Physis
Journal of Marine Science

CIEE Research Station
Tropical Marine Ecology and Conservation Program
Volume XVIII, Fall 2015
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Physis, the Greek word for nature, describes the interactions and cycles in each ecosystem. While nature is exhibited in many places, ranging from Arctic glaciers to hot, dry deserts, little compares to the majesty of the coral reef. Bonaire is fortunate to have some of the most pristine coral reefs in the world, providing an environment able to sustain a great diversity of marine life. Throughout our semester in Bonaire, we have learned about the multitude of interactions between organisms on the coral reef, as well as in other ecosystems. Not only were we able to learn by experiencing the coral reef ecosystem first-hand, but we were also provided with the opportunity to connect with the reef on a more personal level by conducting our own independent research projects. Thus, each of us was able to form a unique relationship with the coral reef ecosystem as well as develop the ability to observe the environment through different, but equally important lenses.

Every ecosystem depends on the organisms within it to help keep its balance and flow. Nature, however, is constantly changing. This variation is essential, as it aids in the growth and healing of the ecosystem. As coral reefs are faced with the threats of human impact, resilience becomes an increasingly important quality. The meaning of Physis is embodied by the ability of an ecosystem to withstand the growing stress and frequency of environmental disturbances. The coral reef is a dynamic ecosystem that is continually growing and evolving. Its cyclic nature allows it to self-rehabilitate after disturbances and respond to changing environmental conditions.

"Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts. There is something infinitely healing in the repeated refrains of nature -- the assurance that dawn comes after night, and spring after winter." -Rachel Carson

Each student who chose to spend Fall 2015 at CIEE Research Station Bonaire came with different goals and expectations. However, we were all united in our intention to further our scientific careers. Physis not only represents our beloved coral reef ecosystem in Bonaire, but it also represents us. Just at the coral reef grows and develops over time, we are growing and developing our identities as scientists. It is with great pride that we present Volume 18 of Physis: Journal of Marine Science. This publication is the product of the learning and personal growth that we have undertaken during our time in Bonaire. We hope that this volume of Physis will serve not just as a culmination of our research, but as a stepping stone into the greater scientific community.

Cheers,

Jessica Hutnick, Courtney Klatt, Rachel Kahn
CIEE Fall 2015
Foreword

The Council on International Educational Exchange (CIEE) is an American non-profit organization with over 200 study abroad programs in 40+ 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.”

The Tropical Marine Ecology and Conservation program in Bonaire is a one-of-a-kind program that is designed for upper level undergraduates majoring in Biology. The goal of the program is to provide an integrated program of excellent quality in Tropical Marine Ecology and Conservation. The field-based program is designed to prepare students for graduate programs in Marine Science or for jobs in Marine Ecology, Natural Resource Management and Conservation. Student participants enroll in six courses: Coral Reef Ecology, Marine Ecology Field Research Methods, Advanced Scuba, Tropical Marine Conservation Biology, Independent Research in Marine Ecology/Biology and Cultural & Environmental History of Bonaire. In addition to a full program of study, this program provides dive training that results in Scientific Dive certification with the American Academy of Underwater Sciences.

The student research reported herein was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Dutch Caribbean. Projects this semester were conducted on the leeward side of Bonaire where most of the population of Bonaire is concentrated. Students presented their findings in a public forum on the 25th of November, 2015 at the research station.

The proceedings of this journal are the result of each student’s research project, which is the focus of the course that was co-taught this semester by Enrique Arboleda, PhD, and Patrick Lyons, PhD. In addition to faculty advisors, a CIEE Intern was directly involved in logistics, weekly meetings and editing student papers. The interns this semester were Sara Buckley, Austin Lin (CIEE Alumni), James Emm (CIEE Alumni) and Nathaniel Hanna Holloway. Astrid de Jager was the Dive Safety Officer and provided oversight of the research dives.

Thank you to the students and staff that participated in the program this semester!

Dr. Rita BJ Peachey
Dr. Rita Peachey has been the Director of CIEE Research Station Bonaire since 2006. Her B.S. and M.S in Biology/Zoology are from the University of South Florida and her Ph.D. in Marine Science is from the University of South Alabama. She is currently leading a long-term research project on the health of Bonaire’s coral reefs and published an article this year about the importance of mangroves for Rainbow Parrotfish in Bonaire. Her current focus is the expansion of the CIEE Research Station and advising CIEE headquarters on a new global initiative to increase access to Science, Technology, Engineering and Math programs for CIEE students. Dr. Peachey is also the Executive Director of the Association of Marine Laboratories of the Caribbean.

Dr. Enrique Arboleda is the Coral Reef Ecology Faculty for CIEE Bonaire and co-teaches Independent Research and Marine Ecology Field Research Methods. He is a Marine Biologist from the Jorge Tadeo Lozano University (Colombia), holds a specialization on Biodiversity and Evolutionary Biology from the University of Valencia (Spain) and obtained his PhD at the Stazione Zoologica di Napoli (Italy) working on photoreception of sea urchins. He worked as a Post-Doctoral fellow at the Max F. Perutz Laboratories (Austria) investigating chronobiology on marine invertebrates before moving to Bonaire. Dr. Arboleda’s research interests include adaptation, plasticity upon disturbance, competition, reproductive strategies and how ecological, molecular and physiological responses, like those associated to an abrupt climate change, can drive evolution by natural selection.

Dr. Patrick Lyons is the Tropical Marine Conservation Biology Faculty and the Outreach Coordinator. His roles include conducting research, coordinating and running marine-themed activities for Bonaire youth, organizing a public lecture series, and teaching three program courses: Tropical Marine Conservation Biology, Marine Ecology Field Research Methods, and Independent Research. He has broad research interests including ecology and evolution of marine mutualisms, predator-prey interactions, predatory behaviors of lionfish, and the impacts of recreational SCUBA divers.
Faculty & Staff

**Astrid de Jager** is the Dive Safety Officer and instructor for Advanced Scuba and Cultural and Environmental History of Bonaire. She came to Bonaire in 2009 and has been working in dive industry ever since. She holds a master in Music History and is a SDI and DAN Instructor Trainer.

**Mary DiSanza** is the Logistics Coordinator for CIEE Bonaire. She was born & raised in Colorado. Bonaire’s early commitment to protecting the environment was what first drew her to the island where she worked as a Dive Instructor, Boat Captain, and Retail Manager for a local dive shop - before branching out to the Resort / Management side of the business.

**Amy Wilde** is the Program Coordinator. She holds a B.S. degree in Business Administration, as well as, a Masters of Science in Management Administrative Sciences in Organizational Behavior, from the University of Texas at Dallas. She has worked in call center management for the insurance industry and accounting for long term care while living in Texas. Amy currently provides accounting and administrative support for staff and students at CIEE Bonaire and she is the student resident hall manager.
**Marc Tsagaris** used to be a contractor in the USA until he traded the New Hampshire snow for Bonaire’s clear waters. He is the facilities manager at CIEE Bonaire, and instructor on the Advanced Scuba course.

**Casey Benkwitt** is the Volunteer Outreach Coordinator and Research Associate for CIEE Bonaire. She received her B.A. from Bowdoin College in Environmental Studies and Sociology with a minor in Biology. Casey is currently in the sixth year of her PhD in Integrative Biology at Oregon State University. Her research focuses on the population dynamics and ecological effects of invasive lionfish in the Caribbean.

**Dushi** (which means ‘sweetheart’ in Papiamentu) is CIEE Bonaire’s service dog. At night she is a fierce guardian of the premises, during the day she guards student and staff’s mental health.
Interns

**Nathaniel Hanna Holloway** is the Intern Coordinator and a teaching assistant for the Culture, Marine Ecology Field Research Methods, and Independent Research courses. He has a BS and MS in Civil and Environmental Engineering from the University of Illinois and an MAS in Marine Biodiversity and Conservation from Scripps Institution of Oceanography. Nathaniel is interested in coral reef spatial ecology, specifically in new and novel coral reef monitoring tools and techniques.

**Sara Buckley** has a Bachelors of Science in Oceanography from University of North Carolina at Wilmington and is a PADI Dive Instructor. She was the program coordinator at Sea Turtle Camp in Wilmington and spent three summers with Broadreach Global Summer Educational Adventures sailing the all over the Caribbean and teaching sailing and diving to youth. She is currently a teaching assistant here at CIEE Bonaire for the Advanced Scuba, Marine Ecology Field Methods, and Independent research courses.

**James Emm** is the Coral Reef Ecology Intern at CIEE Bonaire. He holds a B.S. degree in Ecology/Environmental Science from The University of North Texas. James will continue his education in pursuit of a Masters as well as a Ph.D. He is a PADI Divemaster and a former student. He was at CIEE Bonaire for a five-week summer program in 2014. He has participated in ongoing research throughout his tenure at CIEE Bonaire.

**Austin Lin** is a Tropical Marine Conservation Biology Intern at CIEE Bonaire for the fall semester 2015. Austin completed his B.Sc. degree in Marine and Conservation Biology with a minor in Chemistry at Seattle University in 2015. He was a student at the CIEE Bonaire during fall semester 2013, with his research focusing on groupers abundance in relation to coral ecosystem health. Upon returning to his home institution, he constructed a literature review evaluating the predator interactions across multiple trophic levels in coral reef ecosystem. Austin returned to Bonaire in pursuit for his passion in marine conservation, and desires to educate future marine scientists with a conservation-minded outlook. Currently, he is actively involved in educating, researching, and diving at CIEE Bonaire.
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Abstract Excess nutrients as a result of agricultural, urban, and industrial runoff are major causes to increases in plankton. Coral reefs are nutrient poor environments to begin with; therefore any increase in inorganic nutrients could potentially alter the balance of these ecosystems. Bonaire is suffering from nutrient input in the coastal waters and said trends are expected to increase in subsequent years. Zooplankton diversity and species richness, photosynthetic pigments, water properties and nutrients were measured at two different sites in Kralendijk, Bonaire. The most common taxonomic groups at each site were copepods and siphonophores. The difference in mean turbidity between the two sites was statistically significant (t-test; n = 14; p = 0.002). Excessively turbid water can be explained by an increased plankton population but also by sediment runoff from events such as coastal construction. A possible trend was found between number of zooplankton individuals, chlorophyll a, turbidity, and ammonia nitrogen concentration. This trend could indicate abnormal amounts of runoff entering the waters surrounding Bonaire. Not only is marine management necessary, but also an additional terrestrial aspect to monitor in the form of wastewater and watershed management. Zooplankton taxonomic groups identified during this study could be used as indicators of reef ecosystem health, reproduction success of organisms with planktonic larvae, or predator-prey impact studies such as with pelagic predators of zooplankton. Overall, this study shows important indicators of management for urban areas on Bonaire, but could also contribute to future ecological studies on zooplankton population dynamics around the Caribbean.

Keywords Ammonia • Caribbean • zooplankton

Introduction

Plankton is an essential trophic contributor of almost every aquatic ecosystem. Excess nutrients as a result of agricultural, urban, and industrial runoff are major causes to increases in plankton (Johnson and Harrison 2015). Nutrients such as phosphorous and nitrogen are used by photosynthetic organisms like phytoplankton and algae (Hallock and Schlager 1986). Therefore, an increase in nutrients can cause an increase in the production of photosynthetic organisms such as phytoplankton. Moreover, Striebel et al. (2012) found that increasing primary productivity of phytoplankton leads to increased consumption by zooplankton as well as augmented zooplankton diversity.

Coral reefs are nutrient poor environments to begin with; therefore any increase in inorganic nutrients could potentially alter the balance of these ecosystems (Reopanichkul et al. 2009). Additional inorganic nutrients can cause bottom-up control that increases productivity, macroalgae growth and plankton biomass (Lapointe 1997; Arda et al. 2013). This increase in plankton biomass could have negative affects to the reef ecosystem by increasing the turbidity of the water, and thus stifling sunlight penetration to corals (Hallock and Schlager 1986). Increased macroalgae presence on reefs can also inhibit light penetration to corals by covering their surface and therefore preventing coral growth and adding to competition for primary space (Lirman 2000). These consequences of nutrients in the water can slow coral reef conservation efforts in tropical waters.
The issue of excess nutrients and plankton adds yet another impact to coral reefs that is in need of management, making conservation strategies more complicated. Slijkerman et al. (2014) found that there were excess nutrients and chlorophyll a pigments, an indicator of phytoplankton, present in the waters surrounding Bonaire. More specifically, sites tested near urban areas, like those of Kralendijk (capital of Bonaire), and southern parts of the island showed increased eutrophication and excess nitrogen in the water. This information implies that Bonaire is suffering from a nutrient input in the coastal waters and said trends are expected to increase in subsequent years. Excess nutrients leads to particular concern for the future of coastal water quality and the coral reef dominated ecosystems in Bonaire, but also could indicate ecological shifts for planktonic organisms (i.e. competition between species for resources and survival). However, no recent studies, especially in Bonaire, have tested the affect of nutrient increases on plankton species diversity and richness. It is important to not only know the effects of excess nutrients on coral reef ecosystems, but also how increased nutrients may affect the zooplankton population in Bonaire and elsewhere in the Caribbean. This study proposed the following hypothesis:

H1: In urban areas of Kralendijk, Bonaire, there will be an increase in inorganic nutrients in the surrounding waters than in less urban areas

H2: Excess runoff and nutrients will result in increased primary production in the form of phytoplankton in the urbanized areas of Kralendijk

H3: There will be higher zooplankton diversity and species richness present in the urbanized areas of Kralendijk

Materials and methods

Study Sites

The two sites sampled during this study were Yellow Submarine dive site and Cha Cha Cha dive site in Kralendijk, Bonaire (Fig. 1). These two sites are 1.5 kilometers apart. Cha Cha Cha is a dive site (12°8'42.33"N, 68°16'34.28"W) used in this study as a higher industrialized area in Kralendijk, Bonaire. This area is in close proximity to the waterfront of the west side of the island and is near multiple blocks of restaurants, shops, and shipping docks. In addition, this area is also near the Divi Flamingo Hotel and in the midst of increased road and boat traffic compared to the second sample site. Yellow Submarine (12°9'36.20"N, 68°16'55.25"W) was the second location at which samples were collected. Yellow Submarine is a dive site also in close proximity to the waterfront on the west side of the island, however it appears to be less affected by heavy road and boat traffic. This area is also farther away from any restaurants and shops, more towards a residential area with both apartments and homes.

Sampling took place twice per week on Wednesdays and Saturdays for 5 consecutive weeks between the months of October and November of 2015. In addition, samples were taken within the time span of one hour from 8am to 9am.
One horizontal plankton tow was performed at each study site twice per week. The tows took place at the surface of the water, 10 m from the shoreline and 10 m in total tow length. Before each sub-sample extraction, the container was homogenized, by gently shaking in all directions, in order to randomize the zooplankton densities in the sub-samples and assure that all solids were in suspension. Each sub-sample was extracted using a pipette and analyzed under a microscope. Each zooplanktonic organism was identified down to the lowest taxonomic unit possible within each sub-sample. In addition, the number of individuals within each species was recorded for each sub-sample. The number of sub-samples was determined after the first analysis of samples as well as the species richness and the species accumulation curve, also known as a rank abundance curve. Sub-samples were taken until no new species were discovered. This is because at the point of no new species, the sub-samples were a representative of the entire sample.

After the sub-samples were taken, the total estimated species richness and diversity for each entire sample was calculated using the Simpson’s Diversity index. There was not need to calculate the estimated species diversity and species richness of the entire sample, because it was only necessary to a representative of both richness and diversity.

Measuring photosynthetic pigments

Chlorophyll a was used as a biological indicator of phytoplankton. Chlorophyll a is a common basic pigment of photoautotrophic organisms, like phytoplankton (Suggett et al. 2011). To measure phytoplankton, one water sample was taken at each site, 10 m from the shoreline. The specific size of each sample was 100 ml. A fluorometer was used to analyze the amount of chlorophyll a in each sample. There was no need to standardize each sample because the samples were compared relative to themselves.

Measuring water properties and nutrients

One water sample from each site was taken 10 m from shore in order to measure water property and nutrients. Turbidity was measured using a fluorometer. Turbidity was an essential measurement in this study because it is an important indicator of how much sediment and planktonic organisms are in the water. Turbidity levels indicate how much plankton may be impacting light penetration in the water, especially to coral reefs (Risk 2014). Each nutrient sample was a total of 250 ml. When excess nitrites, ammonium, and phosphates are present in a sample, it can indicate higher levels of inorganic nutrients not readily present in ocean water (Knee 2007). Nutrients were measured using a LaMotte Aquaculture kit. The samples were analyzed by following standard protocol for nitrite and ammonia nitrogen. The samples mixed with reagents were then compared with a color slide of concentration levels to get a reading of nutrients. Phosphate could not be measured because the reagent would not properly mix for analysis.

Data analysis

A Simpson’s diversity index was calculated for species richness and diversity with zooplankton. All variables including zooplankton species richness, diversity, chlorophyll a, turbidity, nitrite and ammonia were all plotted according to site and sampling day. T-tests were then performed between each site for all variables to assess significant differences or trends between Yellow Submarine and Cha Cha Cha. Lastly, all variables were observed together in order to look for trends at certain sites over the seven sampling days.

Results

Measuring zooplankton diversity and species richness

The mean (± SD) Simpson’s species diversity index for Cha Cha Cha dive site was 0.73 ± 0.04. Yellow Submarine had a mean (± SD) species diversity index of 0.72 ± 0.07. The highest species diversity was 0.81 at Cha Cha Cha and 0.79 at Yellow Submarine. The species diversity between both sites was similar on all sampling days, with an inverse
relationship on day two. On day six, Yellow Submarine had a 25% lower species diversity than Cha Cha Cha (Fig. 2). The difference in mean species diversity between both dive sites was not statistically significant over the seven days of sampling (t-test; n = 113; p = 0.64).

The mean (± SD) species richness for Cha Cha Cha dive site was 7.6 ± 1.72 species and 7.1 ± 1.07 species for Yellow Submarine. The highest species richness was nine species at Cha Cha Cha as well as nine at Yellow Submarine dive site. The species richness between both sites did not follow any specific trends (Fig. 3). However, both sites had inverse relationships on days three and four of sampling. On day three, Cha Cha Cha had a species richness ~50% less than that of Yellow Submarine, then continued on to have a ~25% higher species richness than Yellow Submarine on day four. The difference in mean species richness was not statistically significant between the two sites over the seven days of sampling (t-test; n = 113; p = 0.65).

The total number of individuals for Yellow Submarine dive site (n = 1143) was greater than Cha Cha Cha (n = 638). The most common taxonomic groups at each site were copepods and siphonophores (Table 1). At Cha Cha Cha, the most common taxa were Calanoid and Herpactacoid copepods (n = 365) and siphonophores (n = 160). Yellow Submarine had Calanoid, Harpactacoid, and Cyclopoid copepods (n = 740) and siphonophores (n = 328) as prevalent taxa.

The mean (± SD) chlorophyll a for Cha Cha Cha dive site was 35.7 ± 11.75 relative fluorescence units (RFUs). At Yellow Submarine, the mean (± SD) chlorophyll a was 29.3 ± 5.27 RFUs. The difference in means of chlorophyll a levels between sites was not statistically significant (t-test; n = 14; p = 0.2). The highest chlorophyll a reading for Cha Cha Cha was on day five (58.5 RFUs) and on day three for Yellow Submarine (40.7 RFUs). The two sites had similar trends in chlorophyll a levels except for Cha Cha Cha dive site’s peak on day five that was ~50% higher than Yellow Submarine (Fig. 4).

Measuring water properties and nutrients

The mean (± SD) turbidity for Cha Cha Cha dive site was 499.5 ± 130.34 RFUs. Yellow Submarine had a mean (± SD) turbidity of 366.3 ± 87.83 RFUs. The difference in mean turbidity between the two sites was statistically significant (t-test; n = 14; p = 0.002). The turbidity at Cha Cha Cha had a higher trend during all sampling days than at Yellow Submarine (Fig. 5). The mean (± SD) ammonia nitrogen for Cha Cha Cha was 0.06 ± 0.03 parts per.
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**Sample Day** 1-7

**Table 1** Number of individuals identified in each taxonomic group. The numbers of individuals are listed according to the site (Cha Cha Cha or Yellow Submarine) and sample day.
million (ppm) and was 0.04 ± 0.05 ppm for Yellow Submarine. The difference in means between the sites was not statistically significant (t-test; n = 14; p = 0.20). The mean (± SD) for nitrite nitrogen at Cha Cha Cha dive site was 0.03 ± 0.03 ppm and 0.01 ± 0.02 ppm at Yellow Submarine. These means were not statistically significant either (t-test; n = 14; p = 0.20). The detectable limit for nitrite nitrogen is 0.05 ppm and many samples from both sites read below that detection level. Neither ammonia nor nitrite nitrogen showed any trends throughout the seven day sampling period (Fig. 6 and Fig. 7).

![Graph 1](image1.png)

**Fig. 4** Trend in chlorophyll a in relative fluorescence units (RFUs) between each site over the seven-day sampling period. The average (± SD) chlorophyll a was 35.7 ± 11.75 RFUs at Cha Cha Cha and 29.3 ± 5.27 RFUs at Yellow Submarine. The two sites were not significantly different (p = 0.2).

![Graph 2](image2.png)

**Fig. 5** Trends of turbidity in relative fluorescence units (RFUs) between Cha Cha Cha and Yellow Submarine over a time of seven sampling days. The mean turbidity (± SD) was 499.5 ± 130.34 RFUs at Cha Cha Cha and 366.3 ± 87.83 RFUs at Yellow Submarine dive site. The two sites were significantly different (p = 0.002).

![Graph 3](image3.png)

**Fig. 6** Trends between Cha Cha Cha and Yellow Submarine in ammonia nitrogen concentration in parts per million (ppm) over seven sampling days. The average ammonia concentration (± SD) was 0.06 ± 0.03 ppm for Cha Cha Cha dive site and 0.04 ± 0.05 ppm at Yellow Submarine. The two sites were not significantly different (p = 0.2).
Nitrite nitrogen in parts per million (ppm) trends between Cha Cha Cha and Yellow Submarine over a seven-day sampling period. The mean nitrite concentration (± SD) was 0.03 ± 0.03 ppm at Cha Cha Cha dive site and 0.01 ± 0.02 ppm at Yellow Submarine. The two sites were not significantly different (p = 0.20)

Discussion

Zooplankton count, chlorophyll a, and water properties

None of the hypotheses were supported in this study based on the statistical tests performed. The inorganic nutrients quantified in the surrounding waters of the urban site and less urban site were not significantly different. In addition, chlorophyll a abundance was not higher at urbanized Cha Cha Cha dive site in Bonaire when compared to Yellow Submarine. The zooplankton diversity and species richness were not significantly different between Yellow Submarine and Cha Cha Cha.

Despite refuted hypotheses, a possible trend was found between number zooplankton individuals, chlorophyll a, turbidity, and ammonia nitrogen concentration. On day six of sampling, the total number of individuals at Cha Cha Cha increased by over 75% from the previous sampling day (Table 1). Not only did the number of individuals increase, but also turbidity almost doubled from the previous sampling day (Fig. 5). Number of zooplankton individuals and turbidity increases can be explained by the elevated ammonia nitrogen readings at Cha Cha Cha on the fourth and fifth sampling day (Fig. 6). Rain recorded on the fifth day of sampling may have caused ammonia runoff to enter the water. In addition, the levels of chlorophyll a increased on day five of sampling, an effect of the increased ammonia nitrogen in the water (Fig. 4). Nutrients such as ammonia can cause increases in chlorophyll a due to phytoplankton consumption of the excess nutrients (Lallu et al. 2013). As a result of increased chlorophyll a, zooplankton consumption increased and led to an increase in zooplankton individuals. Phytoplankton and zooplankton had a positive relationship, therefore when chlorophyll a or phytoplankton increased, zooplankton did as well (Irigoien et al. 2004). Hallock and Schlager (1986) found that with increased plankton in marine waters, transparency decreases, therefore the increase in plankton at Cha Cha Cha acting as excess sediment, caused the turbidity to increase.

This trend could indicate abnormal amounts of runoff entering the waters surrounding Bonaire. Even in small amounts, increased nutrients such as ammonia could cause bottom-up effects on the rest of the ecosystem as shown in the above trends. Heisler et al. (2008) found that with increased nutrients present, harmful rises in phytoplankton and zooplankton may occur, causing algal blooms. If runoff on Bonaire is not controlled, nutrient concentration could continue to increase and cause minor algal blooms. These algal blooms could then reduce oxygen content of the coastal waters in Bonaire affecting not only corals but also fish health. Anderson et al. (2008) found that with increased nutrient pollution, coastal waters became eutrophic causing fish kills among other affected organisms. In addition, elevated phytoplankton and zooplankton can lead to macroalgal growth. An increase in the macroalgal cover could compete with coral growth and recruitment (Lirman 2000). The consequences of nutrients in the water could slow coral reef conservation efforts in tropical waters. The issue of excess nutrients and plankton adds yet another impact to coral reefs that is in need of management, making conservation strategies that much more complex. Not only is marine management necessary, but also an additional terrestrial aspect to monitor in the form of wastewater and watershed management. Bonaire is a nutrient poor coastal area to begin with, so any increase in nutrients is cause for action to keep the fragile reef environment intact.

Nitrite levels were too sensitive to be detected because the starting detectable threshold might have been above the low levels normally reported for coastal water in oceanic islands. Gavio et al. (2010) found that in a similar coastal Caribbean ecosystem nitrite levels read below 0.05 ppm, as low as 0.002 ppm. In addition, numerous experimental design alterations should be considered when performing this study in the future. Current strength should be taken into account in order to factor in organismal dispersal and flow throughout a coastline (i.e. currents impacting chlorophyll a and...
zooplankton collections). Surface currents can play a major role when measuring plankton distribution (Batten and Crawford 2005). This could explain the distinct increase in zooplankton individuals at Yellow Submarine, a site commonly down current from Cha Cha dive site. In addition, sampling over an entire lunar phase should also be taken into consideration. Lunar cycles can have certain stimulating effect on planktonic organisms where plankton rises and sinks with the moon cycle (Moharana and Patra 2014). Sampling over an entire lunar cycle could ensure that all lunar-effected plankton species are recorded. Lastly, sampling to compare more urban areas to less urban areas should be further away from each other to increase site independence.

Higher turbidity at urban site

The turbidity at Cha Cha Cha was significantly higher than Yellow Submarine. As mentioned previously, excessively turbid water can be explained by an increased plankton population but also by sediment runoff from events such as coastal construction. Perry et al. (2012) found that increased sedimentation can increase the turbidity of coastal waters and have severe impacts to coral reefs. Turbid water can decrease light penetration to corals, stunting growth and accelerating coral death (Hallock and Schlager 1986). In addition, increased sedimentation can smother corals decreasing their ability to gain oxygen and light resources. Heavy sedimentation is associated with unhealthy reefs with less coral cover and slower growth (Niù et al. 2010). If construction and sedimentation continues, along with the nutrient runoff, turbidity at urban areas on Bonaire can drastically alter the coral reefs. Proper management of increased building along the coastline of Bonaire should be heavily monitored in order to assure minimal sediment reaching the surrounding waters.

Copepodia most common subclass in coastal waters of Bonaire

Out of all taxonomic groups identified throughout the course of this experiment the subclass Copepodia was the most prevalent. Lentz (2012) found that copepods, more specifically calanoid copepods, are common in temperate and tropical oceanic ecosystems. However, this study could be of greater importance in showing overall zooplankton taxa found in the southern Caribbean, and more specifically in coastal waters of Bonaire. To date, there are no extensive studies focused on zooplankton species found in the location sampled during this study or elsewhere in the southern Caribbean. Although the identification of zooplankton groups was a small portion of this study, the information could be useful for future studies in coastal Caribbean island ecosystems. Zooplankton taxonomic groups identified during this study could be used as indicators of reef ecosystem health, reproduction success of organisms with planktonic larvae, or predator-prey impact studies such as with pelagic predators of zooplankton. Overall, this study shows important indicators of management for urban areas on Bonaire, but could also contribute to future ecological studies on zooplankton population dynamics around the Caribbean.

Acknowledgements I would like to thank the CIEE Bonaire Research Station and Dr. Rita Peachey for all of the equipment and support in making this study possible. In addition, I would like to thank Dr. Enrique Arboleda and Sara Buckley for their consistent and encouraging support throughout this entire process. Their advice and dedication made the design and execution of this project possible. Margaret Meyer was also an essential part of this project in order to make field and laboratory work run smoothly. She was an encouraging research partner throughout the project development and procedure.

References


Using relative brain mass to better understand trophic interactions and phenotypic plasticity of invasive lionfish (*Pterois volitans*)

Abstract

Understanding predator-prey relationships gives greater insight into coral reef health. A recent study on predator-prey relationships linked the relative brain mass of predators and their prey. Predation pressure forces prey to use decision making skills that require higher cognition by inspecting and identifying predators and then adjusting their behavior to achieve the highest chance for survival. However, the predation pressure that prey face outweighs the pressure predators face to find a prey. This results in prey having larger relative brain masses than their predators. There is little data on relative brain mass of fishes with few natural predators such as *Pterois volitans*. This study compared the brain mass to body mass ratio of *P. volitans*, which have very few natural predators and thus very little predation pressure, to the brain mass to body mass ratio of their prey, possible predators, competitors, and taxonomically similar fish. This study also analyzed the response of lionfish to divers with nets in order to investigate their ability to recognize divers as predators. Lionfish did swim away from divers 56.5% of the time which indicates that lionfish might be able to recognize divers as predators. Lionfish had a significantly smaller relative brain mass than their predators, prey, and competitors, but was not significantly smaller than taxonomically similar fish. These results demonstrate that the morphological anti-predator adaptation of venomous spines cause very little predation pressure. Thus, lionfish are not forced to use the same cognitive skills as other prey or predators and in turn have smaller relative brain masses.

Keywords Cognition • crepuscular hunting • predator-prey interaction

Introduction

Understanding predator-prey relationships gives greater insight into coral reef health because they can impact fish diversity, abundance, and distribution (Hixon and Beets 1993). It has been shown that an increase of predators introduced into a coral-reef can affect the species richness and evenness of prey populations (Hixon 1986). Interest has grown in examining predator-prey relationships through cognition and brain morphology.

Kondoh (2010) furthered the understanding of predator-prey relationships by linking the brain mass to body mass ratio of predators and their prey. Kondoh (2010) analyzed 623 predator-prey relationships and found that 1) there is a strong correlation between log-scaled brain mass to body mass ratios of predators and prey, 2) predator-prey relationships are better identified when based on brain mass to body mass ratios, and 3) prey have a larger brain mass to body mass ratio than their predators. The question of how brain mass to body mass ratios can determine predator-prey relationships is better understood when taking cognitive ability and adaptive behavior (phenotypic plasticity) into account.

Predation pressure and other environmental changes induce learning and phenotypic plasticity within a generation of prey which in turn maximizes their fitness (Kondoh 2010; Murren et al. 2015). Prey must be able to first inspect a fish and then identify it as a potential predator (Lima and Dill 1989; Murphy and Pitcher 1997). Furthermore, not every scenario with a predator is equally dangerous for a prey which means that prey must weigh the cost of energy to escape with
the assessment of risk of predation (Lima and Dill 1989; Ydenberg and Dill 1986). Once the prey has identified the fish as a possible predator and decided that the predator is a legitimate threat, it must adjust its behavior to achieve the best chance for survival (Murphy and Pitcher 1997). If predators must improve their ability to predate just as much as the prey have to improve their ability to escape, why do prey still have larger relative brains? Prey are under stronger pressure than predators because of what is called the life-dinner principle (Dawkins and Krebs 1979). The life-dinner principle states that with every predator-prey interaction, the prey will die if it makes the wrong decision but a predator will only need to find another prey (Dawkins and Krebs 1979). The risk assessments and decision making processes due to greater predation pressure indicate that prey have a higher cognitive ability and thus larger brain mass to body mass ratios than their predators (Kondoh 2010). However, in many cases even predators were prey as juveniles and others are predated upon into adulthood. As a result, predators can still have the same pressure to adapt their behavior to learn to avoid other predators. Kondoh (2010) hypothesized that as a prey’s anti-predator behavior improves, the predator must also improve its predation ability which results in a positive correlation between the brain mass to body mass ratio of predator and prey.

_Pterois volitans_, commonly known as the red lionfish, has very few known natural predators in its native range of the Indo-Pacific as well as in its invaded range of the Western Atlantic (Albins and Hixon 2008). Lionfish have venomous dorsal, pelvic, and anal spines, which is likely why they have few predators (Allen and Eschmeyer 1973). However, whether they have a large or small relative brain mass compared to their prey, predators, and other taxonomically similar species is still unknown. Since their invasion in the Western Atlantic, Gulf of Mexico, and Caribbean Sea, lionfish have been affecting the biodiversity, recruitment, and abundance of coral reef fishes (Albins and Hixon 2008; Green et al. 2012; Côté et al. 2013). Lionfish were first found in Bonaire, Dutch Caribbean in 2009 and as of 2015 are found at a higher density than in their native range (Green and Côté 2008; de Leon et al. 2013). Due to their lack of natural predators, divers began hunting lionfish almost immediately after they were first sited in Bonaire to mitigate the negative effects of this invasive species (de Leon et al. 2013). A study by Côté et al. (2014) found that lionfish reacted sooner to divers after living in an environment where divers periodically culled for lionfish compared to lionfish that were not exposed to hunting divers. Claydon and Calosso (2012) found that not only were juvenile lionfish easier to capture by hand net than larger lionfish but also that adult lionfish actually swam away from divers to evade capture. The ability to recognize potential predators and act accordingly may present the possibility that lionfish have cognition and brain mass to body mass ratio similar to other prey fish. However, there should be a distinction between the predation pressure on a lionfish from a diver with a spear and the predation pressure of fish who have to learn to evade predators every day for survival. As such, although lionfish may learn to evade divers with spears, it may not present the same cognitive ability (and brain mass to body mass ratio) as other prey fish but it could indicate a larger brain mass to body mass ratio than their predators.

This study investigated the brain mass to body mass ratio of invasive lionfish. Brain extractions of lionfish caught in Bonaire were conducted and compared to the brain mass to body mass ratio of their prey. This study also compared the brain mass to body mass ratio of lionfish to possible predators, competitors, and taxonomically similar fish which gave further insight to the predator-prey relationships of lionfish and the level of predation pressure that they face. This study also analyzed the response of lionfish to divers with nets in order to investigate the relationship between age (determined by length) and ability to recognize divers as predators. There is little data about
relative brain mass of fish that have few natural predators (i.e. lionfish) and how it relates to their cognitive ability. The results of this study gives greater insight into using relative brain mass to better understand learning and adaptive behavior (phenotypic). The hypotheses of this study are the following:

H1: Lionfish will have a smaller brain mass to body mass ratio than their competitors because although they have similar prey, their competitors experience more predation pressure.

H2: Lionfish will have a smaller brain mass to body mass ratio than their prey.

H3: Lionfish will have a larger brain mass to body mass ratio than their predators.

H4: Lionfish will evade divers when approached with nets.

Materials and methods

Study site

Lionfish dissections took place at the CIEE Research Station in Kralendijk, Bonaire. Field data were collected on SCUBA dives at Yellow Submarine dive site. Lionfish used for lab data came from dive sites on Bonaire: Yellow Submarine, Bari Reef, Aquarius, Something Special, and Te Amo (Table 1). Yellow Submarine (12°9’36.20”N, 68°16’55.25”W) is located on the west side of Bonaire (Fig. 1). Yellow Submarine was chosen after preliminary dives showed the presence of lionfish at multiple depths. Due to the abundance of massive corals such as *Porites astreoides*, *Orbicella faveolata*, and *Orbicella annularis*, the structural complexity is favorable for lionfish because they are often found hiding in crevasses or under large overhangs when they are not hunting. Yellow Submarine is a fringing coral reef that is a popular site among divers to shoot lionfish which presents the potential for lionfish to learn to avoid divers. Yellow Submarine also has a lot of potential prey items for lionfish such as species of chromis, damselfish, wrasse, butterflyfish, and gobies.

![Fig. 1 Map of Bonaire with the black dot indicating the Yellow Submarine dive site (12°9’36.20”N, 68°16’55.25”W) where field research was conducted](image)

Study organism

*Pterois volitans*, commonly known as the red lionfish, is native to the Indian Ocean and Western Pacific but has been found in the Western Atlantic and Caribbean Sea as an invasive species since 1985 (Schultz 1986; Semmens et al. 2004). Lionfish are found on the reef typically between 0 to 50m (Schultz 1986). Lionfish are crepuscular predators and are considered generalist consumers in both their native and invasive range (Morris and Akins 2009; Cure et al. 2012). With the exception of short periods during courtship, lionfish are solitary reef dwellers in their natural habitat and are usually found in crevasses or under overhangs (Schultz 1986). Lionfish are in the family Scorpaenidae (scorpionfish) which is characterized by having venomous spines (Fishelson 1975). Lionfish have 13 venomous dorsal spines, two venomous pelvic fins, and three venomous anal fins (Morris 2009). There are very few natural predators of lionfish that we know of, but there have been few instances where lionfish are found in the stomach content of larger groupers (Maljkovic et al. 2008).

Lab methods

Lionfish used for dissection were shot and collected at various sites and depths in Bonaire between 2013 and 2015 (Table 1). Lionfish, as well as their brains, were extracted and weighed using a protocol created by the author (Table 2).
Table 1 List of dive sites as well as their latitude and longitude, number of lionfish shot at which depth and what year. Six lionfish were shot at a dive site that was not recorded. The depth that lionfish were shot at Yellow Submarine and Something Special was also not recorded.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Latitude, Longitude</th>
<th># lionfish shot at site</th>
<th>Depth lionfish were shot (m)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquarius</td>
<td>12°5′25.21″N, 68°16′59.84″W</td>
<td>9</td>
<td>8-28</td>
<td>2015</td>
</tr>
<tr>
<td>Bari Reef</td>
<td>12°9′48.67″N, 68°17′13.59″W</td>
<td>1</td>
<td>30</td>
<td>2015</td>
</tr>
<tr>
<td>Something</td>
<td>12°9′45.56″N, 68°17′6.86″W</td>
<td>3</td>
<td>not recorded</td>
<td>2015</td>
</tr>
<tr>
<td>Special</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Te Amo</td>
<td>12°8′3.94″N, 68°16′47.66″W</td>
<td>4</td>
<td>15-20</td>
<td>2013</td>
</tr>
<tr>
<td>Yellow</td>
<td>12°9′36.20″N, 68°16′55.25″W</td>
<td>4</td>
<td>not recorded</td>
<td>2015</td>
</tr>
<tr>
<td>Submarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>Unknown</td>
<td>6</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Table 2 Step by step procedure for extracting a lionfish brain. Also indicates the exact tools needed for dissection (Step 1) as well the neuroanatomy of a lionfish brain (Step 8). The protocol for this procedure was created specifically for this study.

Step 1: The lionfish was weighed using the scale, and its entire weight was recorded in grams. Next, length of the fish from the tip of the mouth to the tip of the caudal fin was measured and recorded in centimeters. Tared weight of petri dish was recorded.

Tools used in dissection:
A. Large forceps with curved tip
B. Scissors
C. Tweezers
D. Small forceps
E. Petri dish
F. Scale
G. Ruler
H. Cutting board
*not pictured: Lab spatula

Step 2: Two incisions were made (indicated by the white dashed lines) using the large forceps to remove the head. The first incision was made directly anterior to the first dorsal spine where the hard plates on the head turn to soft flesh. The second incision was made anterior to the pectoral spines.
Step 3: The mouth was opened and an incision was made (using the large forceps) angled upward to cut off the lower jaw. This incision was made on both sides.

Step 4: Using the scissors, an incision was made between the eyes that was approximately 1 cm. The scissors needed to penetrate through the eye socket and cut through the hard bone that was on the top of the head. The cartilage on the head becomes increasingly harder and thicker on more mature lionfish. On larger lionfish, large forceps were necessary to make the cut.

Step 5: A second 1 cm incision was made perpendicular to the cut made in Step 4. The scissors were used unless the cartilage was too hard, in which case the large forceps were used. The brain is directly underneath this incision, so it was imperative that the scissors did not sink too deep and damage the brain.

Step 6: The scissors were used to wedge open the top of the head at the incision made in Step 5. The brain is located directly underneath and rests in a hard casing made of cartilage.

Step 7 (OPTIONAL): The forceps and the scissors were used to cut away parts of the head around the brain. This step is optional because it was possible to extract the brain without this step, but it does increase visibility of the entire brain and in some cases made the extraction easier. The eyes and mouth were cut off as well as the vertical walls on either side of the brain.
Step 8: Before the brain was removed, I ensured that all of the lobes were present and were not obstructed in the dissection process. Also, the tweezers were used to pull off any flesh or nerves that were still connected to the brain. The cerebrum is the most anterior lobe (B) followed by the two optic lobes (C) and the cerebellum (D). There is also the vagal lobe that is located underneath the brain and is shown in the picture below (F). The most posterior is the medulla (E). The brain was connected to the optic chiasm (A) which is where the two optic nerves merge from the eyeballs. A small incision was made with the small forceps to cut through the optic chiasm (A) to separate the brain.

Step 9: Tweezers were used to pick up the brain by lifting the medulla to place it on the spatula. If the brain wasn’t completely cut from the optic chiasm, it would not freely come out and a second cut was needed to ensure that it was separated.

Step 10: The brain was placed into the petri dish that was weighed in Step 1. The tared petri dish with the brain in it was weighed and recorded in grams. The weight of the brain was converted to milligrams and recorded.
Field methods

Over the course of five weeks, six dives took place. Three of these dives occurred between 9:30-10:30 and three of these dives occurred between 18:00-19:00. These times were chosen because lionfish are crepuscular hunters meaning that they hunt during twilight hours in the morning and evening. Although the morning dives were after twilight hours, studies show that lionfish are still active in the morning after twilight (Côté and Maljkovic 2010). During each dive, I looked for lionfish for 30 minutes between 1-12 meters, and 30 minutes between 12-18 meters. During each dive I recorded 1) the length of each lionfish that I saw (1-10 cm, 11-20 cm, 21-30 cm, etc.) 2) the depth of each siting and 3) their reaction to a diver swimming at them with a net. Behavioral responses were recorded as one of four categories; 1) swam away, 2) did not swim away, 3) defensive towards net, and 4) was hiding. Defensive behavior was recorded when the lionfish flexed their dorsal spines and intentionally swam toward the net or diver. To reduce the chance of observing the same individual on multiple dives, we conducted three dives headed north, each dive starting where the previous dive ended, and conducted three dives headed south using the same protocol.

Data analysis

Lab data analysis

Lab dissection data specifically for lionfish were analyzed in two ways. First, every lionfish was plotted on a graph of ln[body mass (g)] on the x-axis and ln[brain mass (mg)] on the y-axis (Fig. 2). This graph presents the overall trend of the brain mass to body mass ratio for lionfish found on Bonaire. A least-squares linear regression of brain mass against body mass was used to analyze correlation. Second, each lionfish was designated as either a juvenile (< 17.5 cm TL) or a sexually mature lionfish (≥ 17.5 cm TL) (Morris 2009) and plotted on a graph with body mass (g) on the x-axis and brain mass (mg) on the y-axis. A regression line was fit for each data set (juveniles and sexually mature lionfish). An analysis of covariance (ANCOVA) was used to determine whether the slopes of the two lines were significantly different. This analysis gave insight into whether juvenile lionfish had larger or smaller relative brain masses than sexually mature adults.

The brain mass and body mass data of predators, prey, competitors, and taxonomically similar fish came from a study published by Michio Kondoh (2010). Kondoh (2010) published data for over 623 reef fish predator- prey pairs. Over 40 of those species from the families Pomacentridae, Acanthuridae, Scorpaenidae, Apogonidae Acanthuridae, Scorpaenidae, and Labridae were used to analyze lionfish trophic interactions. Prey of lionfish were determined based on a study by Morris and Akins (2009) that investigated the stomach content of lionfish. The brain mass and body mass data of lionfish came from 26 dissections specifically for this experiment. To analyze relative brain mass, every fish (lionfish, predators, prey, competitors, and taxonomically similar fish) was plotted on a graph with ln[body mass (g)] on the x-axis, and ln[brain mass (mg)] on the y-axis. A log-log least-squares regression of brain mass against body mass was used in order to give an equation that represented what the “predicted brain mass” would be. The equation for predicted brain mass was ln[brain mass (mg)] = 0.6118 (ln[body mass (g)]) + 2.7641 (Fig. 3). Finding the predicted brain mass allowed me to address the confounding factor that larger fish have larger brains whether they are a predator or prey. Once I found the predicted brain mass for every species, I subtracted it from the actual brain mass to find a residual. The residual represents how much the actual brain mass deviates from the predicted brain mass for a given body mass fish. The more negative the residual, the smaller the relative brain; the more positive the residual, the larger the relative brain. The residual (referred to from this point forward as relative brain mass) was used to compare the brain masses between lionfish and their predators, prey, competitors, and other taxonomically similar fish.

Field data analysis

Behavioral response of lionfish to divers with nets was the primary focus of the field data
collection. The response of lionfish was compared to their length with the assumption that length and age are related. This data was presented on a clustered bar graph. Total length (cm) represented each bin on the x-axis and the amount of times that the lionfish displayed each response to the diver on the y-axis. Within each bin, individual bars represented each behavior. This graph allowed me to compare total length with the frequency of each behavior. However, the data presented by this graph indicated that age did not have a significant impact on their behavioral response. Thus, the data was pooled together on a graph that presented all of the lionfish together on a bar graph with the behavioral responses on the x-axis and the percentage of the time that they displayed that behavior on the y-axis.

**Results**

Relative brain mass of juvenile and sexually mature lionfish

There was a positive correlation between log-scaled brain mass and body mass of lionfish \((R^2 = 0.94964)\) (Fig. 2). The brain mass to body mass ratio of sexually mature lionfish (>17.5 cm TL) (Morris 2009) was not significantly larger than the brain mass to body mass ratio of juvenile lionfish (<17.5 cm TL) \((p = 0.125, \text{d.f.} = 1)\). Overall, 26 lionfish were dissected including 7 juveniles and 19 sexually mature lionfish.

![Fig. 2](image.png)

**Fig. 2** The log scaled brain mass to body mass of lionfish. There is a positive correlation \((R^2 = 0.94964)\) between ln[body mass (g)] and ln[brain mass (mg)] as shown by the regression line \((y = 0.4704x + 2.2103)\).

Lionfish have smaller relative brains than their prey, predators, and competitors

Lionfish had the smallest relative brain mass when compared to the averages of all other functional groups (Fig. 3 and 4). Lionfish have a significantly smaller relative brain mass (-1.248) than their prey \((0.1044 \pm 3.649\) (mean ± standard deviation) \((p < 0.0001, \text{d.f.} = 1, 21, t = 12.94)\) and their predators \((0.0804 \pm 0.2902)\) \((p < 0.0001, \text{d.f.} = 1, 6, t = 12.12)\) (Fig. 3 and 4). Lionfish also have a significantly smaller brain mass than their competitors \((0.0487 \pm 0.3059)\) \((p < 0.0001, \text{d.f.} = 1, 13, t = 15.86)\) (Fig. 3 and 4). There was no significant difference between the relative brain mass of lionfish and taxonomically similar fishes \((-1.449 \pm 0.2308)\) \((p = 0.640, \text{d.f.} = 1, 1, t = 0.63)\) (Fig. 3 and 4).

![Fig. 3](image.png)

**Fig. 3** The average relative brain mass for predators \((n = 7)\), prey \((n = 22)\), competitors \((n = 14)\), and taxonomically similar fish \((n = 2)\) compared to the residual (referred to as relative brain mass) of lionfish. Negative residuals indicate their average brain mass is smaller than their predicted brain mass. Error bars represent the standard deviation

Behavioral response to divers with nets

Lionfish displayed similar responses to divers regardless of their total length (TL). Lionfish that were 1-10 cm TL were found to swim away from divers 50% of the time and not swim away from divers 50% of the time \((n = 4)\). Lionfish that were 11-20 cm TL swam away from divers 60% of the time, did not swim away from divers 13% of the time, were defensive towards divers 13% of the time, and were found hiding 13% of the time \((n = 15)\). Lionfish that were 21-30 cm swam away from divers 50% of the time, did not swim away


from divers 25% of the time, and were defensive towards divers 25% of the time. Length of

lionfish did not have a substantial affect on behavior towards divers. Thus, I decided to pool all of the lionfish together to analyze the data (Fig. 5). Lionfish swam away from divers 56% of the time, did not swim away from divers 21% of the time, were defensive towards divers 13% of the time, and were hiding 8% of the time (n = 23).

**Discussion**

Lionfish had a significantly smaller relative brain mass than their predators, prey, and competitors. However, they did not have a significantly smaller brain mass than taxonomically similar fish. This supported H\textsubscript{1} and H\textsubscript{2} which stated that lionfish would have smaller relative brains than their competitors and prey. The results of this study did not support H\textsubscript{3} which stated that lionfish would have larger relative brains than their predators. Lionfish did swim away from divers 56.5% of the time which, indicates that lionfish might be able to recognize divers as predators but the predation pressure is not great enough to cause them to have larger brains relative to their natural predators. However, their small brains are mostly likely due to evolutionary constraints which is evident due to other scorpaenids that also have small brains. Standard deviation within the mean was the highest for prey (±3.6488) is most likely due to the wide range of potential prey items that lionfish, as generalists, consume (Morris and Akins 2009). A possible limitation of this study could be in comparing the brain mass of lionfish with every other fish due to different extraction processes. The brain mass and body mass data for fish from the Kondoh (2010) study came from FishBase but the method for extracting the brains was not stated.

![Fig. 4 Comparing the relationship between ln[body mass (g)] and ln[brain mass (mg)] of lionfish to possible predators (n = 7), prey (n = 22), competitors (n = 14), and taxonomically similar fish (n = 2). The lionfish (represented by the red dot) has the smallest relative brain mass. Colored lines represent the boundaries of the most extreme data points for each category of fish.](image)

![Fig. 5 The comparison of different behavioral responses by lionfish to divers swimming at them with nets over the course of 6 dives in 5 weeks. Lionfish swam away from divers 57% of the time, did not swim away from divers 22% of the time, were defensive towards divers 13% of the time, and were hiding 8% of the time (n = 23).](image)
Lionfish and other Scorpaenidae fish (scorpionfish family) had very similar relative brain masses, which suggests that it is a taxonomic constraint rather than predator-prey interactions that is causing lionfish to have relatively small brains. Predation pressure is a large component in forming morphological anti-predator adaptations, such as venomous spines (Lima and Dill 1990). Lionfish and other scorpionfish that were used in this study have venomous spines which are used as defense in result of an anti-predator morphological adaptation (Halstead and Chitwood 1955; Casewell et al. 2013). Thus, lionfish do not have to use cognition to maximize fitness because they rely on their morphology.

Lionfish morphology can also explain why they have smaller relative brain masses than their predators. Even some predators are prey at some point when they are juveniles and thus must still use decision making processes to maximize their fitness. However, due to lionfish’s venomous spines, even as juveniles they do not face the same predation pressure that other juvenile predators do. The lack of predation pressure due to their venomous spines at all life stages never allow lionfish to learn how to identify and avoid natural predators using the same nuance that other prey have to use. However, lionfish did avoid divers with nets as predators 56% of the time (70% of the time when aggression is included as an anti-predator behavior) which indicates that they are capable of some phenotypic plasticity. The probability of being killed during a period of time is called the risk of predation (Lima and Dill 1990). Thus, the low abundance and frequency of hunting divers may apply a smaller predation pressure onto lionfish (due to lower risk of predation) than other predators do onto other prey, and a smaller predation pressure than other predators feel when they are juveniles. Comparing the brain mass to body mass ratio of lionfish to their competitors who have similar prey but may experience more predation pressure gave insight into the degree that predation pressure affects brain mass.

Lionfish morphology may be a fundamental limitation to their phenotypic plasticity. Anti-predator mechanisms can be behavioral or morphological (Lima and Dill 1990) but the findings of this study suggest that only behavioral anti-predator mechanisms contribute to larger relative brain masses than their predators. Further studies could include investigating how morphological anti-predator mechanisms, such as venom, affect their ability to adapt to introduced predators in a controlled environment.

Acknowledgements I would like to thank my research advisor Dr. Patrick Lyons and my co-advisor Nathaniel Hanna Halloway for their insight and support. I would also like to thank Erin Berghahn for accompanying me on my research dives. Austin Lin also was a huge help with helping format my paper and goes down in history as a Microsoft Office Wizard. Finally, I would like to thank Dr. Rita Peachey and the rest of the CIEE staff for making my project possible.

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Species diversity and abundance of Moray Eels (Family: Muraenidae) in Western Bonaire

Abstract

The estimated number of species of Moray Eels (Family: Muraenidae) worldwide is around 200. A majority of morays hide in crevices and holes during the day, but come out to forage at night. The amount of activity during the day and night differs between species. Some are strictly nocturnal or diurnal while others are equally present during both times. Morays are generally piscivores and have large impacts on the biomass of reef fishes due to their maneuverability. In some studies, morays have been found to have the largest impact out of all other piscivores regarding the quantity of fishes consumed. In this study we dove at 15 m for 45 min during the day and at sunset three times each (six dives total). We also snorkeled the same distance covered while diving, but shallower along the shoreline. The number and species of moray within a 2-m band while diving and a 1m band while snorkeling were recorded. Spotted morays were the most common species seen overall (87.3% of all morays recorded). The density of morays was the highest while snorkeling (1.38 ± 0.77/10 m²). The density of morays while snorkeling was roughly 10 times greater than the day dives and almost 14 times greater than the sunset dives. Smaller morays were seen in the shallows while snorkeling and larger morays were seen while diving. The high number of small morays seen shallower indicates that morays may use shallow habitats as juveniles and move deeper on the reef once they mature and can consume larger prey.

Keywords

Behavioral patterns • habitat selection • diet

Introduction

The estimated number of species of Moray Eels (Family: Muraenidae) worldwide is around 200 (Böhlke and Randall 2000). A majority of these species hide in holes and crevices during the day, but come out to forage during the night (Bardach et al. 1959; Gilbert et al. 2005, Humann and Deloach 2014). However, it has been found that spotted morays (Gymnothorax moringa) and goldentail morays (Gymnothorax miliaris) are diurnal, meaning they are more active during the day (Abrams et al. 1983, Humann and Deloach 2014). Morays are generally piscivores, but Young and Winn (2003) found that they also consume crabs and other small crustaceans. Morays are the only piscivores that can move into relatively small spaces and will inhabit them for a given period of time (Hixon and Beets 1993, Young and Winn 2003). In the Abrams et al. study (1983), spotted morays were found to prefer larger holes (~23 cm wide) whereas goldentail morays preferred smaller holes (~10 cm wide). Holes inhabited by morays tended to be in close proximity to “fish pits,” or areas with high fish density and smaller crustaceans such as shrimp, crabs and lobster (Abrams et al. 1983).

As piscivores, morays have a significant impact on the coral reef ecosystem because they do not have many predators (Young and Winn 2003). A study in Hawaii found that out of 16 families of piscivorous fish, morays had the greatest impact on the community due to their abundance and the amount of fish they consumed (Young and Winn 2003). This is partly due to the fact that they are capable of maneuvering into smaller spaces that larger piscivores cannot reach.
The structure and composition of the habitat in Bonaire where the present study was conducted differed from some other studies. The mean coral coverage in Bonaire is over 25% (Sandin et al. 2008), whereas in Abrams et al. (1983), coral coverage at the study site was up to 35%. Although these are more similar, they differ from other studies that took place in areas with little to no coral. For example, Young and Winn (2003) and Gilbert et al. (2005) used coral rubble areas that had small patch reefs, usually less than one meter across. It is possible that the differences in habitat structure where these studies took place might have contributed to different behaviors or numbers of morays.

In the Abrams et al. (1983) study, it was found that different species of moray have will stay in and return to ‘home holes’ for certain amounts of time. They found that goldentail morays will remain in or return to its hole for periods as long as seven weeks whereas spotted morays will stay anywhere between a few days to four weeks. In addition to this some morays travel farther distances from their holes than others. For example, spotted morays were noted to move distances from 25 m to 115 m from their hole, while goldentail morays and purplemouth morays (Gymnothorax victius) moved no more than 2.5 m to 25 m respectively (Abrams et al. 1983, Young and Winn 2003). These studies found that the foraging over greater distances took place during the night.

The activity of morays during the day and night differs with the species. Gilbert et al. (2005) observed spotted morays 1.7 times more frequently during the night while goldentail morays were observed 2.7 times more frequently during the day. They also observed chain morays (Echidna catenata) more during the night than day and two other species; chestnut morays (Enchelycore carychra) and viper morays (Enchelycore nigricans), which were strictly seen during the night (Gilbert et al. 2005). A different study found morays out foraging during the day, but strictly due to baiting them out, otherwise morays were strictly observed foraging during the night (Bardach et al. 1959).

The main purpose of this study was to look at the species diversity and abundance of moray eels during the day and during sunset. Studies on these behavioral trends have not been conducted in Bonaire. The two proposed hypotheses were:

H1: Spotted morays will be more abundant at sunset.
H2: Larger morays will be more abundant at sunset and smaller morays will be more abundant during the day.
H3: A higher number of morays will be seen shallow rather than deep.

Materials and methods

Study Site

Data collection took place over a distance ranging approximately 540 m, from Yellow Sub dive site (12°09'36.5” N, 68°16'54.8” W) to Something Special dive site (12°09'45.6” N, 68°17'06.7” W), located in Kralendijk, west coast of Bonaire, southern Caribbean. The reef starts seaward of roughly 50 m of coral rubble/sand and the reef crest is at a depth of 7-12 m depending on the location (Sandin et al. 2008). The angle of the reef slope is approximately 45°. From Something Special towards Yellow Sub, the angle of the reef slope increases and larger clumps of Orbicella annularis and Orbicella faveolata are noticeable. Undaria agaricites is also another commonly seen coral species.

Snorkeling was done in addition to diving to collect data on the species diversity and abundance of morays along the shore in 0.5 m to 2 m of depth. There is a ledge that follows the shoreline. The top of the ledge is 0.5 m deep and is where small waves break on shore. The ledge only drops another 0.5 m at most, but has openings that go underneath it where fishes move in and out.

Diving

The distance between the two dive sites was split into smaller zones. Each zone was observed by two divers swimming in one direction at a depth of 15 m for 45 min. After 45 min, the divers turned 90° and swam directly back to shore. The divers recorded their exit point to calculate the distance covered and to know where to start the following dive. At each of the smaller zones, a dive during the day at 09:30 and a dive starting 10 min before sunset were conducted.
for five weeks (six dives total). Using a T-bar, the diver had a 2 m wide zone in which they recorded the species of moray seen within the area while the other diver assisted in spotting. Any morays seen outside of the 2 m area were recorded separately as additional data. For each moray observed, the species and estimated length, if the head and tail were visible, were recorded. Pictures of the individuals observed were taken to analyze for possible trends in habitat or shelter.

**Snorkeling**

Snorkeling was added to the study after the first week of diving was completed. As we entered and exited the water we noticed numerous morays along the ledge that followed the shoreline. This lead to the idea of snorkeling alone the ledge for the same zones that were covered while diving.

The snorkeling zones were the same distance as each of the diving zones. The only difference was that the snorkeling took place along the shoreline in shallow water rather than deep. Each snorkel took place at 17:00 once a week for a total of four snorkels. The same data that was recorded for the diving part was recorded for snorkeling if possible. No snorkeling data was collected during the mornings.

Data analysis

**Diving**

Data was compiled into tables numbered for each dive and which time period the dive took place. Two tables (day and sunset) were used to examine the number of species and the total number of morays observed during the dives. A graph was created using Microsoft Excel to show the abundance of each species for the daytime dives and a separate graph for the sunset dives.

**Snorkeling**

A table was created showing the number of species and the total number of morays seen during the snorkels. One graph was created to show the total abundance of each species during the 5 snorkels.

An additional graph compared the total number of each species seen during the day and sunset at the two depths. A final graph showed the total number of morays seen at each time and depth. Appropriate statistical analyses were performed to determine if there was a significant difference in the abundance of eels during the day and at sunset.

**Fig. 1** Map of Bonaire showing the two study sites. The distance between the two sites was about 500 m. Something Special (12°09'45.6"N, 68°17'06.7"W) and Yellow Sub (12°09'36.5"N, 68°16'54.8"W)

**Results**

The density of morays was higher while snorkeling than diving. A Tukey test was conducted and found the difference between the density of morays when comparing day dives and snorkeling to be significant (n = 3 for day and n =4 for snorkeling, p = 0.035). The difference between the density of morays when comparing the sunset dives and snorkeling was significant as well (n = 3 for sunset and n = 4 snorkeling, p = 0.03). The density of morays was about 10 times greater snorkeling (1.38 ± 0.77/10 m²) than during the day dives (0.14 ± 0.072/10 m², Fig. 2). The density of morays snorkeling was roughly 14 times greater than the sunset dives (0.097 ± 0.049/10 m², Fig. 2).
A total of 62 morays were observed while snorkeling and 17 were observed while diving. Spotted morays were the most abundant overall, however greater numbers were seen snorkeling than while diving (Fig. 3). Chain morays were also seen more while snorkeling than diving.

Spotted morays were the most commonly seen species during both day and sunset dives, although two more were seen during the day (Fig. 4). A total of 10 morays were seen during the day dives and seven at sunset. Only two Goldentail morays were seen while diving and only on dives. Only one Chain moray was seen during the day and none at night.

A p-value of 0.002 (n = 29, n = 38, n = 12) calculated from a Chi-Square Goodness of Fit test showed that there was a significant difference between the size of the morays and the depths at which they were observed. The majority of smaller morays were found in shallow water while snorkeling and larger morays were found deeper while diving (Fig. 6).
The first hypothesis that spotted morays would be more abundant during sunset dives was not supported. A total of eight spotted morays were seen during day dives whereas six were seen during the sunset dives. The second hypothesis that larger morays would be more abundant at sunset and smaller morays would be more abundant during the day was not supported. Although the difference was not statistically significant, morays in the second and third size category were seen during the sunset dives while morays in all three categories were seen during the day dives. The third hypothesis was supported. The difference in the density of morays was statistically significant when day and sunset dives were individually compared to snorkeling.

Spotted morays were the most commonly seen species overall, which was also observed by Gilbert et al. (2005) in Barbados, Abrams et al. (1983) in the U.S. Virgin Islands, and Bardach et al. (1959) in the Bahamas. Chain morays were the second most abundant species seen whereas in other experiments, such as those previously listed, golden tail morays were the second most abundant. This also differs from my findings since only two golden tail morays were observed overall. The diurnal behavior that was seen in spotted morays by Abrams et al. (1983) was not supported since a similar number of morays were seen during the day and sunset dives. The findings were more similar to those of Gilbert et al. (2005) who did not observe a difference in the number of morays seen during sunset and the day. It is possible that my findings were more similar to Gilbert et al. (2005) because there are larger abundances of prey available throughout the day and night. Out of the 17 morays recorded while diving, one spotted moray was observed foraging at sunset and one chain moray was observed foraging during the day. Although this is not a large number of sightings, the activity in the morays did not differ much at either time period.

There was a clear trend that smaller morays were seen in the shallows where snorkeling was conducted. The differences in the size of morays between the shallow and deep areas was significant. This could indicate that the juvenile morays stay shallower until they mature enough to go deeper and consume larger prey. I could not find any studies that focused on the size of morays and more so how the size of morays relates to the depth at which they are commonly found. One possible explanation for the high number of smaller morays in the shallows could be related to their small body and jaw size. A study done by Mehta (2008) focusing on the jaws of different morays showed that chain morays are durophagous meaning they mainly crush and eat crustaceans. Spotted morays have a longer jaw which allows them to consume more fish which explains their piscivorous diet. The size of the prey that can be consumed correlates with the size of the moray. This could explain why the smaller morays were seen in the shallows along the ledge where there are large amounts of smaller fishes. It also can help to explain how the majority of Chain morays were seen in the shallows where there seemed to be a larger amount of crustaceans.

Another possible explanation for the high numbers of morays in the shallows could be the availability of food. There are several local fishermen that bring their catch on or near shore and dump the carcasses close to the ledge where the morays were seen. It has been shown in experiments done by Chave and Randall (1971) and Bardach et al. (1959) that morays are likely to stay around dead organisms and come out during the day to feed. The presence of fish carcasses appeared to have a strong influence on the abundance of morays along the ledge because the density of morays decreased the further away I went from the areas containing the carcasses.

Structure also had an effect on the density of morays. The change in structure in the shallows seemed to have more of an impact on the density of morays rather than deeper on the reef. When the
ledge was not present along some of the shoreline, the number of morays decreased. However, once the ledge was present again, the number of morays increased. While diving, the coral cover did not vary greatly between sites so the structure did not seem to have as much of an impact. One observation that stood out was that a majority of the morays (13 out of 17) were either around or sticking out from *O. annularis* or *U. agaricites*. This differs from other experiments like Abrams et al. (1983) and Gilbert et al. (2005) that observed morays near *Montastraea*, *Siderastrea*, or sandy areas with small patch reef. This difference could be due to the difference in the amount of holes in and around *O. annularis* and *U. agaricites* compared to *Montastraea* or *Siderastrea*.

Hixon and Beets (1993) found that morays typically prefer to reside in small hole corals with approximately 24 holes in them. This finding supports what was observed during my experiment since *O. annularis* has many holes for morays to hide in. The *U. agaricites* where morays were found was adjacent to or growing on *O. annularis*. Additional variables that could have been taken into account are the amount of time for data collection, the time of day that data was collected, and the size of the area the research was done. If more dives were done, then the increased amount of data may have shown a more distinct trend in the size difference or abundance of morays during the day and sunset. It is also possible that some of the morays counted in each zone were repeats from previous data collection days.

Future research should be done with morays in Bonaire. Different densities or species of morays could be seen by conducting a snorkel at night. Additional research could be done at different depths to see if there is a difference between the reef crest and then several different depths along the slope. Moray behavior could also be examined by recording the timeframe that morays stay in the holes or area that they are first seen. The results could then be compared to other papers that have done this to see if there are differences in the behaviors of morays at different locations.

**Acknowledgements** I would like to thank the staff at the CIEE Research Station in Bonaire for allowing me to use their equipment. I would also like to thank Dr. Enrique Arboleda, Dr. Patrick Lyons, Sara Buckley and the other interns as well as my dive buddy McKenna Becker for all of their help with my research project.

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The effects of ultraviolet radiation on the covering behavior of the sea urchin *Tripneustes ventricosus*

**Abstract** Recent studies have indicated that levels of solar ultraviolet radiation (UVR) are increasing globally as ozone is depleted. Ultraviolet radiation often has negative effects on organism survival; in sea urchins, UVR has been found to reduce sperm motility (reducing fertilization success) and to increase the occurrence of abnormal embryonic development (decreasing embryonic survival). Several species of sea urchins cover themselves with rubble; this study examined how this behavior was affected by UVR *in situ*. Three types of treatment boxes were placed over *Tripneustes ventricosus* specimens: a control box, a UVR-blocking box, and an opaque box. Specimens were photographed before and after treatment and percent change in rubble cover was calculated for each individual. The mean change in percent rubble cover presented significant reduction under opaque conditions relative to other treatment conditions ($p = 0.005$, $p = 0.010$). There was no significant difference between the latter two treatment groups ($p = 0.980$). The data suggest that covering behavior in *T. ventricosus* is not a response specific to UVR, but is a response to light. Further studies of how UVR affects *T. ventricosus* and as well as how other species cover themselves in response to sunlight and UVR are needed to understand the benefits of covering behavior in elevated UVR climate conditions. Further study on the costs and benefits of covering behavior as well as studies on the covering and sheltering behaviors of other sea urchins is needed to gain a full understanding of how increasing UVR will affect sea urchins.

**Keywords** Echinoderm • photoreception • Caribbean

**Introduction**

Recent studies have indicated that levels of solar ultraviolet radiation (UVR) are increasing globally as ozone is depleted (Andrady et al. 2012). The Caribbean and other areas within tropical latitudes are particularly susceptible to these changes because UVR levels are already much higher in tropics than at latitudes greater than 23° N/S (Lucas et al. 2006). Ultraviolet radiation can be harmful to organisms by acting as a carcinogen in humans and other animals (Lucas et al. 2006) and damaging DNA by causing the formation of non-coding or lethal-coding regions (Andrady et al. 2012). Ultraviolet radiation does penetrate marine waters, though some of it is scattered and absorbed by the attenuation of light waves in seawater. The difference between UVR at the surface and at a given depth is affected by the turbidity (scattering light) and the presence of chromophoric dissolved organic matter (absorbing light) (Tedetti and Sempéré 2006). This means that marine organisms can be affected by the increasing levels of UVR; for example, studies have suggested that UVR may drive coral bleaching or exacerbate the bleaching effects of high temperatures (Lesser et al. 1990; Brown 1997; Richier et al. 2008).

Sea urchins are important invertebrates in many marine ecosystems, particularly coral reefs. These echinoderms are herbivores and eat the macroalgae that compete with coral for space and sunlight, thus sea urchins play an
integral role in coral-algae phase shift dynamics (Ogden and Lobel 1978; Hughes et al. 1987). Studies have shown that sea urchins are susceptible to damage by UVR; this damage includes reduced sperm motility, which in turn reduces fertilization success (Lu and Wu 2005), and increased abnormal embryonic development, which decreases embryonic survival (Bonaventura et al. 2006). In light of these studies and globally increasing levels of UVR, it is important to understand how sensitive marine organisms are to UVR and what behavioral adaptations they have to withstand high levels of UVR.

Several species of sea urchins (Echinus esculentus, Orton 1929; Lytechinus anamesus, Lees and Carter 1972; Sigg et al. 2007; Lytechinus variegatus, Millott 1956; Amato et al. 2008; Stronglyocentrotus droebachiensis, Dumont et al. 2007; Tripneustes ventricosus, Kehas et al. 2005; Amato et al. 2008) cover themselves with rubble (e.g., rocks, shells, Acropora cervicornis fragments, etc.). This behavior has been studied for almost a century (Orton 1929) and continues to be of interest to researchers today. Sea urchins use their spines and tube feet to move rubble from their surroundings to the top of their tests (Millott 1956). Different species of sea urchins may have a preference for certain materials—for example T. ventricosus tend to select leafy items like eelgrass over rocks—however, sea urchins continue to cover themselves with the same amount of rubble in response to an external stimulus regardless of the type of material available (Amato et al. 2008). Some studies have suggested that the external stimulus driving rubble covering behavior is wave surge; the rubble may reduce the effect of wave action on overturning or moving sea urchins by increasing the weight of sea urchins (Lees and Carter 1972; Dumont et al. 2007). Other studies have focused on the effects of light intensity and UVR on the covering behavior of sea urchins; some studies found that the covering behavior is in response to UVR (Millott 1956; Kehas et al. 2005; Dumont et al. 2007; Sigg et al. 2007) while others rejected this conclusion (Lees and Carter 1972).

This study aims to further the understanding of the response of T. ventricosus to UVR by evaluating the covering behavior of sea urchins in situ. Previous studies, cited above, on sea urchin covering behavior have been conducted in ex situ laboratory settings using a mixture of artificial and natural light. An in situ study allows the behavior to be studied in a more natural setting with minimal manipulation of test specimens. By minimizing the handling and the use of artificial environments, the likelihood that a specimen’s behavior is altered by handling is minimized; the behavior of a sea urchin in situ is more likely to be an accurate representation of the organism’s behavior outside of the study. Additionally, this study isolated the effects of UVR from other light radiation, allowing a more accurate assessment of whether the behavior is in response to UVR specifically or instead a response to light in general.

H1: Tripneustes ventricosus cover themselves in response to ultraviolet radiation exposure

Materials and methods

Focus species

Tripneustes ventricosus, the West Indian Sea Egg, was selected as the focus species because 1) it has been used as a focus species in previous studies and 2) it is abundant on Bonaire (Kehas et al. 2005; Amato et al. 2008). They are found at shallow depths between 0 and 9 m, are common to reefs and sandy areas throughout the Caribbean, and are often identified by their characteristic covering behavior (Humann et al. 2013). Tripneustes ventricosus are nocturnally active herbivorous foragers; they graze on mature algae and seagrass on the seabed (Tertschnig 2008; Bodmer et al. 2015). They are reddish-brown to brown or white with short (0.5-3 cm), dense, white spines (Humann et al. 2013).
Study site

All treatments and data collection took place at “Mushi Mushi” on the western side of Bonaire, Dutch Caribbean (12°09’13.2” N 68°16’42.3” W, Fig. 1. This site was selected after a preliminary survey was conducted to assess the abundance of the focus species. The area used is bordered by a cement wall on the windward side and a submerged limestone formation three to four m from the wall on the leeward side. The substrate in this area is primarily rubble and comprises pieces of dead Acropora spp., small rocks, and coarse sand. The depth of the water varies from 0.5 to 1 m.

Field research

Selection of specimens

At 13:30 (peak sunlight), nine sea urchins were randomly selected within a 10 m*1 m area and divided into three groups. Sea urchins on large rocks were not included because treatment boxes could not be properly anchored to flat, hard surfaces.

Specimens in each group had a weighted wire box (30 cm*30 cm*30 cm) with a wire top placed directly over them. These boxes allowed water and nutrients to flow through without blocking light or UVR. Boxes in Group 2 (UVR Blocking) had clear UV-filtering layers on top and boxes in Group 3 (Opaque) had opaque light-blocking layers on top. This setup was based on a study by Lees and Carter (1972) with modifications to allow the study to be conducted in the field and to include a control group without any light blocking filter (Group 1: Control).

Treatment and photograph period

Directly before placing each box, each specimen was photographed with a metric ruler for scale using a Canon S110 camera and underwater housing. Next, any rubble on the sea urchin was gently removed. Finally, the boxes were left over the sea urchins for thirty minutes. After this time, the boxes were removed and each specimen was photographed a second time.

Measurement of abiotic factors

During the thirty-minute treatment period, ambient temperature, UV-A, and UV-B were recorded at the surface and at the 0.5-m depth. Temperature was measured to the nearest degree using a simple mercury thermometer. UV-A and UV-B were measured using a PMA2100 data logger and underwater sensors. At the end of the treatment period, the temperature within each box was also measured using the mercury thermometer to confirm that temperature was not significantly different between treatment groups and therefore not a confounding variable in this study. After the thirty-minute manipulation period, each box was placed over the two sensors to record UV-A and UV-B for each type of treatment and confirm that Groups 2 and 3 were blocking UVR.

Replications

Each treatment group was replicated three times on each day of data collection. Data were collected on seven days over a three-week period in October. Data was not collected for an individual specimen if the box fell over or drifted away over the course of the thirty-minute
treatment period. There were a total of 14, 13, and 13 replicates for the Control group, the UVR Blocking group, and Opaque group, respectively.

Data analysis

Image analysis

Photographs were analyzed using ImageJ. Using the ruler included in each photograph, a scale was set to convert pixels to centimeters. The area of each specimen’s test was measured as well as the area of each piece of rubble on the specimen. From these measurements, percent coverage of rubble was calculated for each sea urchin photographed.

Statistical analysis

The mean percent cover and mean change in percent cover were calculated for each treatment group, as well as the respective standard deviation. A one-way ANOVA was run to detect statistical differences among treatment groups in percent coverage after thirty minutes and in change in percent coverage before and after treatments. The latter was followed by a series of pairwise tests (Tukey’s HSD) to determine differences between specific groups. One-way ANOVA tests were run to detect statistical differences among treatment group temperature, UV-A, and UV-B. A series of pairwise tests (Tukey’s HSD) were run to determine differences between specific groups for both UV-A and UV-B.

Results

Differences in covering behavior

I examined the covering behavior of *T. ventricosus* by photographing specimens before and after being placed in treatment boxes that either 1) exposed the specimens to full light, 2) blocked all light, or 3) blocked only UVR. Analysis of images taken prior to treatment revealed no significant differences amongst treatment groups in the percent of specimens’ tests covered by rubble ($F = 0.41, df = 2, p = 0.668$; Table 1). This confirms that there was no bias in sorting specimens into treatment groups. There was also no significant difference between groups’ percent cover after the thirty-minute treatment period ($F = 1.48, df = 2, p = 0.240$; Table 1).

### Table 1

<table>
<thead>
<tr>
<th>% Rubble Cover (mean ± SD)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.89 ± 25%</td>
<td>23.70 ± 31%</td>
</tr>
<tr>
<td>UVR-Blocking</td>
<td>39.52 ± 17%</td>
<td>26.78 ± 21%</td>
</tr>
<tr>
<td>Opaque</td>
<td>46.45 ± 23%</td>
<td>12.05 ± 12%</td>
</tr>
</tbody>
</table>

All three groups had a negative change in percent cover after the treatment period; that is, the average percent cover was less after treatment than the average percent cover before for all treatment groups (Control: \(-11.41\% ± 0.22\); UV Blocking: \(-12.74\% ± 0.09\); Opaque: \(-34.40\% ± 0.19\); mean ± SD; Fig. 2). A one-way ANOVA revealed a significant difference amongst groups’ change in covering behavior ($F = 6.88, df = 2, p = 0.003$, Fig. 2). The Opaque treatment group had the greatest reduction in percent cover and was significantly different than the other treatments (Fig. 2, Table 2). There was no significant difference in change in percent cover between the Control group and the UVR Blocking group (Fig. 2, Table 2).

![Fig. 2 The mean change in percent cover of rubble on urchins after a thirty-minute treatment period for each treatment group.](image-url)
Measurement of abiotic factors

There was no significant difference in temperature inside of the treatment boxes between groups ($F = 0.05$, $df = 2$, $p = 0.97$; Table 3).

A one-way ANOVA revealed a significant difference amongst treatment groups for both UV-A ($F = 62.52$, $df = 2$, $p = 1.36 \times 10^{-12}$; Table 2) and UV-B levels ($F = 26.24$, $df = 2$, $p = 8.02 \times 10^{-8}$; Table 2). A set of pairwise comparisons showed that the UVR Blocking group and the Opaque group had significantly lower levels of both UV-A and UV-B than the Control group (Table 3).

Discussion

This study aimed to examine the rubble covering behavior of *T. ventricosus* in response to UVR and natural sunlight by placing three types of treatment boxes over the sea urchins and measuring changes in covering behavior. I hypothesized that sea urchins cover themselves in response to UVR. Statistical analyses of the data did not support this hypothesis. Had the hypothesis been supported, there would have been a significant difference in sea urchin covering behavior between the Control and UVR-Blocking groups. Specimens in the Control group were exposed to UVR for the entire treatment period while the UVR-Blocking group was shielded from UVR by the clear filtering material on the boxes. There was, however, no statistically significant difference between these two treatment groups. This may imply that there is no difference in how *T. ventricosus* respond to sunlight in the presence or absence of UVR; the covering behavior is either not stimulated by UVR or is stimulated in a way that is similar to the stimulation of other wavelengths of sunlight.

Because this study was conducted in the field and control of variables was limited, abiotic factors (UV-A, UV-B, and temperature) were measured to detect differences in the conditions within treatment boxes that may have been prompting different responses amongst treatment groups. UV-A and UV-B were both close to zero mW/cm$^2$ for the UVR-Blocking and Opaque treatment groups, while UV-A and UV-B were significantly higher for the Control group. There was a significant difference in the change in the percent cover of sea urchins between the Opaque treatment group and the other two treatment groups. The Opaque boxes blocked all sunlight. By comparing the behavior of specimens in this treatment group to the behavior of specimens in the other two treatment groups, we can see how the sea

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**Table 2** Contrasts ($p$-values) between groups following Tukey HSD tests for change in percent cover after treatment, UV-A levels, and UV-B levels

<table>
<thead>
<tr>
<th></th>
<th>% Change in Cover</th>
<th>UV-A</th>
<th>UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control : UVR</td>
<td>0.980</td>
<td>$&gt; 0.001^*$</td>
<td>$&gt; 0.001^*$</td>
</tr>
<tr>
<td>Control : Opaque</td>
<td>$0.005^*$</td>
<td>$&gt; 0.001^*$</td>
<td>$&gt; 0.001^*$</td>
</tr>
<tr>
<td>UVR: Opaque</td>
<td>$0.010^*$</td>
<td>0.998</td>
<td>0.817</td>
</tr>
</tbody>
</table>

*Bold denotes significant $p$-value

**Table 3** Mean temperature, UV-A, and UV-B levels for each treatment group (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Temperature (°C)</th>
<th>UV-A (mW/cm$^2$)</th>
<th>UV-B (mW/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.29 ± 0.47</td>
<td>0.866 ± 0.39</td>
<td>0.045 ± 0.03</td>
</tr>
<tr>
<td>UVR</td>
<td>28.23 ± 0.60</td>
<td>0.020 ± 0.01</td>
<td>0.005 ± 0.00</td>
</tr>
<tr>
<td>Opaque</td>
<td>28.23 ± 0.44</td>
<td>0.015 ± 0.01</td>
<td>0.001 ± 0.00</td>
</tr>
</tbody>
</table>
urchins’ covering behavior is affected by sunlight in general, rather than specifically UVR from sunlight. The Opaque group had the greatest reduction in mean percent rubble cover. This indicates that *T. ventricosus* may be covering themselves in response to sunlight.

A possible explanation for these findings could be the photoreception in sea urchins. While they lack true eyes, studies have found that sea urchins move in response to particular light cues (Yerramilli and Johnsen 2010), suggesting that they do in fact have photoreceptors. Studies on the physiology of sea urchin photoreception have determined that the tube-feet of urchins contain opsins (light-sensitive proteins) and nerves that could function as photoreceptors, that allow the entire organism to function like a single eye (Lesser et al. 2011; Ullrich-Luter et al. 2011). These studies do not indicate whether sea urchin photoreceptors can detect wavelengths as short as ultraviolet radiation and there is little known about echinoderm UVR detection in general, though UV vision has been found in species of arthropods and chordates (Cronin et al. 1994; Losey et al. 2000). The results of this study, suggest that *T. ventricosus* cover themselves in response to sunlight in general but not to UVR specifically.

Photoreception has been found to vary widely amongst different species of sea urchins (Ullrich-Luter et al. 2011). This study focused on the sea urchin *T. ventricosus*, and its scope of inference is limited to that species. At the study site, two other species of sea urchins (*Lytechinus variegatus* and *Lytechinus williamsi*) were found covering themselves with rubble. Replicating the methods of this study with those species may help increase the scope of inference and understanding about sea urchin covering behavior. Additionally, several other species of sea urchins (*Diadema antillarum*, *Echinometra lucunter*, *Echinometra viridis*, and *Eucidaris tribuloides*) at the study site did not cover themselves with rubble but were found inside crevices in the coral cement or under an overhanging ledge. A modified version of the methods used in this study may be relevant to examine this sheltering behavior to understand if it is prompted by the same stimulus as the covering behavior in *T. ventricosus*.

This research only looked at the stimulus driving sea urchins’ covering behavior, not the costs and benefits of the behavior. Assessment of costs and benefits of rubble covering behavior is important for future studies because it will help us understand what adaptations sea urchins may have to withstand harsh or changing conditions within their environment. The covering behavior may cost significant amounts of energy to move the rubble (reducing the amount of energy that is available to find food or reproduce), but this cost may be outweighed by the benefit of protection from UVR or some other external factor (increasing survival). While costs and benefits may be proposed from detection of a stimulus, further research is needed to rigorously assess these proposals.

**Acknowledgements** I would like to thank all of those who helped me conduct my research and develop this study. Dr. Patrick Lyons and Austin Lin provided support and guidance throughout this project as my advisers. Carlie Sharps and Erika Ascani assisted in data collection. The CIEE Research Station and staff provided equipment, facilities, and additional support. STINAPA granted permission to conduct research within the Bonaire National Marine Park. CIEE and Bennington College made this project possible by supporting my trip to Bonaire for the semester.

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The effect of flash photography on the feeding, reemergence time, and time spent in refuge of Bicolor Damselfish, *Stegastes partitus*

**Abstract** Diving on reefs is a great means for tourism around the world. The impacts of divers differ based on experience and if there is something of high interest to observe. Recreational divers enjoy taking pictures or videos to capture the organisms observed. One organism of great abundance on Bonaire’s reefs is the bicolored damselfish, *Stegastes partitus*. They are a planktivorous reef fish that feeds on plankton in the water column. An increase in flash photography, due to the increase in diving, may affect essential behaviors, such as feeding and predator avoidance. The bite rate for *S. partitus* will decrease under the influence of a stimulus, such as light. The *S. partitus* individual will have the same time in refuge and reemergence times as those affected by a predator. Individuals were observed under three treatment groups: hand (artificial predator), light, and control. For each 10-minute observation period, the bite rate, reemergence time, and time in refuge was recorded. The treatment groups had no effect on the time spent in refuge of bicolor damselfish. The results did have an effect on the bite rate, as well as, show that the presence of a current could affect the reemergence time, depending on the treatment group. This study provided evidence that flash photography can have some effects on fish behavior. Because this topic has not been observed in great detail, further studies on this topic should be conducted.

**Keywords** *Stegastes partitus* • foraging • flash photography

**Introduction**

Coral reefs not only serve as an important ecosystem, but also play a major role in the tourism industry (Lamb et al. 2014). In the past several years, there has been an increase of activity in the diving and snorkeling industry (Lamb et al. 2014). The increase of diving has led to several studies on diver effects on coral health (Rouphael and Inglis 2001; Lamb et al. 2014). Coral diseases are more prevalent in areas of higher diver activity than those of areas with less activity (Lamb et al. 2014). Divers tend to disrupt and touch coral when in the presence of a rare species, such as seahorses or frogfish (Uyarra and Côté 2007). This study took place around the island of Bonaire, and found that not only did divers touch the coral, but the contact was much longer in the presence of these species (Uyarra and Côté 2007). One study showed the effect of using a camera while diving in a coral ecosystem and the increased frequency of physical contact with the coral (Rouphael and Inglis 2001). There was also damage to the coral and benthic substrata, which tended to occur at the beginning of a dive rather than towards the end (Rouphael and Inglis 2001). Additionally, there was a significant difference between genders; men tended to grab the coral, breaking off pieces of it, while woman tended to hold onto the benthic substrata (Rouphael and Inglis 2001). On Bonaire specifically, divers with a camera were more likely to intentionally touch the substrate than divers without a camera in hand (Bertuol and Oliveira 2008). As well as intentionally disrupt the substrate, divers with cameras also accidentally came in contact with the reef than those
without cameras (Bertuol and Oliveira 2008). Formal training of the photographer had no
effect on contact rate, although the average amount of time that the diver came in contact
with the reef was higher with ones with no formal training (Bertuol and Oliveira 2008).

The previous studies looked at diver impacts on a coral reef. However, little is
known about the effect of flash photography on fish. Harasti and Gladstone (2013) concluded
that flash photography had a small effect on the behavior, movement, and site persistence of
Hippocampus whitei, a species of seahorse, but this effect was not significant enough to show
that it was actually the flash and not the divers that caused the movement changes. They
predicted that if flash photography was used at low frequencies, it would have minimal effect
on a species (Harasti and Gladstone 2013). However, recreational diving is becoming more
popular, and with that comes an increase in the frequency of flash photography. The frequency
of flash photography may alter the behavior of fish and other marine organisms, such as
feeding ability or avoidance of predators. It was shown by Feist and Anderson (1991) that
fish are more affected by a strobe light than a continuous light. This study was conducted
mostly on freshwater fishes, which still raises questions about the effect of a strobe light on
coral reef species.

Reef fishes have a clustering of rod pigments in the eye due to the interaction between visual constraints, ambient light, behavior, and predation levels (Sale 1991). The clustered rod pigments help support the “Sensitivity Hypothesis”, which states that targets darker than the background appear as dark images to an individual (Sale 1991). This mainly refers to reflective substances, which are difficult to spot (Sale 1991). The “Sensitivity Hypothesis” shows how complex and specialized fishes’ eyes can be, and a flash of a light could greatly disturb that, especially when feeding on plankton in the water column (Sale 1991).

Planktivorous fish have advanced visual abilities (Sale 1991). A good example of this visual activity is the Bluehead wrasse, Thalassoma bifasciatum. This species has small eyes that limit the resolution to see smaller particles in the water, but make up for this by having high cone densities. A high cone density is helpful in detecting plankton from further away, and this distance increases as the individual grows (Sale 1991). Among the planktivorous and omnivorous fish are pomacentrids, the family commonly known as damselfish (Humann 1992), might then display the same visual activity as the Bluehead wrasse.

Stegastes partitus, bicolor damselfish, is a small pomacentrid (Rilov et al. 2007). It can be
found throughout the Caribbean (Humann 1992; Rilov et al. 2007) and usually inhabits
small patch reefs or reef crests (Humann 1992). This species primarily feeds on zooplankton
found in the water column, plucking them from the current (Helfman and Winkelman 1997;
Deloach and Humann 1999). The feeding height in the water column depends on the size
of the individual (Deloach and Humann 1999). Occasionally, S. partitus will feed on benthic
algae (Samhouri 2009). The avoidance of a potential threat in S. partitus tends to be
stronger in juveniles and those that are present in a school (Helfman and Winkelman 1997).
Juveniles also tend to stop eating in the presence of a predator, while adults tend to
show more curiosity to a potential predator (Helfman and Winkelman 1997). In the
presence of divers, S. partitus will hide if approached closely, with the egg-guarding
males attempting to chase away the diver (Humann 1992). Because of the foraging and
predator avoidance behaviors of the bicolor damselfish, this study will analyze the effect of
a strobe light on these behaviors of S. partitus individuals.

H1: The feeding rate of S. partitus will decrease when flashed with a light
H2: An individual S. partitus affected by the flash of a light will have similar reemergence times and shelter use as one affected by an artificial predator
Materials and methods

Study site

The observations for this study took place at Yellow Submarine dive site (12° 09'36.5"N, 68°16'55.2"W) on the coast of Bonaire. At this site, there is mainly sand with a few patches of coral before the reef crest. The observations of S. partitus took place at the reef crest, which is approximately 6-9 m in depth, where the abundance was a minimum of one individual per coral head throughout the crest.

Methods

Three different behavioral patterns were observed: feeding, reemergence time, and time hidden in coral or rubble. These behaviors were observed at 12:30 two days a week for five weeks. Each individual S. partitus was chosen at random, usually the first one spotted, at sites also chosen at random. Individuals were chosen from coral heads or among rubble that were between five and eight cm in length, located in small groups (< 7 individuals) or as solitary individuals. Once selected, an acclimation period of three minutes was carried out. This allowed the individual to acclimate to the presence of divers. A total of 21 individuals were observed (n = 7 for each treatment group) over the study period. Each individual was filmed for 10 minutes using a GoPro Hero Silver +, mounted on PVC piping attached to a four pound dive weight. The camera was set up in a position to ensure clear and uninterrupted view of the focal fish, and then divers swam away so to reduce observer effects.

The three behaviors were observed under three different treatment groups: a flash of a dive light (1000 lumen), the fist of a diver (artificial predator), and no stimulus (control). Once the individual was flashed with the light, or approached with the fist (~ 7 cm/sec), the 10-minute observation period began. Throughout this 10-minute observation time, the reemergence time, feeding rate, time in refuge, and presence or absence of a current were recorded. The order of treatments remained the same each day: artificial predator, light, and control. After each treatment, the divers changed sites and selected a different individual to observe. The control treatment group, carried out at the last site, was an observation of an individual S. partitus feeding, hiding, and natural reemergence time.

Data analysis

The video software QuickTime Player was used to analyze the videos. Each video was analyzed for 10 minutes. The reemergence time, total time spent in refuge, and the bite rate was recorded. It was difficult to get an accurate count of bites for all individuals due to their orientation towards the camera. Instead, the striking pattern was used to show the bite rate. With better equipment the jaw protrusions could be used to calculate bite rate (Marine 1997). MiniTab was used to run statistical analyses. I used a one-way ANOVA to examine the effect of the treatment groups on the bite rate of S. partitus. A two-way ANOVA was used to examine the effect of the treatment groups and current (present or absent) on the reemergence time of bicolor damselfish. A second one-way ANOVA was used to examine the effect of the treatment groups on the time spent in refuge of bicolor damselfish.

Results

Feeding

The bite rate of S. partitus was measured for 10 minutes after being stimulated by the flash of a dive light, an artificial predator, or no stimulus. Of the 21 individual S. partitus observed, only seven were tested. Three individuals for each light and artificial predator group and one individual for control group were tested for the bite rate, and an average bite rate was calculated for each treatment group (Fig. 1). There was a significant difference between the bite rate and the different treatment groups (F2 = 9.27, p = 0.031).

Reemergence time

The reemergence time of S. partitus was recorded after being stimulated by the flash of a dive light, an artificial predator, or no artificial
stimulus. The “no artificial stimulus” was used to show the natural reemergence time of this species. This natural reemergence time was used to quantify the time it took for an individual *S. partitus* to reemerge after taking shelter from a nonhuman stimulus (light or artificial predator). The reemergence time for *S. partitus* individuals varied among all treatment groups. There was no statistical difference between the three treatment groups and time that it took for the individual to reemerge ($F_2 = 0.29$, $p = 0.751$). The current changed between days of sampling. Because of this, current was factored into the analysis to evaluate for significance against reemergence time. There was no significant difference between current and time ($F_1 = 0.01$, $p = 0.918$).

A two-way ANOVA between treatment groups and current showed a significant interaction ($F_2 = 6.82$, $p = 0.008$, Fig 2). The light and control groups have opposite times when compared to the presence of a current, and the current did not affect the hand group (Fig. 2).

**Discussion**

This study examined the effect of flash photography and an artificial predator on the bite rate of *S. partitus*. As originally predicted, the results revealed that light significantly lowered the bite rate of bicolor damselfish. The stimulus did not affect the time spent in refuge of bicolor damselfish. When presented with a threat, juveniles are more likely to show avoidance, as well as reduced foraging behavior (Helfman and Winkelman 1997).
mature *S. partitus* would be likely to appear less disturbed by the presence of potential threats (Humann 1992), but fed at a lower rate when influenced by a bright light.

Structural complexity of the reef is important when determining the location of greatest bicolor damselfish abundance. Bicolor damselfish are most abundant at the back reef and along the reef crest (Rilov et al. 2007). The complexity was important because if there are too many places to hide, the overall results could be affected. With an increase of hiding places, the individual spent more time foraging, or ventured further away from their gardens (Rilov et al. 2007). When less hiding spots were available, damselfish tended to remain close to home and closer to the substrate or coral (Rilov et al. 2007). A future study could compare sites with a high complexity to those of low complexity in the presence of the treatment groups to observe the behavior of bicolor damselfish related to the structural complexity of the reef. At sites of different structural complexity, the amount of natural light could be different. The presence of a flash from a camera could then alter the foraging behaviors and cause individuals to venture too far from their garden.

This study revealed that the different treatment groups and presence of a current affected the reemergence time. The artificial predator treatment group had similar reemergence times when compared to the presence of a current. The light and control treatment group reemergence times were inverts from one another. This showed that flash photography has a small effect on reef fishes by changing the reemergence time in the presence of a current versus in an absence of current. When no current is present, *S. partitus* naturally reemerged much more rapidly than *S. partitus* individuals under the influence of a light stimulus. However, when a current was present, *S. partitus* individuals naturally reemerged slower than the individuals affected by light stimulus. The individuals could be adjusting to the light in order to be able to feed successfully on the zooplankton in the water column (Helfman and Winkelman 1997). When the current is stronger, the food source may be more abundant, so the individual would not necessarily need to acclimate to the light in order to find food. However, the flow of water impacts how often fish strike due to the angle and amount of prey headed toward an individual (Marine 1997). There may have been an over abundance of prey at one time so feeding was halted because the focal individual was overwhelmed.

Future research would be to further analyze the bite rate of *S. partitus* in the presence of the treatment groups used in this study. Along with bite rate, aggression of *S. partitus* should be further studied by observing interspecific and intraspecific competition. Individuals could be observed chasing smaller individual *S. partitus*, as if guarding eggs or their territory. Other times, the focus individual was seen attacking intruders to their territory. This could be observed in a few videos, but it was not known if the increase or decrease of attacks were due to the light or hand treatment.

Not much is known about the effect on flash photography, or bright lights, on reef fishes. Especially on Bonaire reefs itself, photographers need to be much more careful because of the effects that contact with the reef can cause. The coral and substrate is negatively affected by the presence of photographers (Bertuol and Oliveira 2008). This case study looked at the impacts to the reef, and not the fish living on the reef. If the reef around Bonaire starts to diminish, than that would cause the density of fish species to decline. This shows an indirect effect of photography to the fish on reefs. With the popularity in diving growing, this topic should be further studied.

**Acknowledgements** I would like to thank my advisor Dr. Patrick Lyons and intern advisor Austin Lin for constantly helping me throughout this entire process. I would also like to thank my research partner Alex for helping set up each recording, as well as take pictures of the process. I would also like to thank everyone who helped review and comment on this paper to help improve it every step of the way. Lastly, I would like to thank the entire staff at CIEE Bonaire and the University of Rhode Island for allowing me the opportunity to study here.
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Investigating effects of depth and size on pumping rate and filtering efficiency of the Caribbean reef sponge *Aplysina lacunosa*

**Abstract** Marine sponges provide an important link between the benthic and pelagic environments of coral reef ecosystems, yet there is relatively little known about them. Past studies have indicated that depth and size could be contributing factors in tube sponge filter feeding strategies. This study investigated the effects of depth and size on pumping rate and filtering efficiency of *Aplysina lacunosa*, a common Caribbean reef sponge. At two different depths, several parameters of the sponges were measured: tube length, wall thickness, tissue volume, pumping rate (using fluorescein dye), and filtering efficiency (percent reduction in turbidity between the ostia and osculum). Water samples collected from the water column had similar food availabilities between the two depths. There was a positive relationship between sponge size and pumping rate but not filtering efficiency. Additionally, no relationship was found between depth and sponge pumping rate and filtering efficiency, which is consistent with my finding that food availability did not differ across depths. The filter feeding strategy of *A. lacunosa* may be unique in the context of other benthic filter feeders in that its pumping rate but not filtering efficiency is affected by size and that neither pumping rate nor filtering efficiency are affected by depth. Further investigations are needed to learn more about the biology of *A. lacunosa* and its significance to Caribbean coral reefs.

**Keywords** Tube length • InEx • Porifera

**Introduction**

Sponges play a significant role in coral reef ecosystems. They provide an important link between the benthic and pelagic environments (Bell 2008). By removing food particles containing carbon and other nutrients, sponges can have a substantial impact on pelagic ecosystems due to the large volume of water that they filter each day (Bell 2008). For example, the sizeable sponge population in Florida Bay was found to potentially control phytoplankton blooms, and a study showed that pervasive blooms coincided with sponge community decimation (Peterson et al. 2006). However, relatively little is known about the morphology, physiology, and molecular biology of sponges, making them one of the most understudied marine phyla (Dunn et al. 2015).

As suspension feeders, sponges can filter food particles from the water column both actively and passively through current-induced flow (Vogel 1977). There is a positive relationship between water temperature and pumping rate, but little is known about the relationship between depth and pumping rate (Riisgard et al. 1993). Additionally, a study conducted in Florida indicated that food availability increases with depth, but a review on evidence for food limitation of Caribbean sponges found there to be no patterns in sponge abundance that would suggest depth-dependent differences in food availability (Lesser 2006; Trussel et al. 2006; Pawlik et al. 2015).

Fundakowski (2014) suggested that different sponges have different filter-feeding strategies. This study found that pumping rate
and filtering efficiency of the Caribbean tube sponge *Aplysina lacunosa* (convoluted barrel sponge) decreased from shallow to deep depths (Fundakowski 2014). This indicates that *A. lacunosa* pumps faster and filters more efficiently at shallow depths, suggesting that this species may be specialized for shallow depths across the 10-30 m range.

The results of Fundakowski (2014) suggest that depth influences sponge function, but sponge size was not addressed as a possible confounding factor. Little is known about the effects of sponge size on pumping rate and filtering efficiency. Lesser (2006) found that tube sponges at greater depths exhibit greater biomass, rates of growth, and feeding. Another study on the barrel sponge *Xestospongia muta* suggested that there is a positive relationship between sponge size and pumping rate for this particular species (McMurray et al. 2014). The authors suggested that more tissue could imply the presence of more choanocyte chambers, and therefore greater filtering efficiency (McMurray et al. 2014).

The present study aimed to further the investigation of Fundakowski (2014), specifically on the shallow-specialized filter feeding strategy of *A. lacunosa* and how size influences pumping rate and filtering efficiency of this species. I measured the size, pumping rate, and percent reduction in turbidity (filtering efficiency) of *A. lacunosa* tubes in shallow and deep depth categories and examined the relationships between these variables. The following hypotheses were posed:

H$_1$: Depth will have a negative relationship with the pumping rate and filtering efficiency of *A. lacunosa* (consistent with Fundakowski (2014))

H$_2$: Sponge size will have a positive relationship with the pumping rate and filtering efficiency of *A. lacunosa*

**Materials and methods**

**Study site and study organism**

Data were collected at Yellow Submarine dive site, Kralendijk, on the west coast of Bonaire, Dutch Caribbean (Fig. 1). Sponges used in the study were located within two depth ranges on the reef slope: 6-12 m (shallow) and 20-26 m (deep) (Fundakowski 2014; Trussel et al. 2006). To determine food availability at the site in deep and shallow depths, a 50-mL water sample was taken from each depth category during each data collection session and the relative turbidity of the samples were measured in relative fluorescence units (RFU) using a Turner Designs Trilogy Laboratory Fluorometer (Giampetro 2011). Depth was not found to significantly influence water column turbidity ($t_{1,16} = 0.4125, p = 0.6855$).

**Fig. 1** Map of Bonaire, Dutch Caribbean. Study site (Yellow Submarine dive site) is indicated by the black dot (12°09′36.3″N, 68°16′54.9″W)

*Aplysina lacunosa* was selected for this study due to its abundance at the study site and presence in both depth categories (Fundakowski 2014). *Aplysina lacunosa* is an upright tube sponge, often with multiple tubes extending from a single base, generally less than 0.3-1.0 m in height (Fundakowski 2014). This species is mustard yellow in color and lumpy in texture. Individuals are often covered in filamentous macroalgae, although this is
more frequent in the shallow depth range (Fundakowski 2014).

To decrease the likelihood of sampling the same sponge twice, I used natural landmarks during data collection sessions so that each dive was conducted in a different area of the site. Additionally, photographs of each sponge cluster used in the study were taken to aid in recognition of individuals. Sponge clusters were arbitrarily chosen for data collection. Within each sponge cluster, the most landward tube, the most central tube, and the least landward tube were selected for measurement and sampling. Tube length \( L \), outer diameter of the osculum \( d_{out} \), and inner diameter of the osculum \( d_{in} \) for each tube were measured in situ in centimeters. The wall thickness of each sponge tube was calculated as the difference between \( d_{in} \) and \( d_{out} \) divided by two. An approximate volume of the sponge tube tissue \( V_{tube} \) was calculated assuming the sponge tube was a hollow cylinder:

\[
V_{tube} = \pi \left( \frac{d_{out}}{2} \right)^2 - \pi \left( \frac{d_{in}}{2} \right)^2 \times L
\]

Pumping rate and filtering efficiency

A GoPro Black Hero 3+ was attached to a slate such that the camera faced the slate with adequate space between the camera and the slate for the sponge osculum to be filmed at a rate of 60 fps (frames per second). The portion of the slate in the field of view of the camera was covered in black duct tape and a dark blue ruler was taped to the slate for scale. A velcro strap was added to the slate to secure the sponge osculum, preventing the camera from excess movement while filming. Following Yahel et al. (2005), fluorescein dye was ejected directly into the sponge tube via a syringe and needle poked through the tube wall, and filmed as the dye exited the osculum. Frame-by-frame analysis of the dye front movements in QuickTime Player was used to determine the speed of the excurrent flow. This, along with the circular area of the osculum (calculated using the inner osculum diameter), was used to calculate the excurrent flow rate in cm\(^3\)/s, referred to as ‘pumping rate’.

A modified InEx method was used to determine filtering efficiency (Yahel et al. 2005). SCUBA divers collected 30-mL water samples simultaneously in syringes next to the ostial surface and at the top the osculum, taking care not to touch the sponge with the syringes. Samples were analyzed for relative turbidity and percent reduction in turbidity was calculated.

Data analysis

Six separate analyses of covariance (ANCOVA) were used to examine the effects of a single categorical variable (depth) and three continuous variables (tissue volume, wall thickness, and tube length) on two dependent variables (pumping rate and filtering efficiency). Additionally, three t-tests were used to examine the effect of a single categorical variable (depth) on three continuous variables (tissue volume, wall thickness, and tube length).

Results

The length, outer diameter, and inner diameter of each sponge tube used in the study were measured in order to calculate approximate tissue volume and wall thickness of each tube. Depth was not found to significantly influence sponge tube tissue volume \( (t_{1,48} = 0.4137, p = 0.6810) \), wall thickness \( (t_{1,48} = 1.5496, p = 0.1278) \), or tube length \( (t_{1,48} = 0.7862, p = 0.4356) \).

Sponge tube pumping rate was determined by filming fluorescein dye exiting the osculum. Three dye front movements were measured frame-by-frame for each sponge tube and averaged to obtain the excurrent speed, which was multiplied by the cross-sectional area of the osculum to obtain the pumping rate. Pumping rate was positively correlated with sponge size in both depth ranges (Fig. 2). Pumping rate was affected by tube tissue volume, wall thickness, and length but not
depth (Table 1; Fig. 2). Length seemed to be the most influential size factor, accounting for much of the pumping rate response resulting from differences in tissue volume (Table 1).

Filtering efficiency was determined by calculating the percent reduction in turbidity between water samples taken simultaneously at the ostia and osculum. Filtering efficiency was not significantly affected by tube tissue volume, wall thickness, length, or depth (Table 2; Fig. 3).

**Fig. 2** Effect of sponge tube (a) tissue volume, (b) wall thickness, and (c) length on pumping rate in shallow (gray squares, n=23) and deep (black circles, n=27) depth categories. Linear trendlines were plotted for both depth categories (shallow=gray, deep=black). For tissue volume, deep $R^2=0.58868$ and shallow $R^2=0.45835$. For wall thickness, deep $R^2=0.14025$ and shallow $R^2=0.00048$. For tube length, deep $R^2=0.30431$ and shallow $R^2=0.31394$.

**Fig. 3** Effect of sponge tube (a) tissue volume, (b) wall thickness, and (c) length on % reduction in turbidity for shallow (gray squares, n=23) and deep (black circles, n=27) depth categories. Linear trendlines were plotted for both depth categories (shallow=gray, deep=black). For tissue volume, deep $R^2=0.0047$ and shallow $R^2=0.0005$. For wall thickness, deep $R^2=0.00048$ and shallow $R^2=0.00419$. For tube length, deep $R^2=0.02431$ and shallow $R^2=0.00419$.
Discussions

This study aimed to examine the effects of depth and tube size on the pumping rate and filtering efficiency of *A. lacunosa*. The results show an increase in pumping rate with increasing sponge tube size, supporting the hypothesis that size and pumping rate have a positive relationship in *A. lacunosa*. Tube length had a stronger influence than wall thickness on pumping rate, indicating that the volume of sponge tube tissue and subsequent pumping rate is largely dependent on the length of the tube. McMurray et al. (2014) suggested that greater tissue volume could imply the presence of more choanocyte chambers.

### Table 1 ANCOVA results for the effects of sponge size and depth on pumping rate (n=23 for shallow, n=27 for deep)

<table>
<thead>
<tr>
<th>Tissue volume</th>
<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth</td>
<td>1</td>
<td>124.8</td>
<td>124.8</td>
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<td>0.8354</td>
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<td>Volume</td>
<td>1</td>
<td>156563.2</td>
<td>156563.2</td>
<td>54.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Depth*volume</td>
<td>1</td>
<td>7212.1</td>
<td>7212.1</td>
<td>2.52</td>
<td>0.1191</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Wall thickness</th>
<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth</td>
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<td>4892</td>
<td>0.86</td>
<td>0.3577</td>
</tr>
<tr>
<td></td>
<td>Thickness</td>
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<td>27148.6</td>
<td>27148.6</td>
<td>4.79</td>
<td>0.0337</td>
</tr>
<tr>
<td></td>
<td>Depth*thickness</td>
<td>1</td>
<td>7446.2</td>
<td>7446.2</td>
<td>1.31</td>
<td>0.2577</td>
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</table>

<table>
<thead>
<tr>
<th>Length</th>
<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
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<tr>
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<td>83887.8</td>
<td>18.85</td>
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<tr>
<td></td>
<td>Depth*length</td>
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<td>6727.2</td>
<td>6727.2</td>
<td>1.51</td>
<td>0.2251</td>
</tr>
</tbody>
</table>

Significant p-values (p<0.05) are in bold.

### Table 2 ANCOVA results for the effects of sponge size and depth on % reduction in turbidity (n=23 for shallow, n=27 for deep)

<table>
<thead>
<tr>
<th>Tissue volume</th>
<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>241.5</td>
<td>241.532</td>
<td>0.72</td>
<td>0.4002</td>
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<tr>
<td></td>
<td>Volume</td>
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<td>44.9</td>
<td>44.9</td>
<td>0.13</td>
<td>0.716</td>
</tr>
<tr>
<td></td>
<td>Depth*volume</td>
<td>1</td>
<td>191.3</td>
<td>191.27</td>
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<td>0.4537</td>
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</table>

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<th>Wall thickness</th>
<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth</td>
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<td>239.1</td>
<td>239.061</td>
<td>0.7</td>
<td>0.4059</td>
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<tr>
<td></td>
<td>Thickness</td>
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<td>9.1</td>
<td>9.101</td>
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<td>0.8707</td>
</tr>
<tr>
<td></td>
<td>Depth*thickness</td>
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<td>12</td>
<td>12.012</td>
<td>0.04</td>
<td>0.8517</td>
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<table>
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<th>Sources of variation</th>
<th>DF</th>
<th>Sum sq.</th>
<th>Mean sq.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1</td>
<td>242.9</td>
<td>242.893</td>
<td>0.72</td>
<td>0.4008</td>
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<td></td>
<td>Length</td>
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<td>20.7</td>
<td>20.69</td>
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</tr>
<tr>
<td></td>
<td>Depth*length</td>
<td>1</td>
<td>91.1</td>
<td>91.107</td>
<td>0.27</td>
<td>0.606</td>
</tr>
</tbody>
</table>
enhancing sponge filtering efficiency. Thus, it was hypothesized that size would have a positive relationship with filtering efficiency; however, the results do not support this hypothesis because no significant relationship was found between these two variables. This could be because wall thickness varied relatively little among sponge tubes measured (wall thickness ranged from 0.75-2.25 cm while length ranged from 8-76 cm), hence water that was pumped by the tubes travelled through approximately the same amount of choanocyte-containing tissue before entering the spongocoel and exiting through the osculum. This would result in negligible differences in filtering efficiency given the narrow range of wall thicknesses recorded. Studies on mussels have found pumping rate to be dependent on exhalant siphon area but not shell length (Maire et al. 2007; Riisgard et al. 2011). However, Riisgard et al. (2011) documented a positive relationship between filtering efficiency and shell length for the mussel *Mytilus edulis*. Mussel gill area is also positively related to shell length, so an increased gill area would enhance mussel filtering ability (Riisgard et al. 2011). Because bivalves filter water through one siphon while sponges filter through multiple ostia, it is not surprising that filtering efficiency would increase with size for mussels but not sponges.

Depth was found to have no significant influence on pumping rate and filtering efficiency of *A. lacunosa*. This finding does not support the hypothesis that depth has a negative relationship with pumping rate and filtering efficiency (Fundakowski 2014). Further, this finding is inconsistent with previous findings that pumping rate is slower and filtering efficiency decreases at deeper depths (Fundakowski 2014). This inconsistency could be due to the previous study not controlling for size or accounting for size in its statistical analyses. Additionally, the finding that depth does not influence sponge size is inconsistent with previous findings that suggested that tube sponges (*Callyspongia vaginalis*, *Agelas conifera*, and *Aplysina fistularis* from Florida, Belize, and the Bahamas, respectively) at greater depths exhibit greater biomass, rates of growth, and feeding (Lesser 2006). Because *C. vaginalis*, *A. conifera*, and *A. fistularis* are of similar morphology to *A. lacunosa*, it is interesting that *A. lacunosa* does not exhibit similar trends in feeding. Lesser (2006) also found that food availability increased with depth in those environments, which corresponded with the increased sponge growth and productivity. Because depth was not found to influence food availability in the present study (consistent with Pawlik et al. (2015)), there was no reason to expect depth to affect filtering efficiency of *A. lacunosa*. Different species evolve in the context of different depths. Because the environmental conditions were consistent across depths at the site of the present study, no difference in filtering efficiency was expected. The results suggest that, contrary to Fundakowski (2014), *A. lacunosa* might not be specialized for shallow depths.

An additional factor that is confounded with depth and could influence sponge function is temperature. Riisgard et al. (1993) found that sponges pump and filter faster in warmer temperatures. Coma et al. (2002) found that sponge metabolic rate increases as temperature increases, which would warrant the need for greater energy acquisition and therefore greater filtering efficiency. Since temperature generally decreases with depth, this is in accordance with the finding that shallow sponges expend more energy than deep sponges (Trussel et al. 2002). However, temperature in a geographically and bathymetrically similar island to Bonaire (Curaçao) is consistent across the 6-26 m depth range so pumping rate and filter efficiency were likely not influenced by this factor (Bongaerts et al. 2015).

Size and depth have significant effects on the pumping rate and filtering efficiency of other types of benthic filter feeders, but *A. lacunosa* could be unique within the context of other sponges and benthic filter feeders in that its pumping rate but not filtering efficiency is affected by size and that neither pumping rate nor filtering efficiency is affected by depth.
(Coma et al. 2002; Lesser 2006; Riisgard et al. 2011). Additional studies are needed to examine the effects of depth on \textit{A. lacunosa} feeding in order to learn more about their biology. Additionally, future studies might investigate the effects of time of day on \textit{A. lacunosa} pumping rates and filtering efficiency. More knowledge of sponge filter feeding will increase our understanding of their important role in Caribbean coral reef ecosystems as a link between the benthic and pelagic environments.

Acknowledgements I would like to thank CIEE Research Station Bonaire for providing the resources necessary to complete this project. Thank you to Dr. Patrick Lyons and Nathaniel Hanna Holloway for their guidance and support throughout this project, and to Hattie Lighte for help with my video analysis. Additional thanks to Nathaniel for being my dive buddy and assisting with my data collection.

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Coral bleaching frequency and recovery during the 2015 El Niño- Southern Oscillation event in Bonaire, Dutch Caribbean.

Abstract El Niño Southern Oscillation (ENSO) events are known to bring high sea surface temperatures and in turn cause coral bleaching. The Fall 2015 ENSO event has had record breaking temperatures and has been severely detrimental to Pacific coral reef ecosystems. To gauge the effect this ENSO event would have on the Caribbean, this study looked at the frequency and severity of bleaching and paling, during this ENSO event. The bleaching was measured along a 2 m wide by 10 m long transect. Coral colonies along the transect were observed once a week for four weeks and the water temperature was recorded hourly. At the end of data collection, the overall number of corals experiencing bleaching was recorded and the percent difference in paling and bleaching from week to week was measured. At the end of the four weeks it was found that 60 out of 192 coral colonies were experiencing some form of bleaching. By the fourth week there was no significant increase in bleaching, and paling had significantly increased until week four. This trend followed the decrease of water temperature from week one to week three with signs of coral recovery, but there was also evidence of water temperature starting to increase again by week four. This study shows the resilience of Bonairean reefs and that this ENSO event may have a lesser affect on the Caribbean coral reefs compared to the Pacific.

Keywords ENSO • bleaching • rebrowning

Introduction

El Niño- Southern Oscillation events (ENSO) occur when trade winds, going west across the pacific, stall causing no upwelling to occur in the East Pacific. This is from an irregular oscillation in the tropical ocean-atmosphere system. With this lack of wind pushing warm waters westward, the warm sea surface water flows eastward. This causes anomalies in the ocean sea surface temperature, making the water warmer than average in the Pacific and the Northern Atlantic (Neelin et al.1998). Higher-than-average sea surface temperatures have been shown to be a major stressor on marine life, specifically corals (Li and Reidenbach 2014).

Coral reef bleaching has been attributed to differences in sea surface temperature (Siegel et al. 2011). This event is known as bleaching, for the pure white color the corals become after losing their zooxanthellae. If prolonged, bleaching can result in partial or complete coral mortality, due the loss of the essential energy from their symbiotic relationship (Eakin et al. 2010).
Bleaching is a cause for concern due to the negative effect coral mortality can have on the health of a reef. Dead, bleached coral is the perfect substrate for algae to grow upon. An increase in algae can have a strong impact on coral reef health by outcompeting coral for space, blocking their sunlight for photosynthesis or even causing suffocation from growing onto live coral (Steneck 2015). In any of these scenarios a phase shift may eventually occur if enough coral die, turning the coral reef into an algae dominated reef (Nugues and Bak 2006). Along with coral mortality, macroalgae also inhibits the recruitment of juvenile corals, giving the bleached reef little chance for recovery (Tamai and Sakai 2013). Major bleaching can also have an effect on the future rugosity of a coral reef by exposing coral skeletons to erosion and turning branching corals to rubble (Alvarez-Filip et al. 2011). Rugosity, as a proxy to structural complexity, helps to maintain a rich ecosystem by giving refuge for juveniles and showing greater amounts of diversity and recruitment (Wilson 2015).

Mass coral bleachings have been linked to abnormal spikes in sea surface temperature and high levels of light or photosynthetic active radiation (Sridhar et al. 2012). For this reason the Caribbean is considered a ‘hot spot’ for bleaching events. The Caribbean has experienced a few major bleaching events in 1998, 2005 and 2010 (Jekielek 2011). All these bleaching events occurred during ENSO years. The 2005 event was severe, causing 85% of the coral cover in the Netherlands Antilles to be bleached (Donner et al. 2007). In 1998 Bonaire saw 15% of corals being bleached, but nearly 100% of Agaricia above 30m had some level of bleaching (Wilkinson 1998). The 2010 event was less severe, but in Bonaire there was a 10% coral bleaching mortality (Steneck et al. 2015) which still caused a great decrease in rugosity in certain regions of Bonaire (Wilson, 2015). While this shows that bleaching in the Caribbean does not occur with every ENSO, it still shows that ENSOs increase the risk of bleaching and the consequences that come along with it.

As of right now (Fall 2015) an ENSO event has moved across the Pacific and is reaching the Caribbean. According to the National Ocean and Atmospheric Administration’s (NOAA) Coral Reef Watch, the Caribbean and specifically the Netherlands Antilles have around a 90% chance of having an alert level 1 or higher for coral bleaching in October 2015 through January 2016 (http://coralreefwatch.noaa.gov/satellite/bleachingoutlook_cfs/outlook_cfs.php, September 26, 2015). This ENSO is considered by NOAA to be one of the strongest in the past century. In August, the sea surface temperature was 1.49˚C above average, second to only the 1997-1998 ENSO, which also hit Bonaire. Bleaching has also occurred all over the Pacific. Solomon Islands, Vanuatu, Tuvalu, Fiji, the Samoas, British Indian Ocean Territory and the Maldives all experienced ENSO events as of June 2015 (http://coralreefwatch.noaa.gov/satellite/analyses_guidance/global_bleaching_update_20150602.php, September 26, 2015). With warnings going on around Bonaire and bleaching being observed on site, this study intended to see how progressive this bleaching could be in four weeks time.

Bonaire reefs are considered to be some of the healthiest reefs in the Caribbean, based on coral cover and biodiversity (Hawkins et al. 1999). They also show signs of recovery from bleaching, like the 2010 bleaching event, which has not been seen in many other places in the Caribbean. This can be due to the fact that Bonaire is still dominated by coral reefs rather than algal dominated coral reefs, unlike most other areas in the Caribbean. In the annual Bonaire reef monitoring it was found at the sites observed that coral cover was nearly 50%. It also showed that there has been a steady increase in juvenile corals found since past bleaching events, showing that Bonaire reefs have a steady resilience, which is very important to coral reef health (Steneck et al. 2015).

For this study it was hypothesized that:

$$H_1: \text{There will be a significantly greater number of corals suffering from bleaching by the end of the four week study compared to the beginning}$$
H₂: By the end of the study the bleaching observed will be significantly more severe than the bleaching at the start. It is important to record these findings because they can help to see how much damage the 2015-2016 ENSO will do to even a small part of Bonaire, as well as keep records of how fast this ENSO hit and how the temperature played a role in this effect.

Materials and methods

Study Site

The site used for this study is in downtown Kralendijk, Bonaire (Fig. 1) at a reef currently known as Yellow Submarine (12°9'3620''N 68°16'5525''W). There is a 30 m sand flat before the reef crest can be reached and the reef slope descends at a 45° angle. The reef crest starts at ~10m and the reef ends at around 30 m. The coral reef is dominated by Orbicella faveolata and Orbicella annularis.

Field Methods

At the site one 10m transect was laid. The transect was laid in an area with distinct features and marked with three equidistant rebars. A 2m wide transect was observed twice a week using T-bars. Due to SCUBA limitations, the length of the transect was equally divided into two, 5m sections for the two weekly sampling days. Pictures were taken of all corals with signs of bleaching for further analysis in the laboratory and to keep a record of which corals were previously bleached each time. Records were also taken of coral size, new mortality and old mortality. The bleaching was measured for severity using the Coral Health Chart (Coral Watch non-profit organization), where the severity of the bleaching is ranked from one to six, six being perfectly healthy and one being completely bleached. The most severe point on the coral, the palest point, was the spot measured, as well as the darkest spot on the coral. Water temperature was recorded hourly for the duration of the project using a self-sustained HOBO data logger from Onset.

Data Analysis

A student-t test was done to test if there was a significant increase in the amount of coral suffering from bleaching and if there was a significant increase in the amount of paling the coral colonies were experiencing. Water temperature was analyzed by using a moving average.

Results

Increase in Number of Bleached Individuals

From week one to week four there was an 11.5% increase in the number of coral colonies experiencing bleaching or paling (n = 192) (Fig. 2). Each week the number of colonies experiencing bleaching increased by an average (± SD) of 6.3 ± 4.9. The greatest increase was from week one to week two with a 6.3% increase and a total of 41 to 57 colonies.
Severity

From week one to week three there was an increase in the number of paling and bleached individuals (Fig. 3 and Fig. 4). During week three, the peak amount of paling was reached with an average of 33%. During week four, paling began to decrease with an average of 32.3% (Fig. 3). Week one had the lowest bleaching average at 5.0%, but the highest bleaching average (in week four) was only 0.3% greater than week one (5.3%).

In the fourth week some of the coral colonies also began to show signs of recovery (Fig. 5). Using a student- t test it was found that week three had a significantly greater amount of paling (df = 40) (p < 0.001) compared to week one, but from week three to week four there was no significant difference found. There was also no statistical significant difference found in the estimated percent of bleaching between any of the weeks.

Water Temperature

The average water temperature through all the weeks was 28.7°C. Through the four weeks the average temperature of the water decreased (week one 29.1°C; week two 28.7°C; week three 28.6°C) until week four (28.7°C), where the average began to rise again (Fig. 6).

Fig. 3 Percent difference in paling in coral colonies from (A) week one to week two, (B) from week two to week three and (C) week three to week four. Percent change was only calculated for colonies found in week one of sampling in Bonaire, Dutch Caribbean

Fig. 4 Percent difference in bleaching in coral colonies from (A) week one to week two, (B) from week two to week three and (C) week three to week four. Percent change was only calculated for colonies found in week one of sampling in Bonaire, Dutch Caribbean

Fig. 5 Example of (row A) coral bleaching and (row B) coral recovery from week one to week four observations
Discussion

The number of coral experiencing bleaching increased from 41 to 60 colonies by the fourth week supporting the hypothesis that a frequency increase would occur through the four weeks. However, the increase in the estimated amount of bleaching and paling was no longer significant by the fourth week, meaning that the hypothesis stating that severity would increase was not supported.

While it was found that the ENSO event did raise surface sea temperature it is not likely that it caused severe bleaching within the Bonaire region. From the data found at Yellow Submarine there was a slight bleaching event, but nothing that could be considered severe or detrimental to the reefs overall health. The results found that while bleaching increased from week one to week four it was not a statistical significant amount. Also, paling did increase significantly from week one to week three, but by week four the increase was no longer significant. This could be due to the fact that the temperature decreased from week one to week three and then began to rise again by week four. It was shown by Tolleter et al. (2013) that once temperature decreases, corals can continue to bleach for a period of around a week before beginning to rebrown (opposite of bleaching). The results also showed that while there was rebrowning, some of the corals continued to pale and bleach. This could be because different corals have different tolerance to heat stress from the difference in the clades of Symbiodinium (zooxanthellae) they accept.

It has been found that the C1 clade of Symbiodinium has the highest heat tolerance, producing less toxic reactive oxygen compared to clades A1 and B1 (Hawkins and Davy 2012). This means that with clade C1 the corals have less of a chance of expelling their zooxanthellae under heat stress. Also, some corals may be more successful at recruiting stress tolerant clades once they remove the more ‘undesirable’ Symbiodinium. It has been hypothesized that corals expel zooxanthellae during heat stress so they can gain more heat tolerant zooxanthellae, (Silverstein et al. 2015). It would be interesting to continue with this study to see if the
rebrowned corals would bleach again since the temperature began to rise again by week four.

It was interesting to see rebrowning occur due to the fact that the temperature never returned to the present Caribbean average of 27°C (Sheppard and Rioja-Nieto 2005), but only reached the lowest temperature of 28.27°C during week three. In the Tolleter et al. (2013) paper the corals they experimented on showed signs of rebrowning when they brought the temperature back down to 27°C, under laboratory controlled settings. It may be that corals at Yellow Submarine can still thrive within temperatures greater than 1-2°C above average and perhaps past bleaching events have given them the chance to create more resistant holobionts with more heat tolerant zooxanthellae in order to withstand these greater temperatures.

Varying success among coral species could also explain the presence of continual bleaching and rebrowning. For example Fig. 5 shows the rebrowning of *Porites astreoides*, a thermal tolerant coral (Kenkel et al. 2015), and *Orbicella faveolata*, a coral that requires much more stable environments. This could be the cause for the variation in recovery and further bleaching. While it may not have been done, the data collected could be quantified to test the frequency of bleaching and rebrowning within different coral species. It could be that thermal tolerant corals have better resilience to bleaching, while less tolerant corals have a harder time recovering from heat stress.

Bleaching also leaves corals exposed to disease. Along the transect of this study there were many cases of dark spot disease, which is a common disease on Bonaire. It has been found that dark spot disease is not density-dependent (Mathe 2015). Mathe (2015) believes that the spread of dark spot disease may come from opportunistic pathogens emerging under stressful environmental conditions. The stress of thermal pressure and bleaching may leave corals more susceptible to contracting dark spot disease. If further observations of this site were taken it may have been found that this disease increased in frequency and severity along the transect.

The results of this study concluded that Bonaire experienced record breaking average sea surface temperatures (1.7°C above average) during the 2015-2016 ENSO event, but it did not cause any severe bleaching. It is difficult to say if it did increase bleaching within the area, but future studies should look at bleaching frequencies and severities during years not effected by ENSO events. It is safe to say that the reef studied showed good signs of resilience with rebrowning occurring in some corals and no full coral mortality observed over the course of this four-week study. The results support the theory that Bonaire has some of the healthiest reefs in the Caribbean (Hawkins et al. 1999). Further work should investigate bleaching in the Bonaire region for this reason. If resilience to bleaching can be understood, it can help conservationists understand how to aid coral reef ecosystems in future bleaching events.

**Acknowledgements** I would like to thank SUNY ESF and CIEE for giving me this amazing research experience. Dr. Arboleda and James Emm for their guidance and assistants. My dive buddy, Jess Hutnick, for patiently doing observations with me. I would also like to give my gratitude to the great CIEE team for their endless knowledge. My friends Maggie Myers, Carlie Sharps and Mckenna Becker for giving me endless laughter and a sane mind. Lastly I would like to thank my Mother for helping to give me this opportunity and for her loving support.

**References**


Fluorescent patterns, size, and abundance of the bearded fireworm *Hermodice carunculata* in the intertidal zone on Bonaire

**Abstract** *Hermodice carunculata*, commonly known as the Bearded Fireworm, is a corallivorous Polychaete found throughout the Atlantic Ocean and the Caribbean and is noted for its fluorescence. Studies have found that the highest abundance of *H. carunculata* is in water shallower than 1 m. The present study observed the habitat, size, and fluorescent patterns of *H. carunculata* in the intertidal zone of Yellow Submarine dive site on Bonaire. Three transects were laid at 55 cm and 110 cm deep, at 20 and 50 minutes after sunset. Additionally, fireworms were caught in wire traps to be more closely observed in the laboratory under a dissecting microscope.

There was no significant difference between the depth (110 cm or 55 cm) and the size (less than or greater than 6 cm), nor was there a difference in abundance between the two time periods of data collection (20 minutes and 50 minutes after sunset). Furthermore, there was no significant difference between the fluorescent pattern (GREEN, GOB, OOB, or ROB) and the substrate (algae, coral, rubble, rock, or sand) the individual was found on, or fluorescent pattern and size. There was, however, a significant difference in density of fireworms per square meter over the five-week study period. Fireworm predation can have a large impact on the health of corals. This paper aims to increase the understanding of *H. carunculata*, so that the corals can be better protected, and the interaction between these two organisms can be better understood.

**Keywords** Polychaete • fluorescence • morphology

**Introduction**

*Hermodice carunculata*, commonly known as the Bearded Fireworm, is a Polychaete that is found throughout the Atlantic Ocean and the Caribbean (Ahrens et al. 2013). They are most abundant in water shallower than one meter deep, and are typically found on sand, rubble, coral, and algae (Wolf 2012). However, they can also be seen in the sand flats, on the reef crest, and on the reef slope. According to Wolf (2012), there is an ontogenetic shift in their habitat as they grow, moving from shallow to deeper areas. Trauth (2007) found that at depths of 2 m and 6 m there were worms of all sizes. However, at a depth of 15 m, the abundance of fireworms smaller than 3 cm was significantly less than the abundance of fireworms larger than 3 cm (Trauth 2007).

Very little is known about *H. carunculata* that does not directly relate to their corallivorous nature. These fireworms are nocturnal, and therefore feed on coral polyps during the night and hide under rocks during the day (Fine et. al 2002). Bearded fireworms are omnivorous scavengers that are most active at night (Marsden 1962) and are typically noted for their fluorescence. However, genetic testing has revealed that despite the fluorescence seen in *H. carunculata*, its genome does not contain any known fluorescent proteins (Mehr, et al. 2015). Although the source of the fluorescence is unknown (i.e. biological or mineral), distinct patterns of fluorescence are present.

Bearded fireworms are segmented annelids with setae (bristles) that extend off the segments and create a stinging sensation upon contact. In addition to these bristles, the dorsal...
side of the segments has gills. Together, they form the notopodia (Marsden 1966). Another important structure is the neuropodia, which are the “feet” of the fireworm (Marsden 1966). The neuropodia are on the ventral side of the segments. The whole structure that contains the neuropodia and the notopodia is called the parapodia. The present study provides more information about this corallivorous predator by explaining the fluorescence patterns.

This study is based on the previous research done on Bonaire by Hillenbrand (2013) and Trauth (2007) in which the size, depth, fluorescent patterns, and habitat of *H. carunculata* were observed on the reef and in the sand flats. Trauth (2007) found that fireworms are commonly found on sand, rubble, and coral; however, they are also seen on algae, sponges, and decaying material. The relationship between fluorescence and size was studied by Hillenbrand (2013), in which green fluorescence (GREEN) was seen most commonly in worms that were less than 3 cm, orange segment fluorescence with green fluorescent bands (GOB) was most commonly seen in worms that were 3 to 6 cm long, and the highest proportion of worms displaying the fluorescence pattern of an orange body with orange bands (OOB) were 6 to 9 cm in length.

Through field surveys in the intertidal zones and detailed laboratory observations, this project further examined the relationship between size, habitat, and fluorescence, as well as compared fluorescence between habitats.

H1: *Hermodice carunculata* will be more abundant in the intertidal zone than the sand flats
H2: *Hermodice carunculata* will be more abundant in the intertidal zone than the reef crest
H3: *Hermodice carunculata* will be more abundant 50 minutes after sunset than 20 minutes after sunset
H4: The green fluorescence pattern will be more abundant among individuals that are less than 6 cm long
H5: There will be no difference in abundance of the GOB fluorescence pattern among individuals that are greater than or less than 6 cm
H6: The OOB fluorescence pattern will be more abundant among individuals that are greater than 6 cm long
H7: There will be no difference between fluorescence and the substrate on which the individual is found

### Materials and methods

**Study Site**

The abundance, size class, habitat, and fluorescent patterns of *H. carunculata* were studied in the intertidal zone just north of the Yellow Submarine dive site (12°09'36. 5"N 68°16'54. 9"W) on Bonaire in the Dutch Caribbean (Fig. 1). This site is located on the north side of Kralendijk and is next to the road Kaya J.N.E. Craane. The substrate of the intertidal zone is made up mostly of a sand, rubble, and rock. There is an abundance of macroalgae as well as many coral recruits.

![Fig. 1 Map of Bonaire showing Kralendijk, where this study took place](image)
Field Work

Sampling Transects

Three consecutive 10 m transects were completed at two different depths: 55 cm and 110 cm, for a total of six transects to ascertain the abundance of fireworms (Fig. 2). The transects were performed starting at 20 minutes and 50 minutes after sunset (Hillenbrand 2013). Two researchers performed the 55 cm transects and 110 cm transects simultaneously in order to account for differences in abundance related to time. The first transect began 20 minutes after sunset and the next two transects began immediately following the completion of the one before. The same six transects were repeated beginning 50 minutes after sunset.

The transects were performed at these times in order to compare them to the study done by Hillenbrand (2013), on the sand flats and coral reef at the same site.

Data Collection

Fireworm sightings were recorded within a 1 m wide transect using a t-bar as reference. For each fireworm, the size class (greater than or less than 6 cm), color class (green, green body with orange bands, or orange body with orange bands), and the substrate it was found on (sand, rock, algae, coral or rubble) were recorded. The size class was determined using the ruler at the end of the t-bar and the fluorescent pattern was detected using BlueStar flashlights with yellow barrier filters (Nightsea).

Organism Collection

In order to view their fluorescence more closely, fireworms were collected using wire traps after sunset, approximately 40 m north of Yellow Submarine dive site (Hillenbrand 2013; Trauth 2007). The traps were baited with lionfish meat and placed approximately one-meter deep. After 45 minutes, the traps were removed. The traps were immediately brought back to the laboratory and the fireworms were placed in an aquarium.

Laboratory Work

Organism Examination

Each fireworm was extensively studied to determine its maximum length, fluorescent pattern, and fluorescence related to its morphology. Each individual was placed into a petri dish with seawater and MgCl$_2$ as an anesthetic, and placed under a dissecting microscope with a Stereo Microscope Fluorescence Adapter (Nightsea). The fluorescence on each structure of the body (mouth, gills, segments, neuropodia, and notopodia) was observed.

Data Analysis

The data collected from these transects was analyzed using Chi-squared tests and Analysis of Variance (ANOVA) tests. ANOVA tests were performed to compare the abundance of *H. carunculata* per square meter both dependent and independent of depth. A t-test was used to compare the abundance of fireworms at 20 and 50 minutes after sunset. Chi-squared tests were performed to compare depth and size class, substrate and fluorescent pattern, and size class and fluorescent pattern of the fireworms observed during the transects. The abundance of *H. carunculata* was compared in terms of density per square meter.
Results

Transects

The 12 transects from the 11 days of data collection \((n = 56)\) were analyzed to compare fluorescent patterns, depth, habitat, and size of *H. carunculata* in the intertidal zone. The total abundance of *H. carunculata* in the intertidal zone \((\pm SD)\) was \(1.07 \pm 1.22\) individuals per square meter.

Out of a total of 1052 individuals observed in the field, there were 717 individuals that were less than 6 cm long and 335 individuals that were greater than 6 cm long. Additionally, there were 188 GOB, 805 GREEN, 56 OOB, and 3 ROB fluorescent worms. There was no significant influence of the substrate the fireworms were found on to their size class or fluorescent pattern \((n = 1052, p = 0.99, p = 1)\) independent of depth. However, the majority of the fireworms were seen in the rubble or rock, with 628 and 409 individuals respectively (Fig. 3). When comparing the fluorescent pattern with the size class, there was also no significant relationship \((p = 0.97)\). The depth also had no impact on the size of the fireworms \((p = 0.87)\) (Fig. 4). A t-test revealed no significant relationship between the time after sunset (20 minutes or 50 minutes) and the abundance of *H. carunculata* \((p = 0.37)\) (Fig. 5).

An ANOVA test revealed a significant difference in density of *H. carunculata* per square meter over time \((p = 0.026)\) as well as a significant difference in density per square meter between the two depths (55 cm and 110 cm) over time \((p < 0.01)\). Throughout the data collection period, there was initially a higher density per square meter of *H. carunculata*, followed by a large decrease, and then an increase in density per square meter (Fig. 6).
The density of *H. carunculata* was significantly different from day to day (p = 0.026).

Laboratory

Thirty-four individuals were examined under a dissecting microscope in the laboratory. This observation revealed specific morphological details about the fluorescence of individuals in each color class. On a GOB individual, the orange bands are a distinct color change seen only at the anterior and posterior ends of each segment. The central part of each segment was green, and the ends were orange (Fig. 7A). In an OOB individual, the orange coloration can be seen on the segments themselves, along with the distinct orange band coloration. However, there is a visible change in fluorescence of the tissue at the boundary between the segments and the notopodia, from orange to green (Fig. 7B). The ventral side of the fireworm also contained bands; however, these bands were not necessarily the same color as the dorsal bands, nor were the bands as distinct as the bands on the dorsal side (Fig. 7C). Green bands are also visible on the dorsal side of the GREEN individual (Fig. 7D).

Discussion

Hypotheses 1 and 2 were not supported by the data, as *H. carunculata* was more abundant on the sand flats and coral reef than in the intertidal zone. Hillenbrand (2013) found that the abundance (±SD) of *H. carunculata* on the sand flats was 2.17 ± 1.19 per m² and on the coral reef was 1.97 ± 2.39 per m². However, fireworms were previously found to be more abundant at shallower depths (less than 1 m) than deeper depths (Wolf 2012). The inconsistencies between the results and the literature could be due to the fact that the survey of the intertidal zone was completed two years after surveys of the sand flats and coral reef. Any variation in these ecosystems, such as changes in water temperature, nutrient input, pH, oxygen level, or ocean acidification, could potentially impact the abundance of...
fireworms. These changes to the environment have been seen to have adverse effects on corals, as well as on other marine organisms. This is due to many marine species’ sensitivity to these factors, which can lead to seasonal changes in abundance (Doney et al. 2012).

Hypothesis 3 stated that Hermodice carunculata would be more abundant 50 minutes after sunset than 20 minutes after sunset. This hypothesis was rejected, as the difference in abundance between the two time periods was insignificant. Hillenbrand (2013) found that H. carunculata had no significant difference in abundance on the sand flats from 20 to 50 minutes after sunset (DF 15, p = 0.793). However, there was a significant difference in abundance on the reef crest between these two times. This could be because the sand flats and intertidal zone are less structurally complex, and therefore have fewer places for the fireworms to hide during the day. The places available to hide in the intertidal zone and on the sand flats are a few rocks, coral heads, and rubble. These substrates are fairly small and lack the complexity seen on the reef, meaning that the fireworms would be no require as much time to emerge from hiding in these areas compared to the reef.

Hypotheses 4, 5, and 6 were also rejected, as there was no significant relationship between the fluorescent pattern and size class of the fireworms. However, the only fireworms displaying the ROB fluorescence pattern were greater than 6 cm long. Furthermore, the BOB fluorescence pattern (blue fluorescent segments with orange bands) was only observed in fireworms greater than 6 cm. Hillenbrand (2013) only observed this fluorescent pattern during spawning, and suggested that this may be a terminal coloration related to sexual maturity.

Hypothesis 7 stated that there would be no relationship between fluorescent pattern and the substrate on which the individual is found and was supported by the data. The fireworms were found mostly on the rock and rubble, as that is the most abundant substrate in the intertidal zone of the Yellow Submarine dive site. However, there was no correlation between the fluorescence class and the substrate it was found on. This could be because all fireworms have similar preferences when it comes to substrate, or because some substrates were less abundant than others.

There was no relationship between the size of the fireworms (greater than or less than 6 cm) and the depth they were found at (55 cm or 110 cm), which could be attributed to the fact that the two depths are very similar and in close proximity, and therefore the fireworms could easily move between the two. Furthermore, no ontogenetic shift would be expected between these two depths, as they are both part of the intertidal zone.

There was, however, a significant difference in the density of H. carunculata over the five-week observation period. The density followed a cyclic pattern where it was high initially, then decreased, followed by another increase, and then a final decrease when the observation period ended. This could be a pattern related to spawning, but further research would be needed to better understand these increases and decreases in density. Furthermore, the highest abundance of fireworms was observed at the same time as the new moon, and the following peak in abundance occurred at the same time as the full moon. Further research is needed to determine if the abundance of fireworms in the intertidal zone could be related to the moon cycle.

No known genes encoding for Green Fluorescent Protein (GFP) were found in the genome of H. carunculata, therefore, the fluorescence could be coming from an unknown GFP gene, ingested GFP, or minerals (Mehr, et. al 2015). I would expect that the source of the GFP is biological, based on observation. In the laboratory, some of the segments of a bearded fireworm were heated to 100° C, the boiling point of water. As the temperature increased, the fluorescence became duller and eventually disappeared. The temperature at which the GFP and RFP (Red Fluorescent Protein) disappeared was consistent with studies describing the denaturation of GFP (Alkaabi, et. al 2005).
Further research is needed to better understand the biological source of the fluorescence.

There is little known about the fluorescence of *H. carunculata*, but this study suggests that the fluorescent pattern is not related to the habitat or size of the individual. There was no relationship between the depth (110 cm or 55 cm) and size or abundance and time after sunset (20 or 50 minutes) either. However, this study does suggest that the abundance of fireworms does follow a cyclic pattern over time, which may be related to the moon cycles. Further research is needed to better understand the changes in abundance, the fluorescence, habitat preferences, and differences in fireworms between the intertidal zone, sand flats, and coral reef. Additionally, more studies should be completed to determine if the source of the fluorescence in fireworms is a protein or a mineral. Continuing these studies will increase our knowledge about *H. carunculata*, allowing for a better understanding of the relationship between this organism and the corals they prey upon.

**Acknowledgements** I would like to thank the CIEEBonaire Research Station and Dr. Rita Peachey for providing me with the resources to complete this research project. I would also like to thank Dr. Enrique Arboleda and Sara Buckley for advising me, as well as Madeleine Rhee, Jessica Hutnick, Kate Howard, and James Emm for assisting me with my data collection.

**References**


**Comparing the diversity, total abundance, and richness of fish species associated with two stony corals: Diploria strigosa and Orbicella annularis**

**Abstract** Coral reef environments exhibit numerous ecological interactions between different organisms. The habitat structure of a healthy coral reef is composed of many different coral species, with various fish species inhabiting the reef. Coral reef studies often focus on a large spatial scale rather than smaller local scale environments within the reef. The objective of this study was to compare fish populations associated with the microhabitat surrounding individual coral heads of two different species. The purpose of this study was to determine if there were differences in fish abundance, fish species richness, and fish diversity between two massive stony corals, Diploria strigosa and Orbicella annularis. These two corals are common on many Caribbean reefs but are morphologically different; therefore, it was hypothesized that they would show differences between their associated fish assemblages. By conducting fish count observations on both D. strigosa and O. annularis, I was able to compare means between the coral associated fish populations using statistical tests. No statistically significant differences were found between these two coral species for mean fish abundance, species richness, or diversity. One possible explanation is that the larger scale reef environment and processes may have a significant effect on local fish populations found on individual coral heads. By studying the microhabitats of coral species and the associated fish assemblages, we can gain a better understanding of fish population dynamics of coral reefs across larger ecological scales—both regionally and globally.

**Keywords** Microhabitat • abundance • diversity

**Introduction**

Coral reefs are one of the most biologically diverse ecosystems on Earth, similar to the high diversity seen in tropical rainforests (Connell 1978). The habitat structure of a healthy coral reef is composed of many different coral species. These collections of corals can affect the assemblage of fishes that may be found in the local reef habitat (Harborne et al. 2012). Fish abundance and diversity are important to maintaining health of a coral reef because they fill important ecological roles (Gamfeldt et al. 2008). Different corals affect the rugosity of a reef and change the overall habitat structure (Harborne et al. 2012). Habitat structure of a coral reef can attract and house numerous different fish species in varying abundances (Harborne et al. 2012). Therefore, specific coral morphology (which changes reef rugosity) may have an effect on coral-associated fishes. It has been shown that there is a positive correlation between the diversity of corals on a reef and the associated animals living there (Messmer et al. 2011). Further, certain fish species are seen to have relationships with specific corals, meaning different coral species may attract specific fish species to a reef (Messmer et al. 2011). This suggests that the loss of particular coral species can have an effect on the biodiversity of local fish communities inhabiting a reef (Messmer et al. 2011). Studying how small individual coral heads (microhabitats) attract different fish populations can help understand more about fish population dynamics on a larger spatial scale.
This research leads to the question of whether different coral species attract different species in varying abundances. Therefore, a focus on microhabitat research of coral reefs may allow us to pinpoint specific conservation efforts. Conservation efforts may benefit from focusing on coral species that attract a higher biodiversity or abundance of fish to the reef. By examining fish species on or around specific corals, we may be able to determine how different coral species affect the larger scale reef fish community.

Three parameters are important to analyze to draw conclusions about reef fishes and their relationship with corals: (1) fish abundance, (2) fish species richness, and (3) fish species diversity. Danilowicz (1996) found that most residential reef fish species have limited home ranges. Once they settle on or around a specific coral, they do not travel far their whole life, meaning they must choose a spot that has adequate food and protection from predators (Danilowicz 1996). Therefore, we can hypothesize that certain fish species observed around a coral may have a relationship or association with that specific coral species.

In a study done on damselfish, scleractinian corals, or stony corals, were shown to harbor a wide range of different marine species (Holbrook et al. 2008). Massive stony corals with slow growth rates have been shown to be the primary builders of the reef foundation since they increase rugosity (Alvarez-Filip et al. 2011). *Orcibella annularis* and *Diploria strigosa* are two massive slow growing stony corals commonly found on Caribbean reefs. These stony corals not only help with the reef building process, but also provide protection and food for fish species (Sluka et al. 2001; Cole and Pratchett 2011). *Orcibella annularis* and *D. strigosa* differ in their structure: *O. annularis* forms large lobes with space in between each lobe, while *D. strigosa* grows in a bulbous shape which may provide less structural refuge for larger fishes. Since the morphologies of the species are so different, there may be different fish abundances or species associated with each coral type. Research in the Virgin Islands has shown that inherent properties of coral heads determine settlement of juvenile fish rather than the presence of resident species already there (Wilson and Osenberg 2002). Fish recruits preferred certain inherent properties of different coral species, such as morphological complexity (Shulman 1984). In addition, it has been seen that a greater structural complexity often allows for a greater diversity of fish species (Almany 2004). Therefore, due to the morphological differences between *D. strigosa* and *O. annularis*, there may be differences between the associated fish populations. Based on a collection of previous research, the following hypotheses were developed:

**H₁:** *O. annularis* will have a higher fish abundance than *D. strigosa*

**H₂:** *O. annularis* will have a higher fish species richness than *D. strigosa*

**H₃:** *O. annularis* will have a higher fish diversity than *D. strigosa*

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**Materials and methods**

**Study site**

This study was conducted at “Yellow Submarine” dive site in Kralendijk, Bonaire in the Dutch Caribbean (12°09'36.3"N 68°16'55.0"W) (Fig. 1). Bonaire is located about 65 km north from the coast of Venezuela in the Caribbean Sea. The natural fringing reef at Yellow Sub is easily accessible from a shore entry site approximately 25 m in front of Dive Friends dive shop, and is frequently visited by scuba divers each year. Yellow Sub is similar to other contemporary Caribbean reefs due to stony coral cover and diver frequency. This site is composed of approximately 17% stony coral cover and 45% macroalgal cover (Sommers et al. 2010). This is a slightly higher average of stony coral for a contemporary Caribbean reef (~12% average) (Bodmer et al. 2015). Data for this research were collected along the reef crest (7 m-11 m depth).
Fig. 1 Bonaire in the Dutch Caribbean. The star indicates Yellow Submarine dive site where research was conducted (12°09'36.3"N 68°16'55.0"W)

Study Organisms

*Diploria strigosa* (Symmetrical Brain Coral) and *O. annularis* (Lobed Star Coral) are both massive slow-growing stony corals that were compared in this study. These species were chosen due to the differences in morphology. *Orbicella annularis* forms lobes that may allow for more fish species to dwell inside. Both species grow in colonies. Mean colony size of *O. annularis* is 0.3 - 3 m and depth ranges from approximately 2 - 40 m (Humann and Deloach 2002). *Diploria strigosa* grows in a bulbous dome shape with no lobes. Average colony size of *D. strigosa* is approximately 15 cm – 2 m, and depth ranges from approximately 1 m – 40 m (Humann and Deloach 2002). *Diploria strigosa* and *O. annularis* are both found on the reef crest.

Survey Methods

Fish counts on individual coral heads were conducted using SCUBA. Data were collected in a five-week period (27 Sept. 2015 - 31 Oct. 2015). Fish counts were completed on eight heads of *D. strigosa* and eight of *O. annularis*. On each dive, two fish counts were completed - one for each coral type. The reef crest at Yellow Submarine runs parallel to the shore. Eight observations consisted of corals (four *D. strigosa* and four *O. annularis*) found by swimming north from the entry point, while the other eight observations consisted of coral heads found by swimming south. The study area extended approximately 40 m north and 40 m south from the entry site at Dive Friends dive shop. To avoid sampling the same coral head twice, pictures were taken of each coral head as well as recording measurements (length, width, height) which were reviewed before dives in to differentiate between previously observed corals.

Criteria for coral head selection

Three criteria were used when choosing coral heads. (1) All observed corals were from a similar depth (7m-11m) because fish assemblages are known to vary at different depths (Brokovich 2008). (2) All corals were of a similar size - no greater than 55cm for any measurement (length, width, height), and no smaller than 25cm for any measurement. Coral size, especially height, has been shown to affect fish abundance and diversity (Halborne et al. 2012). (3) Selected coral heads had >80% of the outer surface still living, which was estimated visually. Fish species that normally associate with live corals have shown lower recruitment at bleached (dead) sites (Booth and Beretta 2002). Further, eroded dead corals are less structurally complex, which has been shown to decrease fish abundance and diversity (Rogers et al. 2014). These criteria were selected to reduce variation in fish populations from confounding factors. To reduce sampling bias, coral heads were randomly selected by swimming along the reef crest and sampling the first coral that fit the criteria listed above.

Fish count methodology

On each dive, fish counts were conducted in order to compare abundance, species richness, and diversity for fishes associated with *D. strigosa* and *O. annularis*. Each coral observation lasted 20 minutes. During each observation, data were collected by identifying, counting, and recording every fish that swam
within a 25 cm radius of the coral head. This 25 cm radius was selected to reduce variation in counting fish that were neither attracted to the coral nor utilize the microhabitat. No differentiation was made between resident or transient fish species during observations. Rather, only fish that either remained or passed within the 25 cm radius were recorded.

The first ten minutes of the observation was conducted from a distance of approximately 3 m from the coral head to count fish that might avoid the coral due to diver presence. After ten minutes, we moved in closer to the coral head and continued to identify and count fish for the final ten minutes. During this close observation, special attention was given to looking in all of the cracks and crevices of the coral. During these observations, pictures were taken with a macro camera (Canon S110) to help identify any unknown species.

Data analysis

Three parameters were used to compare the differences in fishes between *D. strigosa* and *O. annularis*: (1) fish abundance, (2) fish species richness, and (3) fish diversity.

*Fish abundance*

After compiling total fish abundances on each individual coral head, a mean value was calculated for *D. strigosa* and *O. annularis*. A *t*-test was performed between the two samples to determine if there was a significant difference in fish abundance between the coral species.

In addition, fish species were categorized into their separate families. Abundances by families (instead of individual species) were determined for each individual coral head. A mean value of abundance by family was calculated for both *D. strigosa* and *O. annularis*. A *t*-test was used for each family to determine if there was a significant difference between abundances by families on the two coral species.

Species richness

Species richness is the number of species present in a sample. The number of species observed on each individual coral head was determined first. A mean value of species observed was calculated for both *D. strigosa* and *O. annularis*. A *t*-test was used to determine if there was a significant difference in species richness between the two coral species.

*Fish species diversity*

The Simpson’s Diversity Index (SDI) was used to analyze differences in fish diversity between *D. strigosa* and *O. annularis*. This index gives the probability that any two individuals drawn at random from an infinitely large community will belong to different species. The SDI equation is as follows:

\[
D = 1 - \frac{\sum n(n - 1)}{N(N - 1)}
\]

*N* represents the total number of fish seen during one coral head observation. The number of fish seen belonging to one species is represented by *n*. This equation gives a diversity value (*D*) between 0 and 1. A value of 1 demonstrates infinite diversity and a value of 0 demonstrates no diversity. A diversity value was calculated for each coral head observed. Once the diversity values were compiled, two mean values of diversity were calculated: one for *D. strigosa* and one for *O. annularis*. A *t*-test was then used to determine if there was a significant difference in diversity between the two coral species.

**Results**

**Fish abundance**

The mean fish abundance observed on a head of *O. annularis* was 50.38 ± 29.08 (mean ± SD), while the mean fish abundance observed on a head of *D. strigosa* was 35.50 ± 20.15. There was no significant difference in fish abundance...
between *D. strigosa* and *O. annularis* (*t*-test: *n*=8, *p*=0.25).

During the observation period, 39 fish species were observed that belonged to 14 different families (Table 1). Abundance by species was calculated for *D. strigosa* and *O. annularis* (Table 1). A mean value of fish abundance per coral head was also calculated for each fish species observed on *D. strigosa* and *O. annularis* (Table 1).

**Table 1** Fish species total abundance and mean abundance per coral head for *D. strigosa* (*n*=8) and *O. annularis* (*n*=8).

<table>
<thead>
<tr>
<th>Families</th>
<th>Species</th>
<th>Total Abundance</th>
<th>Mean Abundance</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>D. strigosa</em></td>
<td><em>O. annularis</em></td>
<td><em>D. strigosa</em></td>
</tr>
<tr>
<td>Gobiidae:</td>
<td>Masked/Glass Goby</td>
<td>158</td>
<td>203</td>
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<tr>
<td></td>
<td>Yellownose Goby</td>
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<td></td>
<td>Orangesided Goby</td>
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<tr>
<td></td>
<td>Sharknose Goby</td>
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</tr>
<tr>
<td></td>
<td>Bridled Goby</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Peppermint Goby</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Bleniidae:</td>
<td>Secretary Blenny</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Spinyhead Blenny</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>Saddled Blenny</td>
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</tr>
<tr>
<td></td>
<td>Darkheaded Blenny</td>
<td>6</td>
<td>5</td>
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<tr>
<td>Pomacentridae:</td>
<td>Bicolor Damsel</td>
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<td>Threespot Damsel</td>
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<td></td>
<td>Dusky Damsel</td>
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<td>Brown Chromis</td>
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<tr>
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<td>Blue Chromis</td>
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<td></td>
<td>Sergeant Major</td>
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<tr>
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<td>11</td>
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<td></td>
<td>Yellowhead Wrasse</td>
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</tr>
<tr>
<td></td>
<td>Spanish Hogfish</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Slippery Dick</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Scaridae:</td>
<td>Princess Parrotfish</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Striped Parrotfish</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Redband Parrotfish</td>
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<td>3</td>
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<tr>
<td></td>
<td>Stoplight Parrotfish</td>
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<td>3</td>
</tr>
<tr>
<td>Chaetodontidae:</td>
<td>Spotfin Butterfly</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Banded Butterfly</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Foureye Butterfly</td>
<td>0</td>
<td>2</td>
</tr>
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<td>Acanthuridae:</td>
<td>Blue Tang</td>
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</tr>
<tr>
<td></td>
<td>Ocean Surgeonfish</td>
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<td>1</td>
</tr>
<tr>
<td>Tetraodontidae:</td>
<td>Sharpnose puffer</td>
<td>5</td>
<td>4</td>
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<tr>
<td>Haemulidae:</td>
<td>French Grunt</td>
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<tr>
<td>Pomacanthidae:</td>
<td>French Angelfish</td>
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<td>2</td>
</tr>
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<td>Serranidae:</td>
<td>Graysby Grouper</td>
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<td></td>
<td>Creolefish</td>
<td>2</td>
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</tr>
<tr>
<td>Mullidae:</td>
<td>Goatfish</td>
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<td>0</td>
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<tr>
<td>Ostraciidae:</td>
<td>Smooth Trunkfish</td>
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<td>0</td>
</tr>
<tr>
<td>Lutjanidae:</td>
<td>Yellow Tail Snapper</td>
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<td>0</td>
</tr>
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</table>
Mean abundance of fish per coral head of *D. strigosa* (*n*=8) and *O. annularis* (*n*=8) was calculated for each family observed (Fig. 2; Fig. 3).

Four families out of the 14 from Table 1 are not displayed on Figure 2 or 3 because their total abundance throughout the observation period was less than 3 (Acanthuridae, Haemulidae, Pomacanthidae, Ostraciidae, and Lutjanidae). Families with lower mean abundances (i.e., mean abundance for both coral species was less than four) are shown (Fig. 2). Serranidae was the only family that displayed a significantly higher mean abundance per coral head of *O. annularis* (1.63 ± 1.30) (mean ± SD) compared to *D. strigosa* (0.50 ± 0.53) (t-test: *n*=8, *p*= 0.040) (Fig. 2; Table 2).

All other families showed no significant difference in average abundance per coral head between *D. strigosa* and *O. annularis* (t-test: *n*=8, *p* >0.05) (Fig. 2; Table 2). Families with greater mean abundances (i.e., mean abundance for both coral species was greater than four) are shown (Fig. 3). No significant difference existed between *D. strigosa* and *O. annularis* for mean

![Fig. 2](image1.png)  
**Fig. 2** Mean fish abundance by family per coral head of *D. strigosa* (*n*=8) and *O. annularis* (*n*=8). These families had lower mean abundances (i.e., mean abundance for both coral species was less than four). Serranidae spp. mean abundance was significantly higher on *O. annularis* vs. *D. strigosa* (t-test: *n*=8, *p*= 0.040) and is indicated with an asterisk (*). All other families showed no significant difference (t-test: *n*=8, *p*>0.05). Error bars show standard deviation of the mean.

![Fig. 3](image2.png)  
**Fig. 3** Mean fish abundances by family per coral head of *D. strigosa* and *O. annularis*. These families had higher mean abundances (i.e., mean abundance for both coral species was greater than four). No significant difference existed between *D. strigosa* and *O. annularis* for either family (t-test: *n*=8, *p*>0.05). Error bars show the standard deviation of the mean.
abundance per coral head for Gobiidae spp. \((t\text{-test: } n = 8, p = 0.439)\) or Pomacentridae spp. \((t\text{-test: } n = 8, p = 0.156)\).

### Table 2

\(p\)-values for two-sample \(t\)-tests. An asterisk (*) denotes a statistically significant result. These tests compare the mean abundance of fish families per coral head to determine if they are significantly different between \(D.\ strigosa\) \((n = 8)\) and \(O.\ annularis\) \((n = 8)\).

Families displayed have lower mean abundances per coral head (i.e. mean abundance for both coral species was less than four but greater than three).

<table>
<thead>
<tr>
<th>Fish Families</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleniidae</td>
<td>0.338</td>
</tr>
<tr>
<td>Labridae</td>
<td>0.699</td>
</tr>
<tr>
<td>Chaetodontidae</td>
<td>0.736</td>
</tr>
<tr>
<td>Acanthuridae</td>
<td>0.554</td>
</tr>
<tr>
<td>Tetraodontidae</td>
<td>0.770</td>
</tr>
<tr>
<td>Serranidae</td>
<td><strong>0.040</strong>*</td>
</tr>
<tr>
<td>Mullidae</td>
<td>0.149</td>
</tr>
</tbody>
</table>

These results indicate that there is no difference in mean fish abundance per coral head between \(D.\ strigosa\) and \(O.\ annularis\). When species were categorized into their respective families \((n=14)\), the abundance of Serranidae spp. was the only family that had a significantly higher mean abundance on \(O.\ annularis\) compared to \(D.\ strigosa\).

### Discussion

The purpose of this study was to examine differences in the fish populations associated with \(D.\ strigosa\) versus \(O.\ annularis\). I hypothesized that \(O.\ annularis\) would have higher fish abundance, species richness, and diversity compared to \(D.\ strigosa\); however, statistical analysis of mean values did not show a significant difference between the coral species. Therefore, all three hypotheses were not supported in this study.

The only significant difference in fish populations between \(D.\ strigosa\) and \(O.\ annularis\) was seen in the mean abundance for Serranidae spp. \((Table 2)\). A higher abundance of Serranidae spp. was associated with \(O.\ annularis\) compared to \(D.\ strigosa\). However, all other abundances by fish families showed no significant difference. The most commonly observed species from the family Serranidae was the Graysby grouper, \(Cephalopholis\ cruentata\). During observations, Graysbys were seen hiding under the overhang of \(O.\ annularis\) lobes. \(O.\ annularis\) tends to have a higher average height (compared to \(D.\ strigosa\)) with overhanging lobes that may offer protection. For data collected for this study, the average height of \(D.\ strigosa\) was approximately 30 cm \((n = 8)\), while \(O.\ annularis\) was 37.5 cm \((n = 8)\) \((t\text{-test: } n = 8, p = 0.029)\). Therefore, the higher mean abundance of Graysbys on \(O.\ annularis\) heads may be due to their association with high relief areas \((Sluka et al. 2001)\).

Larger ecological scales on the reef may have a larger effect than expected on the small-scale microhabitats (coral heads) observed in this study. It has been shown that for a given volume of coral, more fish species are found in regions with high coral species diversity, which
indicates strong regional (larger scale reef) influences on local (microhabitat) diversity (Belmaker 2009). In addition, larger ecological interactions on the reef between coral and fish have been shown to have strong influences on regional microhabitat diversity (Belmaker 2009). Olfactory cues have been shown to effect fish recruitment to a reef on a large scale rather than the individual coral head properties themselves (Dixson et al. 2011). Fish tend to be initially attracted to the smell of a high vegetative environment; therefore, the microhabitat properties themselves may not affect the initial recruitment (Dixson et al. 2011). This research offers an explanation for why there were no significant differences per coral head in fish populations between D. strigosa and O. annularis.

Coral heads were selected with similar size, shape, and depth for this study. However, the substrate surrounding each coral head varied during observations. Some corals were surrounded by sand while others were surrounded by numerous coral species, sponges, and other organisms. Therefore, corals with sand surrounding them exhibited lower local rugosity, which has been shown to be associated with lower fish diversity and abundance (Harborne et al. 2012). Not only can rugosity affect the fish seen in an area, but different organisms surrounding the microhabitat may affect the fish as well. For example, if a sea anemone is close by, it may attract a greater diversity of fish to the area due to the presence of cleaner shrimp (Huebner and Chadwick 2012). These varying environments may explain why there was no difference of fish populations between the coral species.

Further research is needed to understand the interactions between microhabitats and the larger scale reef environment. By comparing these two ecological scales, we can gain further understanding of how microhabitats in a reef interact with the larger regional diversity of a reef. This will allow us to further understand what causes differences in fish populations on coral reefs. Continued research focusing on fishes associated with specific coral species will allow us to focus conservation efforts on certain coral species in order to maintain a healthy reef fish population. Maintaining a healthy reef fish population will enhance the health of the reef as a whole (Gamfeldt et al. 2008). Therefore, it is important to continue to study how corals and their fish populations interact on different sized ecological scales.

Acknowledgements I would like to thank Dr. Patrick Lyons and Nathaniel Hanna Holloway for being awesome advisors and providing encouragement throughout this process. A huge thanks to my super cool dive buddy Erica Ascani for being so flexible when I was running behind schedule! I’d also like to thank Alex Kellam, McKenna Becker, and Carlie Sharpe for providing comradery and comic relief when it was very much needed. Finally, I’d like to thank my dushi Michael Roos for being my anchor when the seas were looking rough.

References
locate settlement habitat surrounding islands. Ecol and Evol 1:586-595
Brain coral bleaching and disease effects on goby population dynamics in Bonaire, Netherlands Antilles

Abstract Certain goby species, including the Peppermint Goby (*Coryphopterus lipemes*), Sharknose Goby (*Elacatinus evelynae*), Glass Goby (*Coryphopterus hyalinus*), and Bridled Goby (*Coryphopterus glaucofraenum*) are known to dwell on brain coral species *Colpophyllia natans*, *Diploria labyrinthiformis*, and *Diploria strigosa*. Coral degradation (e.g., bleaching and disease) can have adverse effects on coral-dwelling fishes, such as these goby species. The purpose of this study was to display how coral bleaching and disease affect the goby populations that live on brain corals. Goby abundance was compared between healthy and bleached specimens for each observed species and specimens in total. Amongst all the coral species, healthy or bleached, the greatest number of gobies was observed on healthy *C. natans* individuals (64 gobies). In total, there was a greater number of gobies dwelling on the bleached corals than healthy corals (71 and 67 gobies, respectively). Goby density was calculated by dividing the number of gobies dwelling on a brain coral by the surface area of each coral head. Average goby density on bleached coral heads (0.0038 ± 0.0040) was found to be significantly greater than average goby density on healthy coral heads (0.0011 ± 0.0006) (t-test; d.f.=12; p=0.0178). Although statistically significant, this result may not be biologically significant. The results imply that gobies can persist on moderately degraded brain corals. This suggests that gobies are resistant to early stages of degradation due to bleaching.

Keywords Coral degradation • coral-dwelling fishes • goby density

Introduction

The coral reef ecosystem and the dynamic relationships involved are fragile to prevalent stressors such as increase in temperature, change in salinity, ultraviolet radiation, sedimentation, aerial exposure, and pollutants (Glynn 1993). These stressors can induce the frequency of coral bleaching, an imminent hazard to coral reefs (Glynn 1993). Coral bleaching occurs when a scleractinian coral expels its symbiotic zooxanthellae and thus its pigment, revealing the calcium carbonate skeleton through its translucent, fleshy polyps (Hoegh-Guldberg 1999). Bleached corals can either regain their zooxanthellae and recover, or they may die, which generally results in overgrowth of algae (Diaz-Pulido and McCook 2002). Bleaching events can increase a coral’s susceptibility to coral disease, another threat to coral reefs. For example, in 2005 a major bleaching event in the Caribbean led to the decline of coral cover at all surrounding sites in the US Virgin Islands at an average of 51.5% twelve months after the event. This bleaching event increased the coral’s susceptibility to disease, which in turn caused a 60% decline in coral cover on the US Virgin Islands’ reefs (Miller et al. 2009).
Climate change and increased water temperature could be the determinant for the increase of severity in coral disease (Bruno 2007). The Caribbean is defined as a “hotspot” for coral disease because of the fast emergence and high prevalence of disease; while the Caribbean only possesses a fraction of the world’s coral reefs, it has 76% of the world’s coral diseases (Miller et al. 2009). Coral disease, like bleaching, is defined as a biological disturbance that can lead to coral mortality (Frias-Lopez et al. 2003; Barneah et al. 2006; Bonin et al. 2009). One prevalent disease, Black Band Disease (BBD), first materialized in the 1970s and is known to commonly affect massive brain corals such as Colpophyllia spp. and Diploria spp. BBD is a biotic disease that grows 3 mm to 1 cm per day resulting from the invasion of a cyanobacterium (Peters 1997; Friaz-Lopez et al. 2003; Barneah et al. 2006). It is characterized by a black band on the edge of the affected area, ranging from 5-30 mm wide leaving the center of the affected area with pale, destroyed, and dead polyps (Frias-Lopez et al. 2003; Barneah et. al. 2006).

Coral heads provide a number of different environments for fish to inhabit throughout the reef. Live corals, especially, are important habitats for coral-dwelling fishes, as they provide shelter while moderating competition and predation (Coker et al. 2014). Coral degradation (e.g. bleaching and disease) can have adverse effects on coral-dwelling fishes. Initial reef fish settlement on corals can be affected by bleaching. Because reef fishes use chemical and visual cues to acknowledge their settlement habitat, loss of pigmentation and physiological damage experienced during bleaching could hinder these cues (Bonin et al. 2009). In one study, reduction of live coral on a degraded colony led to reduction of abundance of newly settling fishes (Feary et al. 2007b). Coral degradation may also prompt coral-dwelling fishes to vacate a host coral after a population has settled on it, thus being subject to predation (Bonin et al. 2009). Coral-dwelling fishes can persist on corals shortly after bleaching and prior to mortality; however there are declines in abundance of the coral-dwelling fishes on degraded corals within this threshold (Bonin et al. 2009).

Certain species of gobies, an extensively diverse group of fishes, are known to dwell on coral heads (Van Tassell et al. 2011). Gobies are opportunistic feeders and have a variety of prey they consume, such as copepods, small invertebrates, algae, and coral tissue (Bonin et al. 2009; Herler et al. 2011). Among the most common species of coral dwelling gobies in Bonaire are: Coryphopterus lipemes, Elacatinus evelynae, Coryphopterus hyalinus, and Coryphopterus glaucofraenum. Moreover, adult gobies in the Elacatinus genus fill an important ecological niche as cleaners; they are the most common obligate cleaner species in the Caribbean (Côté and Soares 2011).

The purpose of this study was to display how coral bleaching and disease affects the goby populations that live on Colpophyllia natans, Diploria labyrinthiformis, and Diploria strigosa. Due to the limited studies done on the correlation between goby density and bleaching and disease, this information will be valuable in the marine ecology field, as bleaching and disease are becoming a more serious threat to coral reef ecosystems. This study attempted to determine if coral bleaching and disease affected the C. lipemes, E. evelynae, C. hyalinus, and C. glaucofraenum goby populations’ density.

H1: Coral dwelling gobies will inhabit bleached or diseased brain corals that are moderately affected, but will not inhabit these bleached or diseased brain
corals that are intermediately or severely affected

H2: Average goby density on bleached or diseased brain corals will be less than the average goby density on healthy brain corals

Materials and methods

Study Site

All research was conducted at the Yellow Submarine dive site, located in downtown Kralendijk, Bonaire (12° 9' 36.20" N, 68° 16' 55.25" W) (Fig. 1). Much of the shallow area is composed of sand with few coral clusters, which stretches out 50 m from the shore until the reef crest begins. The reef crest starts at a depth of approximately 10 m, angled at 40 degrees and continues to a depth of roughly 30 m. Bonaire’s fringing reef is composed of mostly scleractinian corals and macroalgae; the most common scleractinian corals are Montastraea spp. (Sommer et al. 2011).

Fig. 1 Map of Bonaire in the Dutch Caribbean. Indicated is the dive site, Yellow Submarine (12° 9’ 36.20” N, 68° 16’ 55.25” W)

Study Organisms

The gobies observed in this research are C. lipemes, E. evelynae, C. hyalinus, and C. glaucofraenum which are known to live in tropical marine, reef associated environments and inhabit brain coral species such as, D. labyrinthiformis, D. strigosa, and C. natans. Coryphopterus lipemes, commonly referred to as the Peppermint Goby are known to live in waters up to 13 m (Humann and DeLoach 2002). Elacatinus elevynae, the Sharknose Goby, is a cleaner species and feeds on the ectoparasites of fishes. They can be found in water ranging from 1 to 53 m (Humann and DeLoach 2002). Coryphopterus hyalinus, or the Glass Goby dwells in waters 8-52 m (Humann and DeLoach 2002). Coryphopterus glaucofraenum, commonly known as the Bridled Goby is found in depths ranging from 2 to 45 m (Humann and DeLoach 2002).

The brain coral species these gobies dwell on are C. natans, D. labyrinthiformis, and D. strigosa, abundant in Florida, Bahamas, and the Caribbean. Colphophyllia natans, commonly known as the Boulder Brain Coral is found in waters between 6 and 24 m (Humann and DeLoach 2002). Diploria labyrinthiformis, known as the Grooved Brain Coral, is found in waters from 1 to 40m (Humann and DeLoach 2002). Diploria strigosa, or Symmetrical Brain Coral is found in waters from 1 to 40 m and is common to Florida, Bahamas, and the Caribbean (Humann and Deloach 2002).

Sampling Method

Research was conducted twice a week starting on September 30, 2015, and concluding on November 1, 2015. Data collection occurred between the depths of 9 m and 15 m at the Yellow Submarine dive site. On each week of research a colony was randomly selected by swimming using SCUBA diving until a C. natans, D. labyrinthiformis, or a D. strigosa coral head was found. The health of the coral head was then determined by stratifying the relative affectedness of bleaching or disease into four categories. These categories included:
healthy (0% bleached or diseased), moderately affected (1-33% bleached or diseased), intermediately affected (34-66% bleached or diseased), and severely affected (67-100% bleached or diseased). The number of gobies dwelling on each coral head was then assessed by hovering over the colony until the number of gobies was accurately determined. Next, the surface area of each coral head was calculated by measuring the length, width, and height of the coral head with measuring tape. As a standard for all the measurements, the longest given measurement for length, width and height was taken for irregularly shaped coral heads. The density was determined by dividing number of gobies by the calculated surface area of each coral head in which these gobies were dwelling. In order to avoid documenting the same coral head twice, different portions of the study site were observed each day of research.

Data Analysis

Goby presence on moderately affected corals versus intermediately or severely affected corals were counted using a bar graph. Additionally, number of gobies was quantified on healthy and bleached coral for each coral species and added up to have an overall count; this information was illustrated on a bar graph. Moreover, mean goby density between the four categories of coral heads (healthy, moderately affected, intermediately affected, and severely affected) was compared by means of a t-test to quantify if these categories were statistically different from each other, as well as determine if there was a larger goby density on healthy coral.

Results

All 19 C. natans colonies studied had gobies present. For D. labyrinthiformis (n=19) and D. strigosa (n=3) the values dropped to 22.2% and 66.7% of the colonies respectively. Between all the coral species, healthy or bleached, the greatest number of gobies was observed on healthy C. natans (64 gobies) (Fig. 2). Both healthy and bleached D. labyrinthiformis coral heads were found to have the same number of gobies (3 gobies) (Fig. 2). On D. strigosa corals, only bleached coral heads had gobies present (8 gobies) (Fig. 2) In total, there was a greater number of gobies dwelling on the bleached corals than healthy corals (71 and 67 gobies, respectively) (Fig. 2).

![Fig. 2](image-url) Total number of gobies observed on bleached and healthy coral heads stratified between observed coral species. In addition, total number of gobies among all observed bleached and healthy corals is quantified on the far right (n = 138)

Average goby density was calculated for the total number of healthy and bleached corals observed with goby presence. Average goby density on bleached coral heads (0.0038 ± 0.0040) (mean ± std. dev.) was found to be greater than average goby density on healthy coral heads (0.0011 ± 0.0006) (Fig. 3). When a t-test was performed, this difference was shown
to be statistically significant \( \text{t-test; } n = 13; p = 0.0178 \) (Fig. 3).

\[ \text{Average Density (Goby/cm}^2\] 

![Fig. 3 Average goby density on bleached coral compared to healthy coral. There were significantly more gobies found on bleached coral than on healthy coral (t-test; \( n = 13; p = 0.0178 \)). Error bars represent standard deviation](#)

## Discussion

This study revealed that goby species are able to inhabit moderately bleached brain coral species; however, no intermediately or severely bleached brain corals were observed. A possible limitation to the study was that no diseased brain coral specimens were observed. Due to the lack of data, the results did not support the hypothesis that states goby species can inhabit bleached or diseased brain coral species that are moderately affected but cannot inhabit bleached or diseased brain coral species that are intermediately or severely affected. Likewise, the results also did not support the hypothesis that average goby density on bleached or diseased corals would be less than average goby density on healthy corals; the results expressed that average goby density was higher on bleached corals rather than healthy corals. The hypothesis was also refuted by the results because no diseased corals were observed.

Although the hypothesis that gobies can persist on moderately but not intermediately or severely affected brain coral was not supported, multiple studies express that coral-dwelling fishes (e.g. gobies) can persist on corals during early stages of degradation (Feary et al. 2007b; Bonin et al. 2009; Coker et al. 2014). Gobies may be resilient to severe degradation of host corals as well. An experiment conducted by Bonin et al. (2009) found that some gobies did not vacate severely bleached colonies until 50-90% of the host coral had died. Degradation due to bleaching may raise coral-dwelling fishes’ predation rates, but it is not seen to affect settlement patterns (Bonin et al. 2009; Coker et al. 2014). However, coral-dwelling fishes are known to vacate the host coral when the coral tissue is lost and algal cover is present, suggesting that live coral tissue is critical for the habitat of these fishes (Bonin et al. 2009; Coker et al. 2014). Gobies largely persist on degraded colonies perhaps because they have limited motility, face a high risk of predation when migrating from a host coral, and have high interspecific competition for suitable habitat. When all of these factors are considered, they may reduce any benefit of relocating to a healthier host coral (Feary et al. 2007a).

There was a statistically higher average goby density on bleached corals than on healthy corals observed in this study \( \text{t-test; } \text{d.f.} = 12; p = 0.0178 \). Although statistically significant, this result may not be biologically significant. In contrast to my results, a study by Feary et al. (2007b) goby abundance was reduced on degraded coral colonies. Decline of goby abundance on degraded coral was suggested to be due to loss of live coral cover, rather than bleaching (Feary et al. 2007b; Bonin et al. 2009). Alternatively, a different study suggests that coral-dwelling fishes have higher predation rates on bleached corals (Coker et al. 2014). Therefore, it is unclear if degradation due to bleaching itself has an effect on goby abundance. A potential factor of the decline in abundance in these studies is that coral degradation may reduce or negate fish settlement cues (i.e. chemical, auditory, visual).
onto a host coral colony (Feary et al. 2007b). In addition, corals degraded by bleaching may have lower coral-dwelling fish abundance because predators may gain prey perception on pale colonies (Coker et al. 2014).

Although Black Band Disease (BBD) was not observed on brain corals in this study, the effects on goby populations are comparable to the effects of coral bleaching. When a coral contracts BBD, the affected area is left with destroyed and dead polyps (Frias-Lopez et al. 2003). Because live coral is suggested to be a necessary habitat requirement for coral-dwelling fishes, it is likely that gobies may not be able to persist on corals severely affected by BBD (Coker et al. 2014). Additionally, BBD can grow at a rate of 3 mm to 1 cm per day, which may affect duration of goby resistance to host coral degradation (Barneah et al. 2006). Black Band Disease may have adverse effects on goby population dynamics, but due to the lack of data and little research done on the subject, the effects are unclear.

Intermediately and severely bleached corals were not observed, which limits deductions from this study. Further knowledge on the threshold by which gobies vacate degraded coral, cannot be determined. Because average goby density on moderately bleached corals alone was compared to that of healthy corals, the significance of this data may have been skewed. In addition, use of measuring tape is a relatively imprecise method of brain coral surface area estimation, and may have skewed the average goby density results as well. Despite the limitations, the results suggest that gobies can persist on moderately degraded brain corals. This gives further evidence suggesting that gobies are resilient to early stages of degradation due to bleaching.

In order to better understand the effects of goby resilience to degradation due to coral bleaching and BBD, further studies should be conducted at sites with a greater frequency of intermediate and severely affected bleached and diseased brain corals. Additionally, a different methodology should be implemented for surface area measurement of brain corals. Image analysis by 3-dimensional reconstructions have been used to estimate surface area for massive corals such as brain coral species, and could increase the accuracy of the calculations (Jones et al. 2008).

Though goby species appear to be resistant to coral degradation due to bleaching, this does not mean coral-dwelling species are safe from changes in habitat resulting from climate change (Bonin et al. 2009). Recurring bleaching events may lead to coral mortality and algal recruitment, which may reduce available habitat that is suitable for coral-dwelling fishes (Diaz-Pulido and McCook 2002; Bonin et al. 2009). Thus, the abundance of coral-dwelling fishes may decline while the community structure may shift to species that thrive in an algal-dominated habitat (Feary et al. 2007b).

Acknowledgements I would like to thank my advisors, Dr. Enrique Arboleda and James Emm for their encouragement and guidance throughout my project. I would like to thank Kate Howard for being a terrific research buddy and an even better everyday buddy. An honorable mention goes out to Margaret Meyer, McKenna Becker, Alex Kellam, and Erica Ascani for their emotional support and comic relief throughout the program. Additionally, I would like to thank Dr. Rita Peachey and all the CIEE Research Station Bonaire staff for providing me with all the resources necessary to complete my project. Finally, I would like to thank my incredibly strong support system of a family that made this all possible.

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