, only a small number (1-10%) of successful invasions have actually had negative effects on native communities (Lodge 1993). Instead, many non-native species have positive effects on native communities. One type of positive interaction between non-native and native species is facilitation. This is where one organism makes a shared environment more favorable to another organism by decreasing environmental and/or biological stress (Bruno et al. 2005). The presence of a facilitator can increase the diversity of native and non-native species by providing resources such as food and/or protection (Stachowicz and Byrnes 2006). For example, the invasive mud snail (*Batillaria attramentaria*) increased the number of native species around it by creating a hard substrate (its shell) on mudflats (Wonham et al. 2005), and *Potamopyrgus antipodarum*, an invasive freshwater snail, facilitated native invertebrates and increased species richness in its expanded range possibly due to coprophagy (Schreiber et al. 2002).

In the near-shore reef habitats and man-made structures (e.g., docks) of Bonaire, Netherlands Antilles, the azooxanthellate, scleractinian orange cup coral, *Tubastrea coccinea* has successfully invaded from the Indo-Pacific since its introduction during the 1930s (Cairns 1999). Currently, there are no known studies addressing the interactions of *T.
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*coccinea* with native species in the Caribbean basin. Upon preliminary analysis of *T. coccinea* colonies in Bonaire, the coral appeared to facilitate the feeding habits of and provide habitat for several native vertebrate (e.g., blennies, damselfish, etc.) and invertebrate species (e.g., crabs, snails, hydroids). These observations suggest that *T. coccinea* may be facilitating the availability of habitat and food resources for native reef organisms.

In order to address this hypothesis, I asked the following questions: 1) Is *T. coccinea* creating a food source or surface upon which food grows? 2) What organisms utilize *T. coccinea* for food? 3) Are the three-dimensional structures of *T. coccinea* colonies providing a habitat for native species? 4) If *T. coccinea* is providing three-dimensional habitat, what species are utilizing it for protection? This is a novel study because minimal attention has been paid to facilitatory impacts of exotic species on native species in marine systems (although they are as common and just as important as predation and competition) (Bruno et al. 2005), much less the positive effects of *T. coccinea* in reef communities of Bonaire, NA.

Materials and Methods

In order to investigate the effects of *T. coccinea* on the community dynamics of reefs in Bonaire, both observational and experimental approaches were taken. The identification of native species utilizing *T. coccinea* colonies as a source of food and/or habitat was accomplished by observing three haphazardly chosen sites where there were at least four colonies of *T. coccinea* growing on the Harbor Village southern jetty, Kralendijk, Bonaire (N 12º 16’ 27.6”, W 068º 28’ 54.0”). These sites were twice visually observed and video recorded during the morning (8:00), noon (12:00) and at dusk (18:00) (dusk was chosen rather then night observations to avoid nocturnal species with flashlights) using SCUBA at a distance of three meters for twenty minutes. By performing these observations (fish species, invertebrate species and behavior), I was able to document the interaction *T. coccinea* has with native reef species in a more natural setting and thus further compare interactions to those noted during in the experimental portion of the study.

To confirm the potential for positive interactions between *Tubastraea coccinea* and native reef species, I established an experimental caging study in which *T. coccinea* was protected in full cages and exposed in open cages to consumers. I first haphazardly collected 24 colonies of *T. coccinea* (all of comparable size, ~10 cm in diameter) from the Harbor Village southern jetty. The corals were chiseled from the substrate and then transported with water in mesh bags or plastic tubs to the sand flat under the Dive Friends @ Yellow Sub dock, Kralendijk, Bonaire (N 12º 16’ 01.6”, W 068º 28’ 19.4”) where I cemented the colonies to coral rubble using Z-Spar epoxy (Z *Spar, Los Angeles, CA). While submerged, the epoxy was allowed to set over night. All algae and animals were manually removed from around each colony to ensure that all colonies were similar upon the initiation of the experiment.

Twelve of the experimental coral colonies were fully enclosed in 20 cm x 20 cm x 20 cm cages constructed out of 1.44 cm² wire mesh and twelve were enclosed in cage controls constructed in the same fashion without the top side. These cage controls allowed organisms to continue to utilize *T. coccinea* colonies but controlled for any caging artifacts such as changes in hydrodynamics or shading (Summerson and Peterson 1984). These control cages were physically paired with each full cage (~10 cm apart) and secured to the substrate under the protective cover of the Dive Friends @ Yellow Sub dock using two 25 cm long bent rebar stakes (1.5 cm) that were hammered into place. Each pair of cages was placed roughly one meter away from the adjacent pair and treatment cages were uniformly placed in front of control cages (Figure 1). Over a period of 21 days, I periodically (days 3, 5, 17, 21) cleared the wire mesh of any debris and algal accumulation and monitored the percent cover of algae and cyanobacteria as well as the appearance of native species utilizing each habitat.

![Figure 1. Experimental set up under Dive Friends @ Yellow Sub dock (N. 12.16°, W. 068.28°). Treatment (closed) cages are represented as squares and control (open) cages are represented in diamonds. Each pair was separated ~1 m from adjoining pairs and within paired cages were ~10 cm apart.](image-url)
(1999) on reefs in the Florida Keys. A 25 cm chain with 10 randomly marked links was haphazardly placed on the experimental T. coccinea colonies. Any organism on substrate (e.g., coral, algae, rock, cyanobacteria) under each painted point was recorded. This was performed twice for each coral and percent cover for each sample was then calculated by dividing the total counts for each organism on substrate by 20. Two-factor analysis of variance tests (ANOVA) were performed for the percent cover of cyanobacteria and macroalgae, the two most prominent occupiers of space over time.

While collecting percent cover data, the number and identification of species utilizing the colonies of T. coccinea were also recorded. During each monitoring period, I first made a quick pass over all the cages to note those species (e.g., fish) that would quickly leave the experimental units if disturbed. Following these initial observations of fast-moving species, I closely observed and identified slower moving and sessile species (e.g., crabs, snails, hydroids, sabellid worms). The species data was then compiled for open and closed cages and species richness was compared.

Results

The percent cover of cyanobacteria fluctuated over the course of the study; increasing from the initiation of the study and decreasing from day 10 to 21 (Figure 2). Treatment and time had a significant impact on the growth of cyanobacteria ($p=0.001$ and $p<0.001$, respectively) with cyanobacterial growth in closed cages consistently higher than in open cages. However, the interaction of treatment and time did not have a significant effect on overall cyanobacteria growth ($p=0.537$).

Overall percent cover of macroalgae (Rhodophyta and Phaeophyta) on colonies of T. coccinea increased in both treatment and control cages. From the initiation of the study to day 10, algal percent cover increased from 0 to ~15% in both caged and open units, but from day 10-21 percent cover rapidly increased in fully caged units from ~15% to ~45% and stabilized in the controls at ~25% algae cover (Figure 3). Time ($p=0.02$), treatment ($p<0.001$) and the interaction of treatment and time ($p=0.001$) all significantly impacted percent macroalgal cover.

While collecting percent cover data, an increase in species richness was also observed. From the initiation of the experiment until day 5, the number of native species observed in and around both the treatment and control cages were comparable (1-3 species, Figure 4). Between days 5 and 10, there was a 4-fold increase in species richness in the fully-closed cages while control cages were visited by only 3-5 native species (e.g., Malacocentrus triangularis, Acanthurus bahianus and snails of the order Sorveoconcha)(Figure 4). Most organisms (e.g., the sharpnose puffer or Canthigaster rostrata, juvenile trumpetfish or Aulostomus maculatus, and bicolor damselfish or Stegastes partitus) observed in fully-closed cages appeared to be using T. coccinea as a habitat or using the cages themselves as refuge from predators (e.g., trumpetfish and tarpon (Megalops atlanticus)). Upon further investigation, two herbivores (snails of the order Sorbeoconcha and the ocean surgeonfish, A. bahianus) exclusively used the control cages beginning around day 15, when the percent-cover of macroalgae rapidly increased in full cages plots but remained at roughly 25% in open control cages.

The observations of coral colonies on the Harbor Village jetty revealed that several native species interacting with T. coccinea included three herbivorous fish (yellowtail damselfish or Microspathodon chrysurus, longfish damselfish or Stegastes diencaeus and redlip blenny or Ophioblennius macclurei) utilizing the corals as territory. These three fishes remained near particular colonies of T. coccinea and chased away other fish that came near them. Also, the saddled blenny (M. triangularis) and beaded fireworm (Hermodice carunculata) were both observed hanging out around the polyps of colonies at the Harbor Village southern jetty. Two other predators, the adult and juvenile smooth trunkfish (Lactophrys triqueter) as well as juvenile blue headed wrasse (Thalassoma bifasciatum) seemed to be utilizing T. coccinea for protection by hiding under coral colonies and where observed grazing around colonies.

Discussion
The combination of observational and experimental approaches in the study supported my original hypothesis that *T. coccinea* has a positive, faciliatory effect on growth of food resources (e.g., macroalgae) for native species. By removing all algae from experimental coral colonies and caging out potential consumers, I found a significant difference between macroalgal percent cover in fully caged versus open plots over time. In both full and open cages macroalgae percent cover similarly increased until day 10 (Figure 3). This may be explained by a lag period before which species started to utilize the caged areas, thus algal growth remained nearly identical for some time. In the absences of known herbivores (*M. chrysurus, S. diencaeus* and *O. macclurei*) living and feeding and the jetty algae grew uninhibited until new herbivores (*A. bahianus* and snails) moved in to the Yellow Sub dock site.

Fully caged protected plots showed a near-doubling of macroalgal percent cover from day 10 to day 21, whereas in open control plots, macroalgae increased by only 10%. In these open cages, the day 21, whereas in open control plots, macroalgae percent cover from day 10 to doubling of macroalgal percent cover from day 10 to day 21 may have been a result of new coral tissue growth. This decrease in cyanobacteria also correlated with the increase in macroalgal growth, suggesting that the algae were successively out-competing the cyanobacteria. Thacker et al. (2001) also found that total cyanobacterial cover and biomass was negatively correlated with total algal cover in coral reef ecosystem. The significant difference in cyanobacterial between fully-caged and open plots (ANOVA, p= 0.001) may have been a result of the presence of snails in control cages that can consume cyanobacteria

Invasive, habitat-forming species previously have been shown to increase native species richness in coral reef ecosystems (Bruno et al. 2005). This also appeared to be the case for *T. coccinea* in both observations of the coral on the Harbor Village southern jetty as well as during the monitoring of the experimental portion of this study. Juvenile smooth trunkfish (*L. triqueter*) as well as other juvenile fish (e.g., blue headed wrasse, *T. bifasciatum*) seemed to be utilizing *T. coccinea* for protection by hiding under coral colonies. Adult redlip blennies (*O. macclurei*), yellowtail damselfish (*M. chrysurus*) and longfin damselfish (*S. diencaeus*), all herbivorous and territorial (Deloach and Humann 1999), were consistently observed defending *T. coccinea* as either territory/habitat at the Harbor Village southern jetty. Under the Dive Friends @ Yellow Sub dock (sand flat substrate), the fully-caged and open colonies started without natives and increased to 10 and 5 native species, respectively, upon completion of the experiment (Figure 4).
This demonstrates that the presence of *T. coccinea* facilitated an increase in species richness. One possible explanation for higher numbers of native species around fully caged colonies of *T. coccinea* may be the extra protection the closed cages. Three known predators, the saddled benny (*M. triangulatus*), bearded fireworm (*H. carunculata*) (only observed at the jetty), and the bluehead wrasse (*T. bifasciatum*) were observed also utilizing both natural and experimental colonies as habitat. *M. triangulatus* and *L. triqueter* may be indirectly utilizing *T. coccinea* as a food source by consuming organisms such as small crustaceans (Deloach and Humann 1999) that are themselves eating the algae around the coral colonies. *L. triqueter* was observed consuming a non-algal item around colonies at the jetty, however, further observation of this interaction as well as assessment of smaller organisms living around colonies of *T. coccinea* may confirm this indirect positive interaction.

This study strongly suggests that the invasive coral, *T. coccinea* is directly and possibly indirectly facilitating the protection and feeding habits of native species in Bonaire. Numerous fish, crustaceans, gastropods and annelids are utilizing the three dimensional structure of this coral as a habitat and as a food source. These observed positive interaction between native species and exotic habitat building coral indicate that not all invasions are deleterious to the community they invade. This study is an example of how some invasive species can have positive interactions with native species and may, in fact, be beneficial to the invaded ecosystem.

**Acknowledgements**

I first would like to thank CIEE research station and staff for giving me the amazing opportunity of living, learning, and conducting my own research on Bonaire. I gratefully acknowledge Dive Friends @ Yellow Submarine for permission to use the space below the dock and the permission to manipulate *Tubastraea coccinea* from the Bonaire National Marine Park. I thank C. Chang, K. Kozak, A. Parra, C. Wickman, L. VanThiel, and A. Hollebone for their help in the field. I would also like to thank Dr. Amanda Hollebone for her enthusiasm, support, and advice on my study. Lastly, thanks to my parents for all of their support and for helping to fulfill my dream of studying abroad.

**References**


Is larval fish diversity connected to ecosystem level diversity?
A case study in Bonaire, Netherlands Antilles

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ABSTRACT
Greater ecosystem diversity generally results in greater fish diversity. It follows that areas of greater ecosystem diversity would also exhibit greater larval fish diversity during recruitment events. To test the idea that larval fish would be more diverse in areas with greater ecosystem diversity, two sites on Bonaire, Netherlands Antilles were selected for study based on the amount of ecosystem diversity in each area. At Lac Cai, on the windward side of Bonaire, there are fringing reefs, a small barrier reef, seagrass beds, sand flats, and a mangrove lined bay. On the leeward side of the island in front of Kralendijk, the habitat is predominately fringing reef and sand flats. Three collection methods (plankton tows, light traps, and dip netting) were used to test my hypothesis that larval fish diversity at Lac Cai will be greater than that in front of Kralendijk due to greater ecosystem level diversity at Lac Cai. Simpson’s index of diversity (1-D) for Lac Cai (range 0.827 to 0.829) was significantly higher (one-tailed t-test assuming equal variance, p = 0.007, α = 0.05) than the values at Kralendijk (range 0.615 to 0.664) indicating that higher ecosystem diversity does result in higher larval fish diversity.

Introduction
Eutrophication, overfishing, and destruction of fish habitats have increased the importance of understanding how marine environments are connected especially regarding larval transport and exchange (Levin 2006). Studies show that certain marine environments have higher densities of juvenile fish, but have not assessed the diversity of larval fish recruiting to those areas (Nagelkerken et al. 2000; 2002). A study on the importance of mangroves, seagrass beds, and shallow reefs as nursery grounds for important coral reef fish showed that nursery species from the families Haemulidae, Lutjanidae, and Scaridae had greater pooled densities when bay nursery habitats were present (Nagelkerken et al. 2002).

Coral reef fish have a biphasic life cycle. The presence of a larval stage may reduce competition for resources in the adult habitat, increase the distribution capability of the species, or avoid predation pressure in the adult habitat (Swearer et al. 2002). Larval fish disperse through the ocean by currents, tides, and wind in search of a desired habitat for settlement. For many fish species, ecosystems such as seagrass beds, mangroves, and shallow coral reef areas provide ideal nursery sites for larval fish (i.e. ample amount of food, structural complexity for shelter against predation, and reduced levels of predation ensures the probability that higher diversity of fish larvae will utilize these areas in an attempt to minimize mortality rates and maximize growth rates (Nagelkerken et al. 2000). The area that these important biotopes cover may be important in effectively attracting fish larvae to coral reefs (Parrish 1989). Although the relationship between diversity of fish recruiting to areas of high habitat diversity can be assumed by assessing juvenile fish densities in these locations, it is essential that studies are done on the types of larval fish recruiting to low versus high ecosystem diversity areas.

This study investigates the diversity of larval fish recruiting to two sites on Bonaire and the role that ecosystem diversity on larval recruitment. I hypothesize that there will be higher species diversity of larval fish due to the presence of higher ecosystem diversity at Lac Cai on the windward side of Bonaire. Fish larvae were sampled on the windward side of the island at Lac Cai and on the leeward side of the island in front of Kralendijk using 3 different larval collection methods. The use of 3 methods should minimize the biases of different collection methods on sampling fish larvae and maximize the number of species collected.

Materials and Methods
Study Site
Bonaire, Netherlands Antilles is located in the southern Caribbean (N 12°10', W 68°15') approximately 100 km north of Venezuela (De Meyer and MacRae 2006). Due to strong prevailing trade winds, the windward and leeward shorelines differ greatly in terms of wave exposure (De Meyer and MacRae 2006). There are also differences in ecosystem level diversity between the windward and leeward sides of the island.
Study sites were chosen based on differing ecosystem level diversity. The study site on the leeward side of Bonaire, in front of Kralendijk, is characterized by fringing reef, located about 40 m from shore, and sand flat communities (Figure 1a). The study site on the windward side of the island at Lac Cai is characterized by higher wave energy than that of the leeward side of the island. The site was chosen because of its close proximity to several important ecosystems including mangroves, seagrass beds and a small barrier reef which provide inshore habitats for juvenile fish (Figure 1b) (Rooker et al. 1995). The influx of larvae from the open-ocean passes through the channel, where sampling occurred, in order to reach other nursery habitats. If greater ecosystem diversity results in greater diversity of larval fish recruiting to the reef then I would expect there to be higher larval fish diversity on Lac Cai.

Larval Sampling

Lunar periodicity is known to affect larval recruitment (Robertson et al. 1988) with peaks occurring around the new moon. For this study, collections of larvae occurred a day before the new moon on September 28, 2008 and the day of the new moon on October 28, 2008. Plankton tows, dip nets, and light traps were used to collect larvae at the two sampling sites. Various techniques of larval collection were implemented to reduce sampling biases and obtain a more complete representation of larval fish diversity. Biases that have been reduced by having three different sampling techniques are: size selectivity, swimming ability of larvae,

Plankton Tows

Three 5-minute plankton tows were conducted starting at 19:30 h near the reef drop off at about a 15 m depth at the Kralendijk site and in the channel at about a 7 m depth at Lac Cai. A 75 cm diameter plankton tow net with 500 μm apertures was used to collect samples (Figure 2a). The length of towrope was 30 m. A flowmeter was deployed to measure the flow of seawater through the net opening. Volume of water sampled was calculated to determine larval fish density per each tow and GPS readings were taken at each plankton tow location. Plankton tow collections were washed out of the net using a wash bottle, then put into the collection bottle. Samples were fixed with 10% formalin and transferred to 70% ethanol before taxonomic identification using a dissecting microscope.

Light Traps

Light traps were deployed from shore at 19:30 h. The traps were positively buoyant and were tied to a line with an anchor in about 6-7 m of water. The line was adjusted so that the trap remained suspended in the water column at a depth of less than 1 m from the surface (Figure 2b). Deployment lasted for 2 hours. A 14 W, Ikelite C-Lite 8 was attached to the top of the light trap by a PVC adapter, chicken wire, and zip ties as the primary light source. A standard 500 μm mesh net with a collection bottle attached to the bottom of the trap was used to gather larvae. Samples
Data were fixed with 10% formalin and transferred to 70% ethanol before identification of larvae using a dissecting microscope.

**Dip Nets**

Dip netting occurred along the shoreline at each site in 1 m of water from 20:00 h to 21:00 h at 30 min. intervals. A light was used to attract fish larvae for a period of 10 min. before dip netting took place. Five scoops through the water using a dip net with a piece of small aperture netting sewed inside the net was one dip net sample (Figure 2c). The net was emptied into a 20 L bucket. The plankton was washed into the sample bottle, fixed with 10% formalin, and transferred to 70% ethanol before identification using microscopy.

**Data Analysis**

Fish larvae were extracted from the plankton samples, photographed, and separated into generic group types until fish families were determined (Victor 2008). Simpson’s index of diversity (*1-D*) was used to determine the amount of diversity at each location during each month:

\[ D = \Sigma (n / N)^2 \]

In the diversity formula, \( n \) = total number of organisms of a particular family, \( N \) = the total number of organisms of all families and \( D \) = probability that two individuals randomly selected from a sample will belong to the same species. The \( D \) value from the index is subtracted from 1, so that a higher value represents greater diversity. The Simpson’s index of diversity values were tested for a significant difference using a one-tailed t-test assuming equal variance with \( \alpha = 0.05 \). A one-tailed t-test was used because it is assumed that there is higher diversity at Lac Cai than at Kralendijk.

Abundance of fish per family for each sampling technique and fish densities were compared to determine overall effectiveness of the three sampling techniques. Fish densities were determined for each site by multiplying area of plankton tow opening by flowmeter readings to get volume of water sampled. Total fish abundance divided by volume of water per each plankton tow equals larval fish density (fish per m$^3$).

**Results**

A total of 624 larval fish were collected from both sites and 19 fish families were identified (Table 1). Fifty eight percent of the total larval fish were collected from Kralendijk and 14 families were identified. The light trap method collected 7.7% of the total larval fish from Kralendijk. Dip net method collected 8.5% and the plankton tow method collected 83.8% larval fish. Fish families, Carangidae, Lutjanidae, Callionymidae, Pleuronectiformes, Apogonidae, Surinamensis were only found at the Kralendijk site. Fifty six percent of larval fish abundance from Kralendijk were hatchlings and were unable to be identified. Three percent fish were unidentifiable (damaged while collecting) and 1.4% of fish could not be identified to family.

At Lac Cai, 41.7% of the total abundance of larval fish was collected and 11 fish families were indentified from the site. The light trap method collected 4.6%, the dip net method collected 23.8% and the plankton tow method collected 71.5% larval fish. Fish families, Syngnathidae (only *Hippocampus*), Sphyraenidae, Eleotridae, Labridae, Labrisomidae, Engraulidae were only found at Lac Cai. Four percent of the total amount collected from Cai were hatchlings, 0.8% were unidentifiable and 0.4% could not be identified to family.

**Diversity Index**

Lac Cai had diversity index values of 0.829 for month 1 and 0.827 for month 2, which showed greater diversity than Kralendijk (month 1 = 0.615, month 2 = 0.661). Index values from the two locations with varying levels of ecosystem diversity showed a significant difference in larval fish diversity (one-tailed t-test assuming equal variance, \( p = 0.007 \), Figure 3). Larval fish abundance and fish densities showed that the plankton tow method was the most effective technique used. It collected 76.8% of the total fish abundance and 18 families, whereas the dip net method only collected 16.8% of the total fish abundance and 11 families and the light trap method collected 6.4% of the total fish abundance and 5 families (Figures 4a-c). Kralendijk had a mean fish density of 0.142 fish/m$^3$ and Lac Cai had a mean fish density of 0.053 fish/m$^3$ (Table 1). Although higher densities of fish were collected at Kralendijk there was lower diversity.

**Figure 3.** Comparison of mean larval fish diversity (± SD) using Simpson’s index of diversity at two study sites with differing levels of ecosystem diversity. Study sites were chosen based on relative levels of ecosystem diversity (ED). Cai included a variety of different habitats (e.g., mangroves, seagrass beds, fringing reef), whereas Kralendijk was characterized by fringing reef and sand flat habitats. Means are significantly different (one-tailed T-test assuming equal variance, \( \alpha = 0.05, p = 0.007 \). Simpson Index of Diversity (1-D) values show higher amounts of diversity when values are close to 1 and lower diversity when values are close to 0.
Table 1. Types of larval fish families collected at study sites with different levels of ecosystem diversity. The abundance of fish in each family and each study site is determined.

<table>
<thead>
<tr>
<th>Fish Families</th>
<th>Common Name</th>
<th>Lac Cai</th>
<th>Kralendijk</th>
<th>Lac Cai</th>
<th>Kralendijk</th>
<th>Total # of fish in family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherinidae</td>
<td>Silverside</td>
<td>25</td>
<td>12</td>
<td>27</td>
<td>40</td>
<td>104</td>
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<tr>
<td>Clupeidae</td>
<td>Herrings</td>
<td>11</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Carangidae</td>
<td>Jacks</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hatchling</td>
<td></td>
<td>8</td>
<td>142</td>
<td>1</td>
<td>60</td>
<td>211</td>
</tr>
<tr>
<td>*Lutjanidae</td>
<td>Snappers, Seabass</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Gobies</td>
<td>1</td>
<td>36</td>
<td>17</td>
<td>6</td>
<td>60</td>
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<tr>
<td>Callionymidae</td>
<td>Dragonets</td>
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<td>0</td>
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<td>1</td>
</tr>
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<td>0</td>
<td>0</td>
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<td>1</td>
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<tr>
<td>*Scaridae</td>
<td>Parrotfish</td>
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<td>11</td>
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<td>6</td>
<td>35</td>
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<td>Gerreidae</td>
<td>Mojarras</td>
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<td>0</td>
<td>6</td>
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<td>17</td>
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<tr>
<td>*Syngnathidae</td>
<td>Pipefish, Sea Horse</td>
<td>31</td>
<td>0</td>
<td>25</td>
<td>1</td>
<td>57</td>
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<tr>
<td>Eleotridae</td>
<td>Sleeper Goby</td>
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<td>0</td>
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<tr>
<td>Labridae</td>
<td>Wrasses</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Labrisomidae</td>
<td>Blennies</td>
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<td>Gobiescocidae</td>
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<td>3</td>
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<td>Engraulidae</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Unknown 2</td>
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<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
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<tr>
<td>Pleuronectiformes</td>
<td>Flounders</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<td>3</td>
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<tr>
<td>Apogonidae</td>
<td>Cardinalfish</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lobotes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surinamensis</td>
<td>Atlantic Tripletail</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Albulidae</td>
<td>Bonefish</td>
<td>3</td>
<td>2</td>
<td>16</td>
<td>1</td>
<td>22</td>
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<tr>
<td>*Sphyraenidae</td>
<td>Barracuda</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Unidentifiable</td>
<td></td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total # of fish collected at each site</strong></td>
<td>153</td>
<td>239</td>
<td>107</td>
<td>125</td>
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</tr>
</tbody>
</table>

* Families associated with mangrove and seagrass bed habitats (Nagelkerken et al. 2002)
Discussion

Higher index of diversity values for Lac Cai support my hypothesis that species diversity is greater in locations that have higher ecosystem diversity. Areas such as Lac Cai that have high ecosystem diversity may attract higher larval fish diversity because there are more nursery ground habitats available for larval settlement (Parrish 1989). Nursery areas may also have reduced numbers of predators, which increases the chance of larval survival (Nagelkerken et al. 2000). Although fish larvae may not always be able to control their position in the water column, they do respond to auditory, visual, and chemical cues to detect specific habitat locations (Lecchini 2005).

The abundance of certain fish families found only at one site may infer that the types of larval fish recruiting to a specific habitat are not occurring by chance. Nagelkerken et al. (2002) showed that certain fish families (Haemulidae, Lujanidae, Acanthuridae, Chaetodontidae, Scaridae, Gerreidae, and Sphyraenidae) are associated with mangrove and seagrass habitats in the Caribbean. Results from this study show that the fish families, Syngnathidae (only Hippocampus), Sphyraenidae, Eleotridae, Labridae, Labrisomidae and Engraulidae may be associated with mangrove and seagrass areas as well. More replication of this study could provide more evidence on the types of fish larvae recruiting to specific habitats. One species that predominately showed high abundance at Lac Cai was the Syngnathid Hippocampus spp. (1st sample = 31, 2nd sample = 25). This finding was unexpected because very few sightings of sea horses occur in Bonaire.

Larval recruitment at the leeward study site showed evidence of smaller fish recruiting to this area, which has lower ecosystem diversity. A reduction in the size of larvae may correlate with the amount of predators present at the reef. Many studies maturity to reduce their size while passing through areas of high predation (Belk 1998). In fact, 55% of the samples collected at Kralendijk were hatchlings, which supports this idea.

When considering types of methods used for conducting larvae sampling, the plankton tow method worked most effectively. In areas of high and low fish densities, this method collected the most fish families and highest abundance of fish.
The dip netting and light trap methods collected larger larvae and post-larvae, whereas the plankton tow method collected smaller larvae and immature hatchlings. Using three types of sampling techniques maximized the collection of different larvae during recruitment.

The purpose of this study was to test the idea that greater levels of ecosystem diversity would result in greater larval fish diversity. Lac Cai, an area of high ecosystem diversity, showed higher diversity index values than Kralendijk, an area of low ecosystem diversity. An attempt to understanding how high levels of ecosystem diversity relates to higher levels of species diversity may result in humans focusing preservation measures on areas with high ecosystem diversity to ensure the optimum amount of biodiversity being preserved.

Acknowledgements

Thank you to my classmates of fall 2008 for taking the time to collect the great data at Kralendijk and Lac Cai. This project would have been impossible without you guys. Thank you to CIEE for project funding and supplies. Thank you to Rita Peachey, Amanda Hollebone and Albert Bianculli for helping with the plankton tows. Thank you to the CIEE interns, Kelsey Burns and Amy Milman for transportation and overseeing that the project was a success. Thank you to Yellow Submarine Dive Shop, STINAPA and Papy for boat usage. Thank you Caren Ekrich for providing Annie’s larval fish identification key. Thank you to Benjamin Victor for identifying all of the fish larvae to family. Thank you to Dan DeAnda for supplying the Dip nets. And lastly, thank you to Ramon de Leon for allowing us to conduct research in the BNMP.

References


Is #2 the number one problem in Bonaire?
An examination of fecal contamination and sedimentation from runoff

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Abstract
In this study I assessed the extent of sedimentation and contamination by human enteric bacteria Enterococci on the reefs of Bonaire, Netherlands Antilles as well as the relationship of these factors to the prevalence of coral disease and bleaching. Largely the effects of sedimentation and enteric bacteria from wastewater run-off in Bonaire have been relatively unknown. Because of the lack of wastewater treatment in Bonaire, runoff contamination by sewage and nutrient fluxes is common. Assessment sites for this study were chosen based on the intensity of nearby anthropogenic activity. These sites were defined as “More Impacted” (MI, n=2) and “Less Impacted” (LI, n=2). Water and sediment samples were acquired at 12 m on a weekly basis for the assessment of enterococcal concentration using the Enterolert™ fluorescing substrate system and determination of sediment particle size distributions. In addition, the frequency of coral disease and extent of coral bleaching were assessed using Coral Point Count software on data acquired along two 10 m video transect lines laid at 12m for each site over 4 weeks. Overall sediment particle size analysis yielded statistically significant differences between LI and MI particle size distributions, with more fine grained sediments at MI sites and more coarse grained sediments at LI sites. Finer grains suggest greater human impact. Enteric bacteria were found at several sites over time and their concentrations show a positive correlation between human presence and higher bacteria counts. Bleaching and disease did not show any correlation with sediment particle sizes or presence of enteric bacteria.

Introduction
Increasing prevalence of coral diseases and bleaching are currently two of the leading causes of reef mortality worldwide (Voss and Richardson 2006). It has been shown that the presence of human fecal bacteria in reef waters can positively correlate with higher frequency of coral disease (e.g., black band), suggesting that wastewater is an important vector in the transmission of disease to reef corals (Dolenec et al. 2005; Lipp et al. 2001; Lipp et al. 2002; Lipp et al. 2007). Nutrient loading as a result of poor sewage treatment and increased sedimentation in runoff may further increase the frequency and severity of coral disease and bleaching in affected areas (Voss and Richardson 2006). The relationship between stressed corals and sedimentation is not as well understood. However there is evidence that the presence of silty or fine sediments has a more detrimental effect than larger or sandy sediment on corals’ ability to fight off disease and makes the coral more susceptible to bleaching. The smaller particles not only remain suspended in the water column for longer periods of time, blocking out essential sunlight, but are also harder for the coral to remove from surface tissues, inhibiting the corals ability to feed (Voss and Richardson 2006; Weber et al. 2006).

On the southern Caribbean island of Bonaire, in the Netherlands Antilles, there has been concern over waste water runoff and its effects on near-shore fringing coral reefs. Currently, Bonaire does not have a wastewater treatment program. Wastewater and sewage is contained and held in septic tanks which are periodically drained and the contents taken to open, unlined trenches in the center of the island and dumped (Reynolds 2008). Septic tanks may also leak or even overflow directly into the water table. In the absence of lining, which frequently is the case for septic tanks too, the waste seeps into the ground; quickly reaching the water table (Alexander 1961). The bedrock underlying almost the entire island is porous limestone from dead corals; a result of volcanic activity and plate tectonics. This creates an unconfined aquifer—interconnecting both all of the ground water and the ground water to the sea. In either case, once in the water table, the contamination runs directly out to the sea.

In this study, I assessed the effects of two components of contaminated runoff, human enteric bacteria Enterococci, and fine sediment, on the coral reefs of Bonaire. The questions I addressed with this study were: 1) is the frequency of bleaching and the progression of black band disease related to the presence of enteric bacteria and or finer sediments 2) does costal human activity have an impact on sediment particle sizes found in near shore reefs and 3) is Enterococcus present on reefs of Bonaire and is the concentration of bacteria related to coastal human activity?

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Methods and Materials

Set-up

For this study, I selected monitoring sites by distinguishing between “More Impacted” (n=2) and “Less Impacted” (n=2) reef sites in the nearshore waters of the island. “The More Impacted (MI)” sites, were defined as those located <200 m from a commercial establishment (i.e. a scuba diving resort, office building, or restaurant) and included Bari’s Reef, and Eighteenth Palm dive sites (see Figure 1). The “Less Impacted (LI)” sites, were defined as those surrounded on all sides by >200 m of rural or residential land, and included Witches Hut and White Slave dive sites (Figure 1). All of these study sites are currently monitored on a daily basis for temperature and light intensities, (for the assessment of percent organics), at 5, 12, and 20 m depths through the Light and Motion Sensor Program (LMSP). This program provided baseline data for the present study (Reynolds 2008; Sea Monitor Program 2008; Smith et al. 2008). All assessments for this study were performed at 12 m where the reef is close enough inland to be highly susceptible to runoff and far enough offshore to have adequate coral cover (A. Biancoulli personal communication).

Sedimentation Analysis

In order to assess sediment composition, a sediment trap consisting of a 5.08 cm by 17.78 cm round
bottomed PVC pipe attached to a metal rod (secured flush with the substrate) was placed at each site (see methodology in Gleason 1998). Each week, for one month, each trap was collected and replaced using SCUBA. Particle sizes were determined by agitating sediment samples, removing a 1 mL aliquot and placing it on a counting cell (Sedgwick Rafter Counting Cell Slide) (Gleason 1998). Three random gridlines on each slide were chosen and viewed under a light microscope (LOMO, NXE0097). On each of these gridlines the first 50 particles encountered moving from left to right across the slide were documented and the longest dimension of each particle was measured using an ocular micrometer. The particles were classified into six size ranges: <10 μm, 10-50 μm, >50-100 μm, >100-250 μm, >250-500 μm and >500 μm (see methodology in Gleason 1998). These data will be used to determine the average particle size distribution for MI versus LI sites. A 2-way analysis of variance (ANOVA) was used to determine if there was a difference in the composition of sediments between MI and LI sites.

Bacterial Analysis

In addition to weekly sediment sampling over the duration of the study, I also collected one water sample from each site for the determination of enteric bacterial contamination. The water was collected at 12 m using a 250 mL capped plastic bottle. Upon entry into the water the collection bottle was filled with sterilized tap water and once at depth, the bottle was flushed with site specific water three times before being filled and capped for further analysis (see methods in Griffin et al. 1999). The water was returned to the lab within 4 hours of collection and analyzed for the presence and concentration of enterococcal bacteria using Enterolert™. Water samples were diluted to a 10% solution, with Baxter™ Sterile Water. To this, one unit of pre-measured Enterolert fluorescing substrate was added, and then the mixture was poured into an IDEXX Quanti-tray. The tray was then vacuum sealed with the IDEXX Quanti-tray sealer which distributed the mixture into separate wells of the tray. Prepared trays were then incubated in the Thelco Laboratory Oven (model 130DM by Precision Scientific), set to 41°C, for 24 hours. Following incubation, fluorescence of the trays was visually analyzed using a black light. The number of fluorescing large and small wells was recorded and then converted to a Most Probable Number (MPN) using a MPN generator table provided by Enterolert. From this, the parts-per-million concentration of Enterococcus was determined for each site. A weekly mean MPN was determined for MI and LI sites. A 2-way ANOVA with replication was run to determine whether concentrations of enteric bacteria significantly differed between MI and LI sites over time.

Video Analysis

In order to assess the progression of black band disease and bleaching on the reef relative to bacteria counts and sedimentation, each week two 10 m transects from the 25 m north and south of my sediment trap, were randomly chosen to be video recorded each week. Each video transect was trimmed and cut into 10 representative frames using Picture Motion Browser, and each frame was analyzed using Coral Point Count (CPC) software. CPC overlaid each frame with 15 randomly chosen points, under which the substrate was identified and assessed for disease and bleaching where applicable. These data were used to determine the percent of bleached diseased live coral on a weekly basis. These data were analyzed using an unpaired t-test to determine whether the frequency of disease or bleaching differed between MI and LI sites.

Results

Sedimentation Analysis

![Figure 4](image-url) **Figure 4.** The mean (± SD) overall Enterococcus concentrations for MI and LI sites over the course of three weeks with standard deviations. The x-axis shows the study site and the y-axis shows the average concentration (ppm) of bacteria. Two-way ANOVA. (P > 0.10).

![Figure 5](image-url) **Figure 5.** The mean (± SD) Enterococcus concentrations for MI and LI sites for each of three weeks. The x-axis shows the time in weeks and the y-axis shows the concentration (ppm) of bacteria.
Analysis of overall sediment particle size distribution for the four sampling periods combined showed that the particle size distribution significantly differed between MI and LI sites (ANOVA, p < 0.001). The sediment composition at MI sites contained more finer, siltier particles (<50 μm) whereas LI sites contained more larger or coarser particles (>50 μm) (Figure 2). Upon closer examination, particle distributions for each week show a trend consistent with the overall distribution (Figure 3).

**Bacterial Analysis**

Enterococcal bacteria was found in the water at coral reef sites of Bonaire. An unpaired t-test showed that the overall enterococcal concentrations did not differ between MI and LI sites (t-test, p = 0.34) (Figure 4). However, visually, there appears to be a trend. The concentration for MI sites was consistently higher than LI sites over the duration of the study (ANOVA, p = 0.91, Figure 5).

**Video Analysis**

Upon analysis of videos for coral disease and bleaching, I found limited instances of both. Of the 4,800 points analyzed by CPC only 3 were tagged for black band disease. Therefore, no further analysis on disease was performed. Bleaching occurred relatively infrequently, (~1-7% of live corals). Based off 2-way ANOVA, the bleaching relationship between MI and LI sites was not statistically significant (p = 0.44), (Figure 5). Over time, percent bleaching fluctuated more dramatically at LI sites than at MI sites however no meaningful trend could be determined.

**Discussion**

Based off this study the frequency of coral bleaching and the progression of black band disease is not related to the concentration of enteric bacteria or sediment particle size. However, *Enterococcus* bacteria is present on the reef. These data did not show a relationship between bacteria concentration and coastal human activity. Though sediment particle sizes were found to be smaller at MI sites and larger at LI sites as predicted.

Unfortunately, the information gathered via video transects was insufficient in order to speculate about the relationship between coral diseases, specifically Black Band disease and fecal contamination, or particulates. The coral bleaching data collected would seem to suggest that, overall, more rural sites are experiencing more bleaching than more urban sites. Perhaps these particular LI sites were receiving too much light and thus bleaching more than the MI sites in this case.

The assessment of sediment particle size distribution showed that the MI sites had significantly finer, siltier particles than the LI sites as predicted. There are several possible explanations for this result. There are many construction sites located along the coast where both of my defined MI sites are located (Figure 6). Activities such as sanding, cutting, and on-site concrete mixing may create large amounts of dust which settles directly in the water or on nearby roadways from which it is washed directly into the sea during heavy rain events. The presence of roads, parking lots and sidewalks alone near the water increase runoff because the water from rain may not soak into the ground. Rain running over these surfaces picks up dust and dirt particles and carries them out to the sea. Along the leeward side of the island in Kralendijk, (where the MI sites are located), there are few trees and grasses which may act as filters of particles traveling in runoff from rainwater. In fact, Bari’s Reef (MI) is located at the end of a slab of pavement which extends from the road to the waters edge and Eighteenth Palm (MI) is located just off shore of a manmade beach with imported sand. In contrast, the mangroves and other plant life located near the LI sites may filter out smaller particles before they reach the sea.

Assessment of the presence of human enteric bacteria revealed that it is in fact present in the coastal waters of Bonaire. Although the Enterolert bacterial analysis did not show a statistically significant impact of site or time on bacterial concentration, visual analysis of the data suggests that *Enterococcus* concentration is consistently higher at MI sites than at LI sites (Figure 3). This may be explained by the presence of underground septic tanks for nearby coastal establishments which increases the likelihood of direct contamination, via carelessness while pumping or as a result of leaks. Also, if more terrestrial runoff is entering coastal
waters, the likelihood that a sewage spill or leak would enter the water is higher. Overall, the confirmation that enteric bacteria occurs in coastal waters requires further examination. The current study only assessed two MI and two LI sites over the course of one month. However expanding this study to incorporate several more sites over a longer period of time (months to years) may provide a more complete picture of runoff composition impact on reefs. Such a study may also provide useful information concerning island locations which are more susceptible to contamination and thus greatest risks to nearshore reefs.

Acknowledgements

I would like to thank Amy Milman for all of all your Sundays and Wednesdays, Christina Wickman for helping me with CPC, the cameras and hanging out with me for the past two months. I want to thank Amanda Hollebone for all your help and advice about my project. Thank you to Caren Eckrich for the use of the cameras. I want to thank Ramon DeLeon and BNMP for allowing me access to the reef. Finally, this project would not have been possible without CIEE program and staff. Thank you for the funding and support to facilitate my research.

References


Good fences make good neighbors: Habitat partitioning by spinyhead (*Acanthemblemaria spinosa*) and secretary (*Acanthemblemaria maria*) blennies.

Christopher Savage*
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Abstract
Spinyhead blennies (*Acanthemblemaria spinosa*) and secretary blennies (*Acanthemblemaria maria*) are abundant, tube-dwelling fishes on the reefs of Bonaire, Netherlands Antilles. In other areas of the Caribbean *A. maria* are relatively rare and although habitat partitioning has been described for *A. spinosa*, little is known concerning habitat partitioning in *A. maria* or about the competitive interactions between the two species. This study determines the species distribution and density of *A. spinosa* and *A. maria* in the nearshore reefs of Bonaire and documents the competitive interactions between the 2 species using a manipulative experiment. The distribution of *A. spinosa* and *A. maria* was determined using transects on the reef slope and by observations of patch reefs on the reef flat. Experimental condos were constructed and placed on sand flats at 6 m depth, where there is overlap of the ranges. Five individuals from both species were captured and placed on a condo to observe the competitive interactions between *A. spinosa* and *A. maria*. *A. maria* are more abundant on the reef flat while *A. spinosa* are primarily found on the reef slope (> 6 m). In the condo experiments *A. maria* were dominant over *A. spinosa*. It is hypothesized that the slightly larger size and increased competitive nature due to higher densities of *A. maria* give them an advantage over *A. spinosa*. This study supports previous findings on the distribution of *A. maria* and *A. spinosa* on coral reefs and documents competitive dominance of *A. maria* over *A. spinosa*.

Introduction
Two species of *Acanthemblemaria*, a genus of the blenny family Chaenopsidae, commonly occur in Bonaire, Netherlands Antilles (REEF surveys 2008). Members of the genus *Acanthemblemaria* are tube-dwelling, planktivorous blennies that reside in commandeered holes, either dug into the coral matrix or in tubes constructed on the coral surface (Clarke and Tyler 2003) (Figure 1). *Acanthemblemaria* only leave their holes to quickly capture copepods or to compete for a hole superior to that which they currently occupy, a lifestyle referred to as “hemisessile” (Kotrschal and Lindquist 1986).

*A. spinosa* is the most widespread species of the genus *Acanthemblemaria* in the western Atlantic (Smith-Vaniz and Palacio 1973), while *A. maria* is less common and less studied in the region. Bonaire is unique because it has the highest sighting frequency of *A. maria* in the western Atlantic and *A. maria* occur twice as frequently as *A. spinosa* (C. Savage, analysis of REEF surveys 2008). Bonaire is an ideal location to study the competitive interactions and habitat partitioning of *A. spinosa* and *A. maria*.

In the US Virgin Islands Clarke (1994) described habitat partitioning of blennies including *A. maria* (in low numbers) and *A. spinosa*. According to Clarke (1989; 1994) the density of *A. spinosa* is low in waters above 3 m and increases with depth while *A. maria* density is highest in shallow waters and decreases quickly with depth. I hypothesized that *A. maria* will occur at higher density than *A. spinosa* in depths of 6 m and less and that *A. spinosa* will occur at higher density than *A. maria* below 6 m.

There is very little known about *A. maria* habitat partitioning, but several studies have characterized habitat partitioning by *A. spinosa* (Clarke 1992; 1994; Clarke and Tyler 2003; Greenfield and Greenfield 1982). Better food resources and reproductive success at greater height above the substrate drive dominant, larger blennies to reside in superior holes higher above the substrate (Clarke 1992; Clarke and Tyler 2003; Deloach and Humann 1999; Greenfield and Greenfield 1982). According to Greenfield and Greenfield (1982), when *A. spinosa* and *Acanthemblemaria greenfieldi* compete for tube dwellings the larger of the two species, *A. greenfieldi*, occupies the holes on horizontal
spaces while the smaller species, *A. spinosa*, occupies holes on vertical surfaces located below *A. greenfieldi*. In a study implemented in Belize and the Virgin Islands *A. spinosa* were found to be most abundant on vertical surfaces even when other species were not present (Clarke 1994). I predict that *A. maria*, the larger of the two species (Deloach and Humann 1999) will outcompete *A. spinosa* for superior holes when in close proximity due to the larger size of *A. maria*.

**Materials and Methods**

The study was conducted on the leeward side of the island of Bonaire, Netherlands Antilles. The southern boundary of the study area was the pier at the Yellow Submarine Dive Shop (N 12º 15’ 1”, W 68º 28’ 1”) and included the area 100 m north of the pier. The study consisted of two parts: distribution surveys conducted from the reef flat to the reef slope at depths from 3 to 12 m, and experimental tests of species partitioning placed at the depth of maximum overlap of *A. spinosa* and *A. maria*, at 6 m.

**Distribution**

To determine changes in density of *A. maria* and *A. spinosa* with depth, 5 randomly selected 1 m wide transects 10 m long were inspected at 9 m and 12 m depths. At 3 m and 6 m depths, where transects were impractical due to sparseness of blenny habitat, the haphazardly chosen patch reefs were inspected and the area estimated based on measurements of the patch reef. Fish within transects or on patch reefs were identified to species, height of the hole above the substrate was measured and hole orientation was recorded using a range from vertically facing the surface to vertically facing the substrate (Clarke 1989; 1994). A 2 factor (density x depth) analysis of variance (ANOVA, \( \alpha = 0.05 \)) was used to determine if the density of *A. maria* and *A. spinosa* varied with depth. To compare the differences with height above the substrate and hole orientation of *A. maria* and *A. spinosa*, \( t \)-tests were used (\( \alpha = 0.05 \)).

**Competition Experiment**

In order to understand the competitive interaction between *A. maria* and *A. spinosa* in Bonaire, dead coral rocks similar in size to observed patch reefs were collected and made into blenny condos (Figure 2). Ten holes, spaced 2.5 cm apart, were drilled on each condo in a vertical line to force height competition. Each hole measured 6 mm in diameter and 5 cm deep in accordance to the average size of natural holes (Buchheim and Hixon 1992). Any naturally occurring holes were destroyed.

Two sites were selected for blenny condos at a depth of 6 m where both species occur naturally. Condos were placed 5 m from other potential blenny habitat, for *Acanthemblemaria* will not move into open water of that magnitude (Buchheim and Hixon 1992). Site #1 was 40 m south and Site #2 was 60 m north of the pier at the Yellow Submarine Dive Shop. Four experimental condos and 2 control condos spaced approximately 5 m apart were constructed at each site.

Individuals were collected by placing a small net over the blenny hole and squirting ethanol through the net into the blenny hole. Blennies would swim out of their holes and into the net. Five individuals of *A. maria* and *A. spinosa* were placed on each experimental condo. Ten of a single species were released on the control condos. Hierarchal equilibrium takes time to be established (Sale 1984), so
observations of condos were made 2, 24, and 72 h after transplantation. Data analysis of percent species remaining after 72 h was conducted using a t-test (α = 0.05).

**Results**

**Distribution**

Two hundred and ten blennies were observed in an area of 106.4 m². Densities of *A. maria* and *A. spinosa* were calculated and compared by analysis of variance (ANOVA). *A. spinosa* was found to be more abundant on the reef slope (9 m, 0.66 fish/m² and 12 m, 0.62 fish/m²), and *A. maria* was more abundant on patch reefs on the reef flat (3 m, 16.60 fish/m² and 6 m, 18.18 fish/m²). ANOVA (p < 0.001) indicated that the density of *A. maria* was greater than the density of *A. spinosa* at depths less than 6 m and less than *A. spinosa* at 6 to 12 m (Figure 3).

Height above substrate was found to be an average of 7.7 cm higher for *A. spinosa* than *A. maria* (t-test, p = 0.002, Figure 4). When fish hole height above the substrate was compared within each depth (3, 6, 9, 12 m), no significant difference was found between *A. spinosa* and *A. maria*. The difference in species height above the substrate is attributed only to greater relief heights on the reef slope than on the reef flat.

**Competition Experiment**

After 72 h, 60% of *A. maria* remained on the condos whereas only 10% of *A. spinosa* remained (t-test, p < 0.001, Figure 5). *A. spinosa* left the condos within the first 2 hours after transplant even when empty holes were available for occupancy. As a single species control, 4 condos were constructed. One received 10 *A. maria*, and 3 received 10 *A. spinosa*. Observations revealed retention of both species when the other was not present. When a single *A. maria* settled on an *A. spinosa* control condo after observations were complete, all 4 *A. spinosa* that had been occupying the condo were gone within 24 hours.
Discussion

A. maria were found in greater densities in shallow water (3-6 m) whereas A. spinosa were found in greater densities > 6 m. This study provides information previously unknown on competitive interaction between A. spinosa and A. maria. The results support previous reports on depth distribution for A. spinosa (Clarke 1994; Greenfield and Greenfield 1982) and A. maria (Clarke 1994; Deloach and Humann 1999) indicating that even though the population of A. maria is much larger in Bonaire than elsewhere, the distribution with depth is similar to that found in the US Virgin Islands (Clarke 1994). The retention of preferred habitat for A. maria may be maintained by its ability to outcompete A. spinosa.

A. maria are found on patch reefs regardless of depth suggesting that habitat selection is based not on depth, but on type. A. maria prefer to live on small patch reefs in sand flats as opposed to the reef slope. This study shows that A. spinosa occupy the reef slope, but that it is not necessarily their preferred habitat. All experimental control condos showed equal retention of fish until an A. maria displaced all A. spinosa from the controls. Based on the results of the competition experiment it is likely that A. spinosa inhabit the reef slope only in submission to A. maria.

An unexpected result was the lack of species differences in height above substrate in the natural environment as well as hole orientation. Interspecifically, the experiment showed a significant difference in hole height above the substrate; A. maria above A. spinosa. It was expected that this finding of species hierarchical superiority would show in natural habitats as well as manipulative tests. The lack of height habitat partitioning in nature may be attributed to a lack of species interaction due to spatial separation. These fish may live on the same coral head but may be too far away from one another to interact. Observations of A. spinosa in natural habitats showed no intraspecific or interspecific competitive acts (i.e. taking over another fish’s hole), and only intraspecific competition was observed in A. maria suggesting previous establishment of hierarchy. When in forced close interaction, A. maria resided in holes above those occupied by A. spinosa, further supporting dominance of A. maria over A. spinosa. It seems that the superiority of A. maria seen on the experimental condos is manifested in partitioning of water depth, not hole height above substrate. Whether this is due to better food sources in shallow depths is unknown.

Few studies have recently been done concerning the Acanthemblemaria genus (see Clarke and Tyler 2003), and even fewer have looked at habitat partitioning between species (see Clarke 1994). Competition within and among coral reef fish is well documented (Sale 1991), however the study of reef fish ecology is still largely unknown for many fish. Since A. maria and A. spinosa occupy holes created by a variety of organisms, sponges, gastropods, bivalves and polychaetes (Clarke and Tyler 2003), there is little chance for lack of hole space on the reef. But continuation of reef destruction due to anthropogenic and natural factors (Norse and Crowder 2005) is quickly destroying patch reef habitat. As this continues, either A. maria will be forced to deeper depths, forcing A. spinosa down as well, or A. maria will encounter habitat limitation due to inability to succeed at deeper depths.

Acknowledgments

Thank you Kelsey Burns, Chiu Cheng, and Kara Kozak for your role as dive buddies and specimen collectors. Thank you Dr. Rita Peachey, my advisor, and Amanda Hollebone for data analysis assistance. Thank you, Jerry Ligon for your help in identification of these two, very similar species. Thank you to BNMP for allowing me to collect and transplant the blennies. Lastly, thank you CIEE for making this study possible.

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To eat or be eaten: Consumer induced behavior in variegated feather duster worms (*Bispira variegata*)

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Abstract
Predator-prey interactions are a driving force for many identifying features of ecosystems. Predation pressure not only strongly influences population structure and distribution but also influences prey behavior. Predator avoidance behavior may play a large part in self-defense. In marine systems, there are many cases of organisms which are not capable of changing physical location as a predator avoidance technique (e.g., sponges, corals). In this study, *Bispira variegata* was used as an indicator for consumer impact on behavior in a benthic, sessile organism. Primarily, population structure (i.e. size, density) was assessed. Paired clipped and unclipped populations were used to quantify an increase of potential predators based on exposure and the impact that they may have on worm response. In order to determine the impact of predation pressure, worms were observed and video recorded to assess the distance from simulated predation that caused worms to react. There was a mean density of 12 worms m⁻². The population was variably distributed with no bias towards the large nor the small end of the size range represented. There was significant difference in the number of potential predators which entered the clipped plots (p = 0.005). For the predator simulation, there was extreme variation on the distance from the simulated predator that triggered a reaction, specifically retraction. The results of this study can be used as a stepping stone for understanding the effect of predation pressure on benthic, sessile organisms by showing the impact of three-dimensional habitat as well as the behavioral reactions to simulated predation.

Introduction
Biological interactions can have a significant impact on the population structure of organisms in soft-substrate environments; more specifically, predator-prey interactions influence many of the species assemblages and distributions within benthic, marine communities (Woodin 1974). The effects of these interactions impact not only population densities for both consumer and prey but also extend to phenotypic adaptation by the prey species (Agrawal 2001; Sharp et al. 2005). These adaptations can be developmental, morphological, physiological or behavioral (Main 1987; Preisser et al. 2005). In particular, predator avoidance behavior may come as a cost to the prey species by altering the organism’s ability to obtain food or find mates (Nelson et al. 2004). Predator deterrence or avoidance is essential for organisms that are sessile and unable to actively escape predation via physical change in location.

In terrestrial systems, the mobility constraint is limited to vegetation. However, in marine and freshwater systems there are a number of organisms which are restricted to a sessile existence and thus have evolved novel traits to escape consumption. For example, sponges rely on calcareous or siliceous spicules and chemical defenses (Pawlik 1993; Jones et al. 2005), gorgonian corals utilize sclerites and chemical compounds (Pawlik 1993; Puglisi et al. 2002) and bryozoans have inducible spines (Harvell 1998). Previous work has shown that worms of the order Sabellidae are an ideal species for investigating the effects of predation pressure on morphology (Kicklighter and Hay 2006) and chemical deterrence (Kicklighter et al. 2003; Kicklighter and Hay 2007), but studies investigating the effects on worm behavior are relatively rare (but see Dill and Fraser 1997; Kicklighter and Hay 2007). Dill and Fraser (1997) assessed hiding as a predator avoidance strategy in *Serpula vermicularis* of Belcarra Park in British Columbia, Canada using an *ex situ* methodology. They found that there was high variability in individual reactions (i.e. retraction, hiding) and that anti-predator behavior was affected by food availability; where hiding time was increased when food was less abundant and decreased when more abundant. Kicklighter and Hay (2007) investigated the reaction times of the variegated feather duster worm, *Bispira variegata* from soft-sediment mangrove environments in the Caribbean and tropical Atlantic in order to assess the combination of behavioral and chemical strategies for predator avoidance as part of a larger study which also determined palatability of worms and strength of worm tubes. They found that *B. variegata* did not

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retract until they had almost been touched and with a retraction time of 84-154 ms (Kicklighter and Hay 2007).

The island of Bonaire, Netherlands Antilles hosts a wide variety of benthic, sessile species that reside in the diverse array of ecosystems found between the leeward and the windward sides of the island. The leeward (west) side of the island has only sand flats and fringing reefs, whereas the windward (east) side of the island houses similar habitats as well as the island’s only mangrove forest. Between the mangroves and the open ocean on the Sorobon peninsula at Lac Bay (N 12° 05’ 64.8”, W 068° 14’ 27.8”) there is an expanse of seagrass which supports a population of B. variegata. Unlike the highly visible polychaetes found on the sand flats and within the reef structures of the leeward coast, the individuals in the seagrass environment are nestled inconspicuously among vegetation, including seagrass, Thalassia spp., and the calcareous green alga, Halimeda spp. Scattered throughout the seagrass are areas of sand, which are not populated by B. variegata (L. Van Thiel personal observation), although the sparsely vegetated areas might present fewer obstacles to filter-feeding, respiration, and mating. Predation pressure may be higher in the areas dominated by seagrass because the structure provides protection to prey, thus attracting and concentrating the number of potential predators (Peterson et al. 2001). Potential predators of polychaetes known to reside in seagrass beds throughout the Caribbean, include wrasses, gobies, and trunkfish (Huh and Kitting 1985; Clifton and Motta 1998; L. Van Thiel personal observation).

This present study will assess predation pressure on B. variegata in exposed areas of seagrass habitat versus those that are completely surrounded by vegetation. We expect increased predation pressure on worms in the sparsely vegetated areas of the seagrass environment to cause worms to retract quickly upon sensing danger within ~15 cm and spend more time retracted in their tubes; thus, feeding and respiring less, and, in turn, impacting population structure (e.g., sizes, distributions).

Materials and Methods

In order to investigate the hypotheses posed in this study three experimental procedures were carried out using SCUBA. First, the population structure (e.g., size, distribution, population density) of B. variegata in seagrass beds of Lac Bay was quantified. The second procedure assessed the importance of three-dimensional structure for feather duster worms by comparing predation pressure in exposed versus vegetated areas. The third procedure documented feather duster worm reactions to naturally occurring potential predators as well as simulated predation pressure.

Population Structure and Distribution

Feather duster population structure and distribution were determined using a 0.25 m² quadrat (divided into 0.16 m² compartments) whenever populations were encountered along three 30 m haphazardly chosen transects that ran parallel to the mangrove prop root line on the Sorobon peninsula in Lac Bay, Bonaire, Netherlands Antilles (N 12º 05’ 64.8”, W 068º 14’ 27.8”). Each side of the transect tape was analyzed independently (n=9). Assessment of population structure and distribution included mean density (m⁻²), percent cover of the area sampled (60 m²), diameter of worm feeding-respiratory apparati, and the height of the tube. Sampled populations were no less than 0.25 m apart from one another along each transect and included no fewer than 2 individuals and no more than 10 individuals. After a population had been located along the transect, the 0.25 m² quadrat was placed around the population. The individuals in the population were then allowed to acclimate for approximately 10 minutes. After the individuals had emerged from their tubes, the number of individuals was counted, the diameter of their feeding apparatus was estimated using the subdivisions of the quadrat and the tube length of each individual was measured to the nearest centimeter.

Impacts of Habitat on Predation Pressure

The effects of three-dimensional seagrass habitat on the predation pressure experienced by B. variegata were also assessed by pairing clipped and unclipped plots of seagrass bed. Eighteen individual worm populations (~2-10 individuals) were haphazardly identified, paired (n=9), and randomly assigned a status of “clipped” or “unclipped” within each pair. The seagrass vegetation around the “clipped” populations was cut down only to the substratum (to avoid disturbance of worm tubes extending below the surface) in order to expose worms to consumers. The clipped plots were marked with a 0.50 m² quadrat and vegetation removed using a pair of garden shears within a 0.25 m² quadrat placed inside the larger quadrat guideline. The plots were then left undisturbed for ten minutes. Counts, identification of species, and behaviors of the organisms moving into or through the marked area were made.

The reaction of the worms to the “visitors” within the plots was also noted. Species diversity of organisms utilizing the plots was determined using the Shannon-Weaver Index and compared between
clipped and unclipped treatments using a paired t-test.

**Behavior Assessment**

Eleven populations (~2-10 individuals) of *B. variegata* were haphazardly selected within the seagrass beds of Lac Bay in order to simulate predation pressure. These plots were marked with PVC pipe and the boundaries of the 0.50 m² area were designated with string tied to nails at 3 of the 4 corners. The plots were placed 2-18 m apart. Following the selection of the sites, the worms were allowed to recover from any perturbation for ≥15 minutes, before being simulated and video-recorded. The recording camera was oriented directly behind the PVC marker, ~15 cm away to prevent inducing an undesired reaction from the worms under observation. Predation activity was simulated using a water gun filled with sea water dyed with red food colouring. The water gun was pumped three times and aligned perpendicular to the left of the video camera at a distance of ~15 cm from the edge of the plot. The trigger was squeezed for 3 seconds and the response of the worms was recorded until all of the individuals in the plot had retracted or until the dye had settled evenly over the entire area. During a second set of filmed observations, the diver with the predator simulator was positioned to the right of the camera to ensure no bias due to the direction of stimulation. The plots were allowed to settle for 15 minutes between stimulation and recording events to prevent any residual effects on worms behavior. In any case where individuals within a plot were retracted, the plot was recorded for five seconds but disregarded during analysis. In order to ensure that the worms were reacting to the movement of water and not to the food colouring, five individuals were haphazardly selected and immersed in dye utilizing ambient flow.

Upon return to the lab, the videorecorded plots were paired and the distance between the leading edge of the food colouring plume and the closest edge of the worm crown was recorded. The measurement was taken immediately preceding the videoframe in which the worm began retracting. The percent of the worms within the plot that reacted by retracting was also determined. The distances between the food colouring and the worm crown (immediately preceding retraction) was compared using an analysis of variance (ANOVA) between observation day, among observations, and among plots.

**Results**

*Population Structure and Dynamics*

In seagrass beds of Bonaire, NA there were on average 12 *B. variegata* m⁻² within the sampled area (~10,800 m²). In the course of assessing the population structure of *B. variegata*, 203 individuals were measured. The mean crown diameter was 1.83 cm (± 0.91) with a range of 0.25-4 cm. The mean tube height was 3.21 cm (± 2.06) with a range of 0.5-18 cm (Figure 1).

**Impacts of Habitat on Predation Pressure**

There was a significant difference between the diversity of species that entered the clipped plots versus those that entered the unclipped plots (paired t-test, p = 0.005). The clipped plots had an average Shannon-Weaver diversity index of 0.786 (± 0.525) while the unclipped plots had an average diversity index of 0.139 (± 0.085) (Figure 2).

The dominant groups of fish swimming into the clipped plots included predatory wrasses (*Halichoeres* spp.) and gobies (Family: Gobiidae) while the dominant group found in the unclipped

![Figure 1](image1.png)

**Figure 1.** The relationship between crown diameter (cm) and tube height (cm) of *Bispira variegata* within the analyzed plots. The bold points indicate the mean tube height for each crown diameter.

![Figure 2](image2.png)

**Figure 2.** Mean Shannon-Weaver Diversity Index (± SD) for clipped and unclipped plots of *Bispira variegata*. (paired t-test, p = 0.005)
plots was omnivorous damselfish (Family: Pomacentridae). The mean number of potential predators utilizing (e.g., swimming, resting, feeding) clipped plots was overall significantly higher than in unclipped plots (Figure 3, paired t-test, p=0.020).

**Behavior Assessment**

There was no statistically significant difference in the response of worms to simulated predation between days or among observations so all of the data were pooled (n=11) (ANOVA, p = 0.113, F = 3.96; ANOVA, p = 0.107, F=2.66, respectively). There was, however, a significant difference among plots (ANOVA, p = 0.002, F=3.96). Of the total 42 observations for 11 plots, there were 19 in which worm feeding appendages, were not extracted when the observation period began and thus the data were omitted. Of the remaining 23 observations, a mean simulated predation pressure.

![Figure 3 Mean (± SD) number of potential predators present in the clipped versus the unclipped plots of Bispira variegata. The p value represents the difference overall for potential predators present (paired T-test, p = 0.02).](image)

**Discussion**

As with other marine, soft sediment organisms, there does not appear to be a simple, distinct factor that limits the distribution of *B. variegata* within the seagrass beds of Bonaire (Woodin 1974). Individuals are concentrated throughout the *Halimeda spp.* dominated areas of the seagrass suggesting that the worms are food and space limited. The relatively normal size class distribution (the majority of the population falling on or about the mean) of the Lac Bay worm population suggests that there is no preference by the predators in the area. This distribution eliminates any possible population biases for the other aspects of this study by naturally providing an array of size classes to potential predators; this was unlike Dill and Fraser (1997) which limited their study to “medium” size worms (33 ± 0.10 mm SE in tube aperture, inside diameter).

Reactions (i.e., retraction) of individual worms to simulated predation pressure varied greatly which are supported by previous research on other polychaete species (e.g., Evans and Downie 1986; Dill and Fraser 1997). The variation in reaction times could be due to size variation and, thus, different energy requirements. Hiding, as a predator strategy, may decrease the opportunity to obtain food; thus, individuals in areas exposed to predators will be on average smaller than those in more protected areas (Dill and Fraser 1997). In future assessments, it might be beneficial to assess the time worms spend retracted inside their tubes following predator simulation. Considering that smaller individuals may be food limited, it would also be valuable to investigate the variation in reactionary behavior among size classes. Another predator avoidance technique to be explored is the dependence of worms within population on the population as a whole. Preliminary observations suggest that some worms are more sensitive to the reactions of worms in close proximity than others (L. Van Thiel personal observation). This suggests that there may be an adaptive strategy in which individuals work together as a population to avoid predation.

Observations of clipped and unclipped plots provided a preliminary look at the potential predators of *B. variegata* within the seagrass environment and supported the hypothesis that there are more potential predators utilizing exposed areas versus unexposed areas. The higher diversity of potential predators passing through clipped (e.g., wrasses, gobies, trunkfish) versus unclipped plots (e.g., gobies) may be attributed to both the physical exposure of the worms to consumers as well as the disturbance of epiphytes, algae, and associated organisms. Disturbance may send these organisms into the water column where they, themselves attract consumers (L. Van Thiel personal observation).

The implications of this study not only apply to behavioral adaptations such as reactionary responses of sessile, benthic organisms, but also to recruitment by suggesting recruitment is limited in exposed areas where predation is higher. It would be valuable to assess the recruitment behavior and patterns of *B. variegata* to determine the fate of individuals settling outside areas protected by seagrass and algal structures. Ultimately, comparisons of interactions among predator and prey populations as well as behavioral adaptations of worm individuals on sandy reef flats versus those in the seagrass would provide a better idea of the factors determining successful *B. variegata* recruitment. Further assessments of the resident predator communities in sandy reef flat and
seagrass areas may indicate the level and type of consumer pressure worms within the two environments experience. Ideally, a larger scale study assessing recruitment, retraction time (time spent in versus out of the tube as an indicator for food acquisition), and individual dependence on a population would provide a more complete picture of the B. variegata population and the factors controlling its distribution.

Sabellid polychaetes are an ideal species for assessing responses of organisms to predation pressure due to both their sessile nature and the ease of assessing behavioral responses. Although this study offers merely a glimpse into the responses of polychaete worms elicited by predation pressure, it can be used as a preliminary step in determining larger scale ecological processes in seagrass habitats such as the impact of predator-prey interactions on community structure and organismal diversity.

Acknowledgements
I would like to thank C. Kicklighter for her assistance with identification. K. Burns for transportation and her extensive help in the field. A. Parra and D. Binky for their assistance in the field. My advisor, A. Hollebone, for all of her feedback and encouragement throughout the project. This project would not have been possible without CIEE support and funding.

References
Is it possible to predict which areas of Bonaire are more susceptible to coral bleaching?

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ABSTRACT
Bleaching is caused by the combination of light and temperature stress on corals, and can result in coral mortality. On the island of Bonaire, a monitoring program continuously records light and temperature data at 13 sites along the leeward shore of the island. In order to predict where bleaching would occur a light and temperature index was developed. Natural ranges of temperature and light were determined from the monitoring program. Ranges were coded and codes were multiplied to calculate the index. Four sites were chosen. Two that were assumed to be impacted by anthropogenic modifications and two sites where there is little modification of the shoreline. Corals at the four sites were surveyed for bleaching three times during October, and then compared to the index, which was a mean of six values from the two weeks prior to sampling. Impacted sites were hypothesized to have the highest index numbers due to anthropogenic influences. However, a higher index corresponded to a lower percentage of bleaching. Impacted sites did not seem to have a significantly higher amount of bleaching than low-impacted site. An interesting trend is noted in light levels that may be related to runoff or nutrient addition. The prevailing current along the west coast of Bonaire is from south to north. Light levels at 12 m and 20 m decreased from a high at the southern most site.

Introduction
Coral bleaching has become a mounting concern as global sea surface temperature rise (Donner et al. 2005). When a scleractinian coral bleaches, it expels its symbiotic algae known as zooxanthellae, which are pigmented and provide corals with color as well as a majority of their energy needs. As zooxanthellae are lost, the white calcium carbonate skeleton becomes visible through the transparent coral tissue giving the organism a 'bleached' appearance (Joikel and Brown 2004; Harvell et al. 2007). If symbiotic zooxanthellae are not replenished within a short period of time corals lose their photosynthetic potential, causing cessation or reduction of growth and a decrease in reproductive output (Jones 1997; Cox 2007). Bleaching of corals is thought to be due to stresses caused by a variety of environmental conditions outside of the local normal ranges (Littler and Littler 2006). Unusually high or low temperatures, low light levels, constant light, high levels of UV radiation, salinity shock and other chemical insults can result in bleaching (Buddemeier and Daphne 1993).

It may be possible to predict which areas of Bonaire are more susceptible to coral bleaching. A light and temperature index was developed using data currently being generated by the Light and Motion Sensor Program (LMSP). Thirteen arrays of HOBO Pendant Light/Temperature Data Logger sensors are presently deployed at dive sites along the west coast of Bonaire (Jones et al. 2008). Four sites with sensor arrays were chosen for comparisons based on the level of impact expected due to specific threats of warm water entering the reef. Two sites, White Slave and Witches Hut, were selected because they are located furthest away from marinas and extensive shoreline modifications. The other two sites, Eighteenth Palm and Bari’s Reef (Figure 1), were selected because of their proximity to marinas and in the case of Eighteenth Palm, extensive coastal modifications have been made where seawater enters a system of man-made canals.

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Figure 1 Map of Bonaire, Netherlands Antilles showing study sites.
At both sites, it is possible that warm water is draining out onto the reefs from the marinas and canals that could be detected by the sensor and reflected in a light and temperature index. It is also possible that increased sedimentation due to development near the marinas could be decreasing light levels in the water column at Eighteenth Palm and Bari’s Reef.

Temperature and light have synergistic effects on corals and when combined the effect of elevated temperature and exposure to sunlight causes high levels of stress in corals and bleaching is more prevalent where both factors are found (Ferrier-Pagès et al. 2007). My hypothesis is that the site with the highest light and temperature index will be the site where the most coral bleaching will occur and that the impacted sites will have the highest index values and bleaching. This study is unique because it will allow predictions to be made about exactly what areas in Bonaire are at risk for bleaching. If the light and temperature index predicts bleaching using data from the sensor program, this study may identify specific anthropogenic impacts that need to be remediated in order to protect corals in Bonaire.

Materials and Methods

Study Site

White Slave, Bari’s Reef, Witches Hut, and Eighteenth Palm (Figure 1), were chosen for bleaching surveys. Eighteenth Palm and Bari’s Reef were chosen as higher water temperatures are expected due to the presence of marinas and extensive coastal modifications that result in warm water entering the reef. White Slave and Witches Hut were chosen because they were the LMSP sites with the least coastal modifications due to their geographic locations to the north and south of the main residential and business area of Bonaire.

Bleaching Surveys

Sampling for bleaching occurred every two weeks beginning at the end of September and ending at the beginning of November. Transects at the four sites were surveyed using underwater video cameras. A 50 m wide area at each site was randomly sampled at two depths (12 m and 20 m) using the sensor mooring as the center of the sampling area (Figure 2). On each side of the sensor, 25 m transect tapes were extended along the depth contours at 12 m and 20 m. 10 m transects were videoed at constant distance above the reef surface. The starting points for the transects were selected using random numbers. Video data frames were sampled using Coral Point Count software, by cutting each video into ten non-overlapping frames. Coral Point Count then overlaid each frame with 15 random points under which the substrate was analyzed. Percent cover of live corals, bleached corals were determined using this software.

Light and Temperature Index

Each study site has a LMSP sensor array with temperature and light sensors at three depths (5 m, 12 m and 20 m). At each depth there is a sensor measuring green light and one measuring blue light. For the light and temperature index, data from the
blue light sensor was used because blue light penetrates seawater deepest.
A temperature and light index was created using the data from the sensors. First, the natural range of temperature and light was determined. The range was then divided into eight temperature categories and nine light categories that were coded from one to nine, the lowest category being one and the highest being nine. Multiplying the light and temperature codes created index values. In order to test the idea that the index could predict bleaching, mean index values were calculated for three consecutive two-week periods at each dive site with data collected at two depths, 12 m and 20 m by the LMSP.

There is higher average percent bleaching at 20 m than at 12 m (Figures 2 and 3). The low impacted sites at 12 m depth (White Slave and Witches Hut) showed similar levels of bleaching between week 1 and 3, then bleaching decreases at week 6 (Figure 2). The impacted sites (Eighteenth Palm and Bari’s Reef) have increased mean percent bleaching over time though data for week one was not available (Figure 2). The low impacted sites at 20 m depth show steadily increasing mean percent bleaching over time.

**Results**

**Bleaching survey**

**Figure 5.** Mean light levels versus time at 12 m depth for all four sites.

**Figure 6.** Mean light levels versus time at 20 m depth for all four sites.

**Figure 7.** Percent coral bleaching compared to the calculated index at four dive sites. 7a. Eighteenth Palm, 7b. Bari's Reef, 7c. White Slave, 7d. Whitches Hut.
while impacted sites show increased mean percent bleaching between weeks 1 and 3, and a decrease at week 6 (Figure 3).

**Index**

Percent bleached coral was compared to the index between the four sites (Figures 7a-d). All trends show that as the percent of bleaching increased the index number decreased. This is the same trend reflected between depths (Figures 8a and 8b) and overall at both depths among sites (Figure 8c).

**Light**

Light over time between the impacted sites and the non-impacted sites did not give a trend for either depth, however light intensity decreases as it moves along the shore line (Figures 5 and 6).

**Discussion**

Field and laboratory studies on bleaching in corals have established a causal link between temperature stress and bleaching (Lesser 1996). Additionally, ultraviolet radiation has been shown to cause bleaching either alone or synergistically with elevated temperatures (Lesser et al. 1990). This study created an index that would reflect the effects of both temperature and sunlight stresses on corals. I hypothesized that as the light and temperature index number increased, the percentage of bleached corals would increase. In addition, I hypothesized that the sites with more anthropogenic impact would be the sites where the most bleaching would occur.

The temperature at all study sites of study decreased over time at both depths (Figure 4). This shows that the warmest part of the study was at the beginning of the study near the end of September and no peak of temperature was exhibited during the month of October, which was thought to be the warmest month.

Light over time does not reflect areas of high or low amounts of bleaching (Figures 5 and 6). However, light levels decrease from south to north along the coastline. White Slave is the furthest point south and light levels at 12 m and 20 m are higher than the other sites (Figures 5 and 6). The current in the Caribbean has a general northern trend and this is the case in Bonaire. As water moves from south to north, it could be picking up sediments or nutrients. As one moves from south to north development increases and then decreases further to the north. Sediment picked up and suspended in the water column would reduce the amount of light available to corals. Scleractinian corals utilize their zooxanthallae to gain energy through photosynthesis and a decrease in photosynthetic efficiency results in bleaching which can lead to necropsy (Riegl and Branch 1995).

Percent bleached coral was plotted against the light and temperature index to see if a high index number corresponded to a high percentage of bleaching (Figures 7a-d). Among the four sites, increased percent bleaching corresponded to a lower index number. This is consistent among all four sites as well as both depths (Figures 8a-c). It is unclear why this trend is exhibited, but it’s possible that other variables that were not measured in this study such as changes in salinity, UV radiation, and exposure to pollutants (Glynn 1991) were affecting percent bleaching.

During the course of this study, Bonaire was impacted by the effects of Hurricane Omar which
passed approximately 100 miles from its shoreline. Studies from previous hurricane effects on Caribbean islands showed an increased amount of bleaching post-hurricane due to conditions of reduced light caused by the turbidity of the water (Klomp and Kooistra 2003). If this is the case in Bonaire then the bleaching documented in this study may be the result of Omar and not the direct effects of temperature and light.

In conclusion, the temperature and light index did not seem to be an indicator of where the most bleaching would occur. There also did not seem to be a difference between high impact and low impact sites and the amount of bleaching that occurred between those sites. This may be due to salinity, weather, and sedimentation. It may also be due to the short sampling period that this study was conducted over. A longer sampling period showing where the peak of bleaching is exhibited by the index may be more effective. This study needs to be conducted on a larger time scale because data from this study may not be an accurate representation of bleaching over time.

Acknowledgements

I would like to thank my advisor Rita Peachey for all her hard work and dedication to my project. Caren Eckrich for showing me the ropes with the cameras. Amy Milman for coming with me on every dive. Annemarie Rini for diving, moral support and listening to me complain. Also to BNMP without which this would not have been possible. And to CIEE for the support.

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