Physis

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Physis

Journal of Marine Science

CIEE Research Station Bonaire

Tropical Marine Ecology and Conservation

Volume XIX • Spring 2016

PHYSIS Φύσις

"When one tugs at a single thing in nature, he finds it attached to the rest of the world."
– John Muir

Physis (φύσις) is an ancient Greek word for nature, associating the natural world with disorder and death. But as ancient humans gained understanding of the Earth's processes, they discovered that the seemingly chaotic natural world is full of patterns. As scientists, we explore these patterns that are often invisible to the naked eye. We know now that each living organism is intricately connected, and that all creatures are important to the framework of nature.

Comprising seventy percent of Earth's surface, the oceans are an enormous part of the web of life. Phytoplankton, some of the smallest organisms in the sea, form the foundation for this framework by sustaining other oceanic life forms, from zooplankton to whales. These creatures are so often overlooked; yet they provide us with half the oxygen on earth. In recent years, phytoplankton populations have been declining at alarming rates, in part due to an increase in atmospheric CO_2 levels. The chemistry of the ocean is changing, sea surface temperatures are rising, and these factors are seriously affecting all the ocean's inhabitants. It is no longer acceptable to deny that humans are the main cause for shifts in Earth's climate, and that these changes will soon impact us in profound ways.

With continuing advances in science, we are better able to understand the fragile links that keep ecosystems in balance, while discovering ways to preserve these vital connections. At the CIEE Research Station Bonaire, we work towards identifying and understanding complex relationships that exist in the marine environment. It is our role to communicate our findings to the public, with the hope that, by sharing our research, we can inspire our readers to respect and preserve what is beneath the surface of our mysterious and beautiful oceans. After all, we are a part of nature, just as nature is a part of us. We present Volume XIX of *Physis: Journal of Marine Science* as a tribute to environmental conservation and awareness.

Taylor Carlin and Victoria Cassar

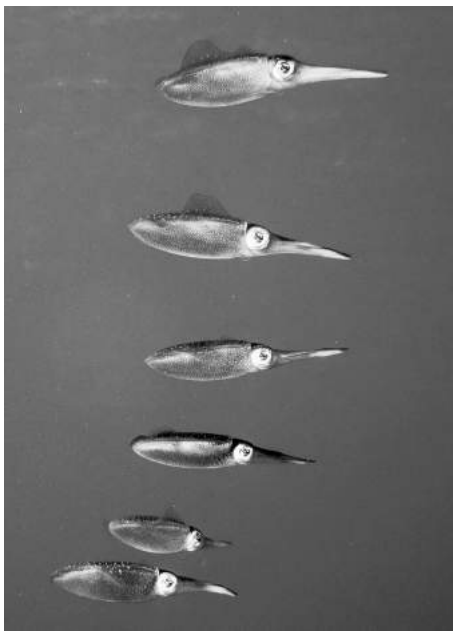
Foreword

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 4th of May, 2016 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, Rita Peachey, PhD, Nathaniel Hanna Holloway, MS, and Fadilah Ali, PhD Candidate. 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, BS, Austin Lin (BS; CIEE Alumni), and Amy Gosney, BS. Astrid de Jager was the Dive Safety Officer and provided oversight of the research dive program for the course.

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

Dr. Rita BJ Peachey

Faculty

Dr. Rita Peachey is the Founding Director of CIEE Research Station Bonaire. 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. 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 and co- teaches Independent Research. He is a Marine Biologist, holds a specialization on Biodiversity and Evolutionary Biology (University of Valencia, Spain), and obtained his PhD at the Stazione Zoologica di Napoli (Italy). Dr. Arboleda's research interests at CIEE Bonaire include behavior, ecology and marine water quality.

Fadilah Ali is the Tropical Marine Conservation Biology Faculty for CIEE and co-teaches Independent Research. She is an ecologist with a specialty in invasive species biology, control and management and has been researching lionfish for the last 5 years. Originally from Trinidad and Tobago, she has a Masters degree in Environmental Science and is currently completing her PhD in Ocean and Earth Sciences at the University of Southampton.

Nathaniel Hanna Holloway is the instructor for Marine Ecology Field Research Methods course, co-instructor for the the Independent Research course and the Intern Coordinator. 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 novel coral reef monitoring tools and techniques.

Astrid de Jager is the instructor for the Cultural and Environmental History of Bonaire course, and Dive Safety Officer. She came to Bonaire in 2009 and has been working in dive industry ever since. She holds a masters degree in Music History, and is a SDI and DAN instructor trainer.

Staff

Mary DiSanza was born and raised in Colorado, a state with a long-term commitment to protecting the environment. Computers, banking, & law gave way to scuba diving and travel, and skis were traded in for dive gear. Bonaire was an island far ahead of its time. Mary worked as a Dive Instructor and Retail Manager for a dive shop on Bonaire for several years, before branching out to the resort / management side of the business.

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 Research Station Bonaire, and instructor on the Advanced Scuba course.

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 and she is the student resident hall manager.

Interns

Amy Gosney is a teaching assistant here at CIEE Bonaire for Tropical Marine Conservation Biology, Marine Ecology Field Research Methods and Independent Research courses. After completing her B.Sc in Environmental Science, she travelled to the Indo-Pacific to work on marine conservation projects in Indonesia and the Philippines. Her main work in these countries focused on teaching species identification and survey methods to monitor coral reef ecosystems. She also worked with community engagement to increase the success of local marine resource management.

Sara Buckley is currently a teaching assistant here at CIEE for the Advanced Scuba, Coral Reef Ecology, Marine Ecology Field Methods, and Independent Research courses. Sara is also a SDI and PADI Dive Instructor; she helps train our CIEE students to become AAUS research divers. Sara completed her B.Sc in Oceanography at the University of North Carolina at Wilmington in May 2015. Her undergraduate research focused on sea level reconstruction from salt marsh agglutinated benthic foraminifera as well as measuring tidal lags around Wrightsville Beach North Carolina's intracoastal waterway and surrounding creeks. Sara is currently working on a research project focusing on the effects of UV light on zooplankton diversity and density in Bonaire.

Austin Lin is a Research Laboratory Intern at CIEE Bonaire for the spring semester 2016. He is assisting with the Independent Research and Marine Ecology Field Research Method courses. 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 research station Bonaire during fall semester 2013, with his research focusing on groupers abundance in relation to coral ecosystem health. He is currently managing a research project focusing on box jellyfish reproductive ecology and post-spawning survival. Austin returned to Bonaire in pursuit for his passion in marine research, conservation, and education..

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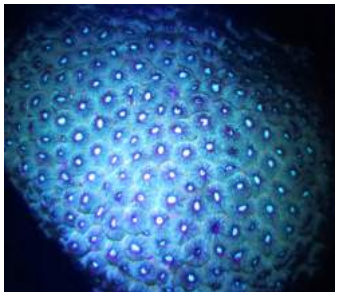

Bonaire
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5 mi
5 km
Caribbean Sea

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REPORT

Sam Barrett • Washington & Lee University • barretts158@gmail.com

Temporal dynamics of fish assemblage on artificial reefs with varying distances from a coral reef ecosystem

Abstract Increasing coral reef degradation worldwide is putting more reef fish under stressful circumstances. A possible solution to mitigating the effects of degraded coral reefs is the implementation of artificial reefs. An ongoing question of effective artificial reef utilization asks how far from a natural coral reef ecosystem should these structures be placed. Some studies support the theory that structure isolation attracts larger fish diversity in terms of aggregation and recruitment. In order to test such a hypothesis, four identical artificial structures were deployed next to a coral reef ecosystem in Bonaire, Dutch Caribbean. The impact of position relative to the natural reef was measured, placing two structures close to the reef crest and two 30 meters inshore on the sand flat. After conducting fish counts over a five week period, it was found that artificial reef isolation attracted a higher assemblage of fish, particularly juveniles. Total fish assemblage counted for 1021 fish at the sand flat and 364 fish at the reef crest, with higher fish diversity at the reef crest (1 - 0.77 = 0.23). An interesting temporal trend of French Grunt recruitment was observed at the sand flat. These results demonstrate fish preference for isolated artificial reefs, but the range of species recruited may be limited.

Keywords Recruitment • aggregation • *Haemulon flavolineatum*

Introduction

Coral reefs are a vital habitat for a high diversity of reef fish. They provide a complex habitat structure that the fish populations can utilize in order to have a readily available resource of food, shelter and living space (Komyakova et al. 2013). This structural and topographic complexity of the reef often influences the species richness and abundance of reef fish communities. A high reef complexity is correlated to high fish abundance and diversity because of the reduction of predation and competition (Komyakova et al. 2013). Graham and Nash (2013) conducted a report on the importance of structural complexity in coral reef ecosystems and also found very strong evidence of increasing structural complexity on both increasing overall fish density and biomass. The consistent relationships in both the Caribbean and Indo-Pacific regions suggests reef fish density is dependent on the structural complexity of coral reefs in both regions and is important to consider when analyzing the integrity of a coral reef ecosystem (Graham and Nash 2013). Furthermore, Graham and Nash (2013) recommend that maintaining and even enhancing a reef's structure by creating additional habitat should be a key objective of environmental managers.

Because of what is known about coral reefs and the rich ecosystem they support, the topic of environmental stress, both anthropogenic and natural, has become a serious concern (Stokes et al. 2010). The degradation of the world's coral reefs has shown to directly affect reef-associated fish communities (Komyakova et al. 2013). The loss of structural complexity and coral cover from coral reef degradation are due to the combined effects of coastal development, agricultural land use, climate

change, artisanal and industrial over-fishing (Stokes et al. 2010; Komyakova et al. 2013).

Artificial reefs exhibit significant potential as a tool in the rehabilitation of coastal ecosystems and are defined by the European Artificial Reef Research Network as submerged structures placed on the seabed deliberately to mimic some characteristics of natural reefs (Pickering et al. 1998). It has been shown that artificial reefs are successful tools in fisheries enhancement because of their efficiency in fish aggregation (Pickering and Whitmarsh 1997). Artificial reefs are also important in filling a variety of roles beyond aggregation, including: nature conservation, the provision of additional specific habitat, aquaculture, tourism, mitigation and habitat restoration (Pickering et al. 1998). It is also known that the presence of artificial reefs in natural ecosystems enhances the diversity and production of fish species (Pickering and Whitmarsh 1997; Pickering et al. 1998). The proper utility of artificial habitats in aquatic systems has been a topic of debate in scientific circles for many years, but increasing numbers of artificial reef projects are providing evidence that there is a valid role for artificial reefs in marine ecosystem restorations (Seaman 2007). Sherman et al. (2001) re-examined peer reviewed data on site selection of artificial reefs in coral reef ecosystem restoration and concluded that there needs to be a better understanding of artificial reef design, key environmental determinants of artificial reef function, and interactions between artificial reef design and those environmental determinants of function. Previous research done on artificial reefs has focused mainly on structural preferences of fish, long-term fish behavior associated with isolated artificial reefs, importance of structural complexity, specific materials to use, and how artificial reefs can strengthen fisheries (Walsh 1985; Hixon and Beets 1989; Lowry et al. 2014).

For decades, the fringing reefs of Bonaire in the Dutch Caribbean have held the status as a protected marine park. The goal of this environmental management framework is to conserve Bonaire's coral reefs through restricted zoning while at the same time providing enough areas for responsible ecotourism (Thur 2010). Recent surveying has shown that reef species and assemblages have been undergoing major changes due to coral reef degradation on Bonaire, which follows a trajectory similar to other Caribbean reefs (Stokes et al. 2010). This is a problem because Bonaire's economy depends heavily on artisanal fisheries and ecotourism by scuba divers (Stokes et al. 2010). If the majority of the reef fish population ends up failing, most of the revenue towards the island's economy will be lost. This problem is applicable to many islands throughout the Caribbean and the Indo-Pacific. A possible solution to this emerging problem is the design and deployment of artificial reefs within a natural coral reef environment to help strengthen reef fish communities by providing additional structural complexity and habitat for future fish recruitment. Healthy fish recruitment and aggregation dynamics within a coral reef ecosystem are imperative to the sustainability of future populations as well as species richness and diversity of the reef fish community. This healthy recruitment and aggregation is threatened by the increased degradation of corals, which is an important habitat to fish larvae and juveniles (Rilov and Benayahu 1998). This is where the creation of new and well-planned artificial reefs may offer alternative shelters, which would be expected to recruit juveniles and thus enlarge the overall pool of reef fish (Rilov and Benayahu 1998). Spatial variation in recruitment, growth and mortality rates interact to explain the distribution of adult fishes within reef systems (Jones 1997), but information on how fish recruitment responds at different temporal and spatial scales is limited (Wilson et al. 2006). This study aims to add more understanding on the proper placement of an artificial reef as an extension of a natural reef system and how this can maximize long-term stability to reef fish populations by providing additional habitat to juvenile fish.

The purpose of this study is two-fold: (1) to provide more data on the temporal dynamics of

fish assemblage to non-coral structures, and (2) to better understand how distance from a coral reef influences fish assemblage behavior to a deployed artificial reef. Although artificial reefs are often monitored following deployment, the temporal variability of fish assemblages is seldom measured (Willis et al. 2005). Studies have found that number and species of fishes on patch reefs increased with isolation, but could be limited by 20 meters to 30 meters around coral reefs (Schroeder 1987). Causative factors suggested for the occurrence of more recruits on well-isolated reefs include preferential recruitment, lower predation risk and less interference by neighboring reef fishes (Schroeder 1987). This study tests the following hypotheses using artificial reef structures (ARSs):

H_1: ARSs placed 30 meters from the reef crest on the sand flat will have greater fish abundance than ARSs placed three meters from the reef crest

H_2: ARS placed 30 meters from the reef crest on the sand flat will have greater species diversity than ARSs placed three meters from the reef crest

The results from this study may help future environmental managers in deciding where to properly place ARSs in order to maximize habitat effectiveness.

Materials and methods

Study site

The ARSs were deployed at Yellow Submarine dive site (12°09’36. 2”N 68°16’55. 2”W) located on the western coast of Bonaire, Dutch Caribbean (Fig. 1). The fringing reef surrounding Bonaire is a marine park, protected by the National Parks Foundation, STINAPA. The section of reef at Yellow Submarine appears to be in good condition, exhibiting high biodiversity and structural complexity (i.e. rugosity). The sand flat at Yellow Submarine extends approximately 60 m from shore to the reef crest, where it transitions to reef slope at a depth of 7.5 m. Yellow Submarine dive site is located on the waterfront of Kralendijk. Many mooring blocks placed by STINAPA are situated throughout the sand flat and have recruited live corals and attract a variety of fish species showing that artificial structures have been successful in providing habitat at this study site in the past.

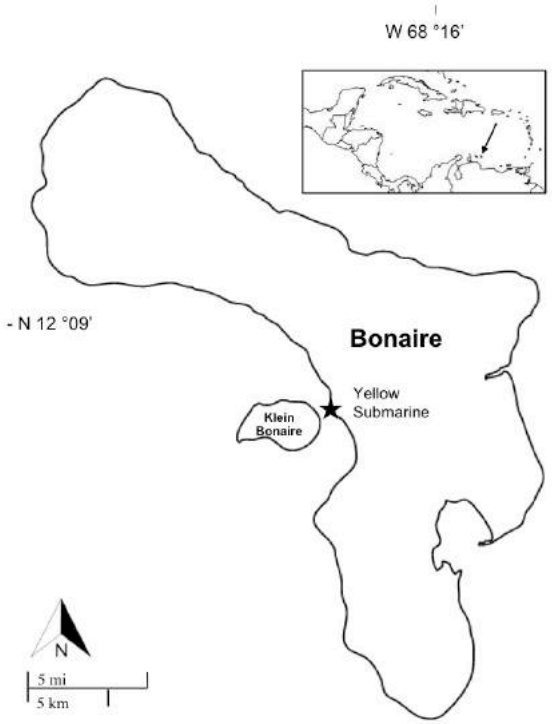

Fig. 1 Map of the island Bonaire, marking the study site Yellow Submarine (12°09’36.2”N 68°16’55.2”W) as a black star

Artificial reef structures

Four identical ARSs were constructed out of various materials including: one meter of cut PVC pipe with 40 2-cm diameter holes and two square 10 cm x 10 cm openings, four open ended cages wrapped in duct tape placed at 90° increments around the PVC pipe and a taped wire mesh placed into one of the square openings to create an overhang. The entire structure was supported by two steel rods that were hammered into the sandy substrate. The design (Fig. 2) was engineered to utilize several variables found in the literature that attract fish recruitment and aggregation

behavior such as vertical height, holes and void space (Hixon and Beets 1989; Seaman 2007). These structures were removed from the study site after five weeks of data collection.

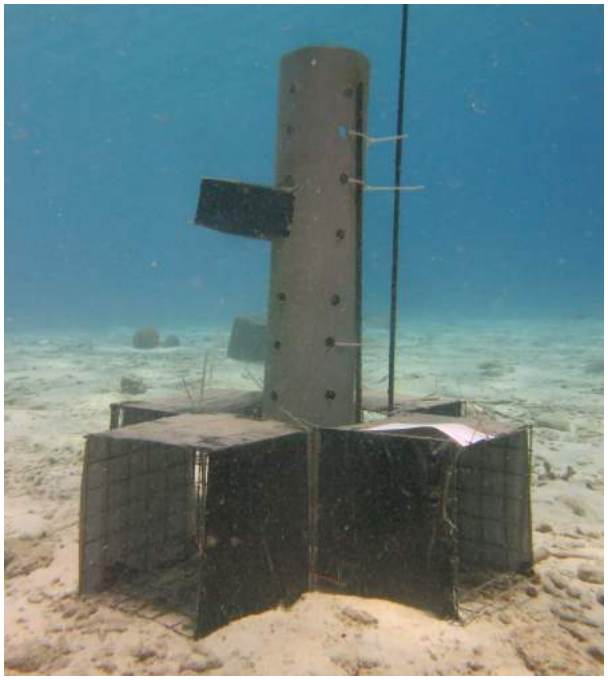

Fig. 2 One of four identical artificial reef structures (ARS) deployed on the reef crest and sand flat at Yellow Submarine, Bonaire

Sampling design

ARSs placed three meters from the reef crest were at a depth of seven meters and ARSs placed 30 meters were at a depth of three meters. One pair of ARSs were placed 35 meters north of the Yellow Submarine dock at their respective distances from the reef crest. The other pair of ARSs were placed 35 meters south of the Yellow Submarine dock. This allowed for 70 meters of separation between both pairs of ARSs to ensure independence of sampling units. A total of six samplings were carried out by SCUBA diving over the course of five weeks and always at 13:00 hrs. Each dive surveyed all four ARSs. At each ARS fish abundance, species, and size were recorded by conducting fish counts for a five-minute period. The first two minutes of the observation were made at a distance of approximately three meters from the ARS. All species within 25 cm of the ARS were identified and counted. Fish abundance and species within the ARS were recorded for the last three minutes. Size was also recorded for identified fish in order to track recruitment.

Data analysis

Bivariate relationships between fish abundance, species richness, diversity, fish size and the ARS distance from the reef crest was determined by averaging the reef crest and sand flat fish count data (n = 2). To estimate biodiversity, Simpson's index was used (1 - D). When measuring species composition between the reef crest and sand flat, fish that made up more than 1% of the total composition were grouped into families. Fish that made up 1% or less of the total composition were classified as 'other'. Temporal length and assemblage data of grunt juveniles at the sand flat sites was averaged (n = 2). Because of its particular abundance, a cohort analysis was done on grunt juvenile size frequencies over time from the structures placed on the sand flat.

Results

Fish abundance

A total of 1383 fish were counted between all four ARSs. The fish counted at the reef crest ARSs were compromised of 253 (68%) adults and 111 (32%) juveniles. The fish counted at the sand flat ARSs compromised of 31 (3%) adults and 990 (97%) juveniles (Fig. 3).

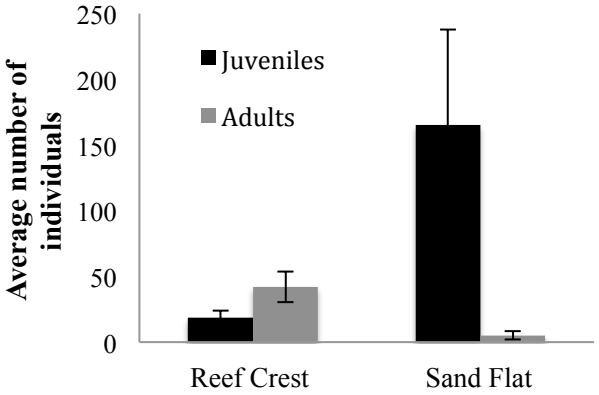

Fig. 3 Average number (± SD) of juvenile and adult fish counted at both reef crest and sand flat sites (n = 2 for each location)

Biodiversity and species composition

In terms of species richness, 27 different species of fish were observed at the reef crest ARSs and 12 different species were observed at the sand flat ARSs. Between both sites, 11 species were observed at both. The reef crest had high biodiversity with a Simpson's index value of 0.77 while the sand flat had low biodiversity with an index value of 0.27.

When analyzing species composition between the two sites (Fig. 4) fish were grouped into Gobies (Gobiidae), Damselfishes (Pomacentridae), Wrasses (Labridae), and Grunts (Haemulidae). Gobies include the Bridled Goby (*Coryphopterus glaucofraenum*) and Goldspot Goby (*Gnatholepis thompsoni*). Damselfishes include the Bicolor Damselfish (*Stegastes partitus*) and Brown Chromis (*Chromis multilineata*). Wrasses include the Puddingwife (*Halichoeres radiates*), Slippery dick (*Halichoeres bivittatus*), Bluehead Wrasse (*Thalassoma bifasciatum*) and Yellowhead Wrasse (*Halichoeres garnoti*). Grunts include the White Grunt (*Haemulon plumierii*) and French Grunt (*Haemulon flavolineatum*). The grunt population observed at the sand flat ARSs consisted of only *Haemulon flavolineatum* juveniles. Other species observed at both the reef crest and sand flat ARSs included the Sharpnose Puffer (*Canthigaster rostrata*), Banded Butterflyfish (*Chaetodon striatus*), and Ocean Surgeonfish (*Acanthurus bahianus*). The reef crest ARSs had additional species of fish that weren't observed at the sand flat ARSs. These species included the Smooth Trunkfish (*Lactophrys triqueter*), Trumpetfish (*Aulostomus maculatus*), Greater Soapfish (*Rypticus saponaceus*), Stoplight Parrotfish (*Sparisoma viride*), Yellow Goatfish (*Mulloidichthys martinicus*) and Sergeant major (*Abudefduf saxatilis*). The 306 fish counted over the data collection periods at the reef crest ARSs was mainly composed of 132 (43%) Bridled

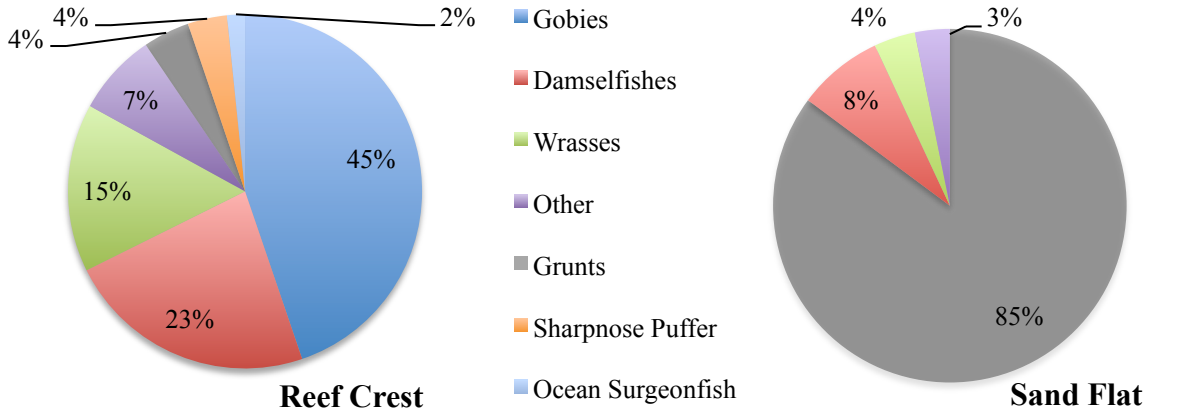

Fig. 4 Species composition among the reef crest and sand flat sites. 'Gobies' include the Bridled Goby and Goldspot Goby. 'Damselfishes' include the Brown Chromis and Bicolor Damselfish. 'Wrasses' include the Puddingwife, Yellowhead Wrasse, Bluehead Wrasse and Slippery Dick. 'Grunts' include the White Grunt and French Grunt. The 'other' category includes species with an abundance of 1% or less

Gobies, 53 (17%) Brown Chromis, 23 (7.5%) Bluehead Wrasses, 17 (5.5%) Puddingwife Wrasses, 17 (5.5%) Bicolor Damselfish, 11 (4%) Sharpnose Puffer and 5 (2%) Ocean Surgeonfish. The 1007 fish counted over time at the sand flat was mainly composed of 856 (85%) juvenile French Grunts, 72 (7%) Brown Chromis and 25 (2.5%) Puddingwife Wrasses.

Haemulon flavolineatum juvenile assemblage over time

Large average numbers of *H. flavolineatum* juveniles were present during all data collection periods at the sand flat structures. An increase in the average number of *H. flavolineatum* juvenile assemblage over a four-week time period was observed (Fig. 5), peaking at 128 fish on average on 16th March

2016 and dropping to a low of 67 fish on 26th March 2016.

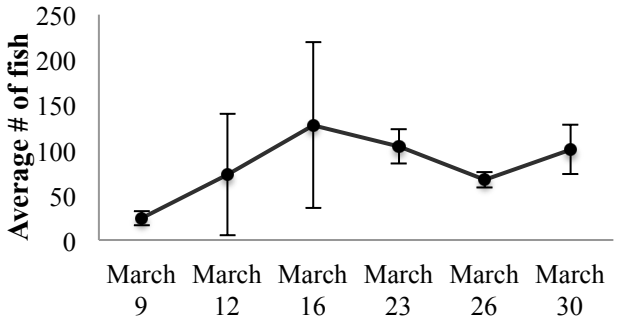

Fig. 5 Average abundance of *Haemulon flavolineatum* juvenile assemblage (± SD) over time at the sand flat artificial reef structures (n = 2)

Haemulon flavolineatum juvenile growth over time

Haemulon flavolineatum juveniles observed at the sand flat sites show a positive trend of increasing average length over a four-week period, with no fish over three centimeters observed (Fig. 6).

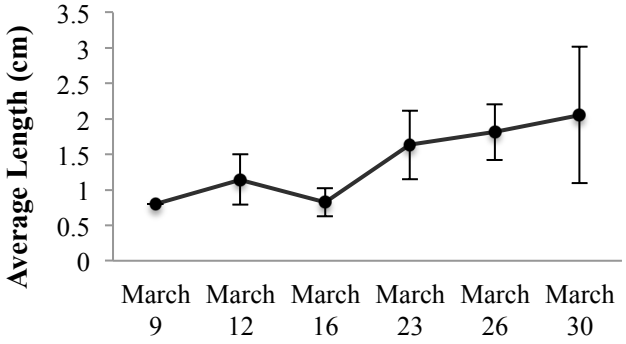

Fig. 6 Increase in average length (± SD) of *Haemulon flavolineatum* over time at the sand flat artificial reef structures (n = 2)

Cohort analysis of *Haemulon flavolineatum*

A cohort analysis was conducted by separating the *H. flavolineatum* population counted during all data collection dates into one-centimeter size classes (Fig. 7). On 9th March, counts were 31 fish <1 cm. The 12th March counts were 107 fish of 1 cm and 18 fish of 2 cm. The 16th March counts were 101 fish of <1 cm and 131 fish of 1 cm. The 23rd March counts were 66 fish of 1 cm and 112 fish of 2 cm. The 26th March counts were 21 fish of 1 cm and 91 fish of 2 cm. The 30th March counts were 20 fish of <1 cm, 40 fish of 1 cm, 39 fish of 2 cm and 80 fish of 3 cm.

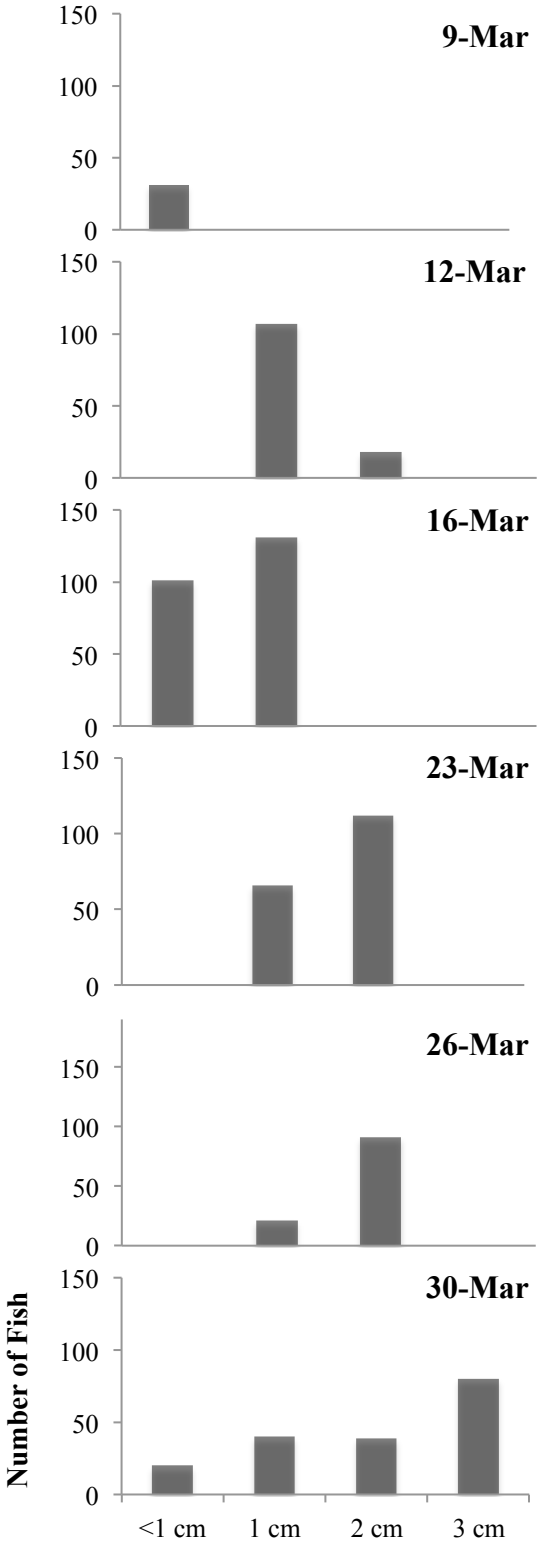

Fig. 7 Cohort analysis of *Haemulon flavolineatum* sizes recorded from sand flat artificial reef structures over the six data collection periods

Discussion

This study supports the hypothesis that ARSs placed 30 meters from the reef crest on the sand flat will have greater fish abundance than ARSs placed three meters from the reef crest. The sand flat structures combined attracted 1021 fish over the five week period compared to the 364 fish attracted to the reef flat structures. These results agree with a previous study that examined increased fish assemblage on isolated patch reefs within a larger coral reef system (Schroeder 1987). The higher percentage of juvenile fish at the sand flat structures suggests several positive causative factors to this outcome. The first is preferential recruitment by these juvenile populations towards the sand flat structures, the second is lower predation risk by having the sand flat act as a preventative barrier from the natural coral reef system, and the third is less interference by the high abundance of neighboring reef fishes on the reef crest. These factors all enhance the isolation of the sand flat structures, therefore increasing fish assemblage to the area (Schroeder 1987).

Species diversity was higher at the reef crest ARSs, not supporting the hypothesis that structures placed on the sand flat would have higher species diversity. This result contradicts other studies that have found isolation of artificial reefs to have higher fish biodiversity relative to less isolated ones (Walsh 1985). The exception to this contradiction however is that even though fish biodiversity of the reef crest structures was higher, the overall fish abundance surveyed was a lot lower when compared to the sand flat structures. This supports further observations in the same study that isolation might offer selective advantages in terms of reduced predation, competition, and disturbance (Walsh 1985). The healthy reef system at Yellow Submarine may have been the ultimate reason for less species being found at the more isolated structures. This is because most fish assemblages at this reef system do not need to find additional habitat if they already have sufficient resources within the established coral reef ecosystem. These important spatially-driven processes continue to be studied and a lot of research has helped us understand that reductions in the abundance of dominant habitat forming taxa (e.g. coral) interrupt trophic pathways and have wide-ranging impacts across a multitude of organisms (Wilson et al. 2006). Coral reefs and their high species richness, dynamism and evident susceptibility to a range of factors, both natural and anthropogenic, mean that these ecosystems are integral in the development of diversity-stability theory (Wilson et al. 2006).

Another important finding from this study is evidence of fish recruitment at the sand flat structures. French grunt juveniles made up 85% of the total fish assemblage observed at the sand flat. Evidence of recruitment is seen through a cohort analysis of the various size assemblages of French grunt juveniles over time. An increase in average length as well as average assemblage of French grunt juveniles over time was also observed. New cohorts of French grunt juveniles measuring less than one centimeter assembled to the sand flat ARSs in addition to already existing, larger French grunt juveniles. This shows the possibility of active fish recruitment taking place. Previous studies support this possibility and have found the highest total recruitment by coral reef fishes to be in lagoonal areas consisting of isolated patch reefs (Schroeder 1987). Fish recruits might be drawn to the sand flat ARSs because of the increased distance from a busy reef system, which provides more protection, enhancing the survival of post-larval fish cohorts through the vulnerable stage of recruitment (Schroeder 1987).

When environmental mangers consider using artificial reefs in order to mitigate coral reef degradation, they should consider the artificial reef design and location relative to the coral reef system. Based off this study, the design should implement different physical variables found to attract a wide array of fish species. These variables include holes, vertical relief, overhangs and void spaces (Walsh 1985; Hixon and Beets 1989; Lowry et al. 2014). When considering location, reefs that utilize the sand flat as a protective buffer will help

support future fish recruitment and provide additional habitat and structural complexity. Future studies should examine artificial reef effectiveness with more distance intervals from the natural reef to collect data on what distance is most effective in terms of fish assemblage. Further improvements and experimentation could also be done on artificial reef design, which could be implemented to protect future reef fish populations if coral reef ecosystems were to continue to be degraded from the increasing negative impacts on our oceans.

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REPORT

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Effects of sediment grain size on abundance of marine nematodes in Bonaire, Dutch Caribbean

Abstract Free-living marine nematodes are a functionally and morphologically diverse group of animals. They have important ecological functions, many of which are not currently well understood, and may be bioindicators of climate change and pollution. Nematode abundance is impacted by many factors; the focus of this paper is to study the effects of sediment composition on nematodes. Within a study site on the west side of the island of Bonaire in the Dutch Caribbean, three stations with different sediment compositions and mean grain sizes were selected. Endobenthos samples were taken at each station and abundance of nematodes was recorded and compared between the stations. Mean grain size was not found to have a correlation with the density of nematodes, non-nematode organisms, or total organisms across the study stations. The overall average density of nematodes found at the site (1.71 ± 0.32 nematodes per cm^3, ± SD) is lower than values found in comparable studies, which could be related to pollution or change in temperature affecting the endobenthic community in the study site. This data may have been insufficient to support the hypothesis due to having a small sample size and too narrow a range of mean grain size between the stations. Despite this, this paper provides the first published data on nematode communities in Bonaire, and is an important foundation for future study of the ecological functions of marine nematodes in this area.

Keywords
Endobenthos • meiofauna • sediment analysis

Introduction

The phylum Nematoda, also known as roundworms, represents an estimated 70-90% of all metazoan animals, and includes parasitic and free-living; marine, freshwater, and terrestrial species (Heip et al. 1982; Boucher and Gourbault 1990). Parasitic nematodes can cause disease in many organisms, including humans and crop plants, and thus inflict considerable personal and economic damage (Heip et al. 1985). However, nematodes may also be very useful, as in the case of the free-living terrestrial nematode *Caenorhabditis elegans*, which has been adopted as a genetic model organism to study developmental biology and neurobiology, and is frequently used in biomedical research (Kaletta and Hengartner 2006).

Free-living marine nematodes (from here on referred to as "nematodes" for simplicity) are part of the endobenthos, meaning they reside within the oxygenated top layer of sediment on the sea floor, where they can be among the dominant meiofauna (Heip et al. 1982, 1985). They are a morphologically and functionally diverse group of animals, and fill many ecological niches (Heip et al. 1985). Some nematodes feed on algae, dissolved organic matter, or detritus, while others actively hunt for their prey, with each feeding behavior being closely linked to a different morphology (Heip et al. 1985; Bongers and Ferris 1999). Free-living species range in size from 100 μm to 5 cm in length (Heip et al. 1985). Their tough exterior skin, or cuticle, can be very smooth or highly ornamented, including bumps, ridges, and hair-like

structures, which may be adaptive for specific habitats (Warwick 1971).

Nematodes' extreme overall abundance in the endobenthos suggests that they perform important ecological functions, but these are not fully understood. Danovaro et al. (2008) found that high nematode diversity is correlated with high overall benthic diversity and high ecosystem functioning and efficiency in deep-sea ecosystems. Sultan Ali et al. (1983) concluded that nematodes are involved in maintaining energy flow through sediments. Additionally, nematodes may be relevant to current global issues such as climate change and pollution. Bongers and Ferris (1999) suggested that shifts in nematode diversity patterns can be used as a bioindicator for climate change, since some species are more or less adaptable to changes in temperature, while Lambshead (1986) found that assessment of nematodes can reveal localized contamination caused by sewage and industrial waste. All of these findings support the ecological importance of these small animals, and provide more reasons for research to focus on them.

Abundance and diversity patterns of nematodes have been found to be dependent on salinity, sediment type, depth in sediment, horizontal distance from shore, other animals or algae in or on the sediment, tides, and human or naturally caused disturbances in the sediment (Heip et al. 1985). Nematodes are particularly sensitive to differences in sediment when compared to other meiofauna (Wieser 1959). Greater abundance of nematodes has been found in finer sediments; but a higher diversity of species is associated with coarser sediments (Wieser 1959; Heip et al. 1985).

This research investigated the marine nematode community in the shallow waters of Bonaire, Dutch Caribbean. There is limited research on free-living marine nematodes in most of the Caribbean, and no previously published works have been focused on Bonaire. Prior to the 1990's, much of the study of nematodes has been concentrated on the northwestern coastal regions of Europe (reviewed by Boucher and Lambshead 1995), but since then has been expanded to a greater range of habitats, including Cienfuegos Bay in Cuba (Armenteros et al. 2009), the Guadeloupe Islands (Boucher and Gourbault 1990), and deep waters of the eastern Mediterranean (Danovaro et al. 2001). This study provides a baseline from which other research in the area can be built.

This research seeks to assess whether abundance patterns previously seen in marine nematodes in other areas are also found on Bonaire by testing the hypothesis:

H_1: Areas with finer sediment have a greater abundance of nematodes

Materials and methods

Study site

Bonaire is an island in the Dutch Caribbean, about 50 km east of Curacao and 100 km north of the northern coast of Venezuela. The study site is along the waterfront of downtown Kralendijk on the west side of Bonaire (Fig. 1), between the Yellow Submarine (12° 09' 36.4536" N, 068° 16' 55.0056" W) and Something Special (12° 09' 45.4644" N, 068° 17' 06.7596" W) dive site entrances, a distance along the shore of about 300 m. This area is on the leeward side of the island, and generally does not experience strong winds or currents. Immediately to the north of the study site is the entrance to a marina, which may influence current, sediment distribution, and pollutants in the area. Kralendijk is the most highly populated area on the island, and the nearby waters are subject to water runoff and other human influences.

The study site includes a large, gently sloping sand flat of no more than 10˚ inclination, containing scattered mooring blocks and other large debris. The sand flat extends from the shoreline to the reef crest, about 40 m from shore, with a maximum depth of about 5 m. Beyond the reef crest is a coral reef slope of about 45˚ inclination, which continues to a depth of about 30 m, where it levels off into another sand flat.

Three stations were selected within the study site based on sediment composition. Out of nine potential stations examined, stations 4 (12° 09' 35.1360" N, 068° 16' 56.2872" W), 6 (12° 09' 37.1052" N, 068° 16' 58.2060" W) and 8 (12° 09' 39.3840" N, 068° 17' 00.5136" W) were chosen for further sampling. These stations were all at approximately five meters of depth, and no closer than three meters to the nearest coral head, mooring block, or other structure. Because the stations were all within a 300 m range, it was assumed that the impact on abundance of nematodes by other factors, such as salinity and temperature, was minimal.

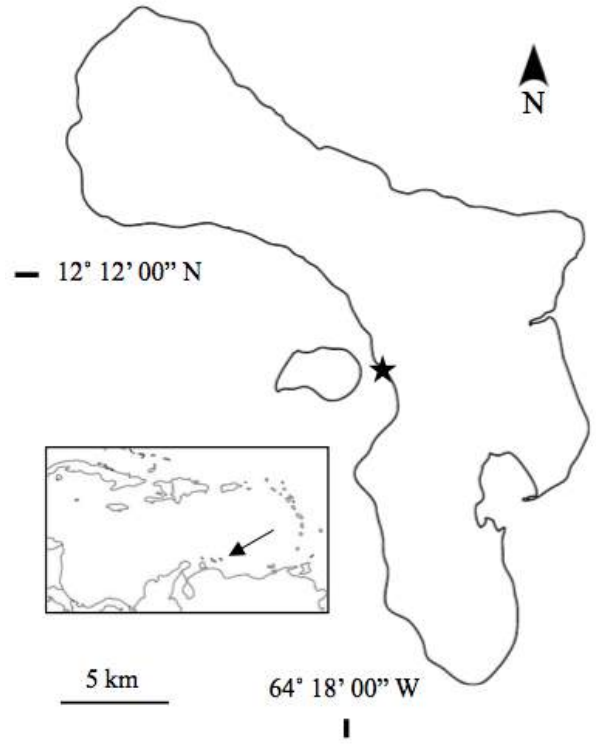

Fig. 1 Map of Bonaire, Dutch Caribbean. Star indicates study site location. Arrow in inset indicates location of Bonaire in the Caribbean Sea

Sample collection

All samples were collected via SCUBA diving. Sediment and benthos samples were both collected using a PVC hand corer of 3 cm interior diameter, inserted 6 cm deep into the sediment. A small, flat piece of plastic was slid underneath the open end of the corer and held there firmly before removing the corer from the sediment to reduce loss of sediment particles. Samples were taken from sandy patches, avoiding pieces of rubble or larger organisms such as tubeworms living on the sand. Samples were placed in labeled zip-top plastic bags for transport back to the laboratory. Sediment and benthos samples were collected on different days within a four-week period.

Granulometry and selection of study stations

Grain size analysis was performed on sediment samples collected from nine potential stations within the study site. One sample was collected at each station. Samples were first air dried in trays until no visible pools of water remained, and then oven-dried at 38˚C for 48 hours, or longer if necessary, until completely dry. Dried samples were manually broken up if they had formed hardened clumps in the oven, and then placed into a sifter, which was set to an amplitude of 30 for 10 minutes, or longer if necessary for sediment to be thoroughly sifted. The sifter has meshes with openings of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm, and 63 μm in diameter, arranged in descending order from top to bottom, with any particles smaller than 63 μm being collected in a tray at the bottom. Sediment collected at each level was separately weighed.

This data was used to determine the cumulative mass retained above each mesh as a percentage of the total mass of the sediment sample. The mesh opening diameters were transformed into phi by taking the negative base two log of the diameter in millimeters. Phi was plotted versus cumulative mass retained for all nine stations, and these plots were visually compared to select the three stations most representative of different sediment compositions.

Nematode extraction and analysis

Most of the methods described here are adapted from Armenteros et al. (2009), with some influence from Heip et al. (1985). Benthos samples were collected at each of the three selected stations via the method described above. Two or three replicates were collected

from each station to ensure sufficient material for analysis.

In the laboratory, benthos samples were dyed with Rose Bengal and thoroughly mixed. Tap water was added if necessary such that the entire sample was submerged. Samples were left in the refrigerator for at least 12 hours to allow the dye to stain any organic matter present. Samples were randomly selected for subsampling such that three subsamples were analyzed per station. The 10-cm^3 subsamples were rinsed over a 30-µm mesh to wash away excess dye. What remained on the mesh was combined with water and commercial white sugar crystals to reach a concentration of approximately 1.17 g cm^{-3}, thus changing the water density so that organic material floated to the top.

After thoroughly mixing the sample and allowing sediment to settle to the bottom, 200-µL aliquots were taken from the surface to be viewed under a microscope on glass slides. Aliquots continued to be taken until further aliquots yielded few or no organisms and there were no visible pink-dyed meiofauna in the sample. Total number of nematodes was recorded across all aliquots, as well as number of individuals in other broad categories of meiofauna.

Data analysis

Nematode density was calculated for each station by taking the total number of individual nematodes found in each subsample and dividing by the subsample volume to get a value of number of nematodes per cubic centimeter. This was averaged across all the subsamples analyzed for each station.

Sediment composition data was analyzed using the particle analysis software GRADISTAT (www.kpal.co.uk/gradistat.html). Logarithmic mean grain size was used as the single variable against which to compare nematode density.

Pearson's correlation test was used to determine the significance of the relationship between mean grain size and nematode density.

Results

Granulometry and selection of stations

To determine the location of study stations from which to collect endobenthos samples, sediment samples from nine potential stations were analyzed by plotting particle diameter in phi versus cumulative percent mass retained at that diameter. Visually comparing all nine potential stations in this way showed that stations 4, 6, and 8 were representative of the three most different sediment compositions present among all nine stations (Fig. 2). This data is also visualized in Fig. 3. The mean grain size at stations 4, 6, and 8 was 1.36, 0.85, and 0.65 phi, respectively. The average mean grain size across all three stations was 0.95 ± 0.37 phi (± SD).

Endobenthic organism density analysis

To determine if density of nematodes in the endobenthos is correlated with grain size, average abundance of nematodes per cm^3 across three subsamples was compared to mean grain size in phi for each station (Fig. 4). The average number of nematodes per cm^3 (± SD) for stations 4, 6, and 8 was 1.77 ± 0.86, 1.37 ± 0.31, and 2.00 ± 0.66. The average nematode

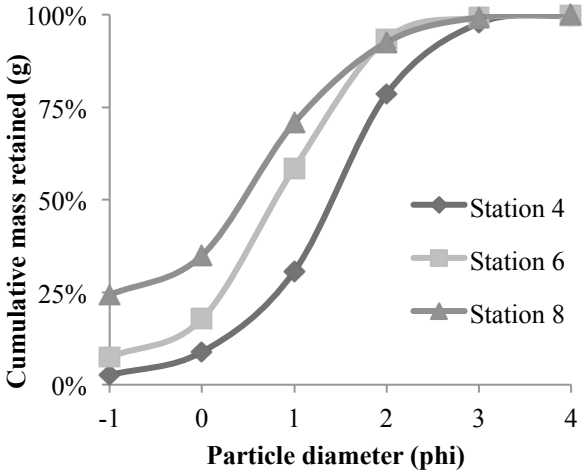

Fig. 2 Cumulative mass retained, as a percentage of total sample mass, above sieves with openings of the given diameter in phi for sediment samples taken from three stations in the study site

density across all stations was 1.71 ± 0.32 nematodes per cm^3. Pearson's correlation test resulted in a non-significant correlation between average nematode density and mean grain size (R^2 = 0.01699, p = 0.9168, n = 3). The same test was used to determine if there were significant correlations between mean grain size and average number of non-nematode organisms per cm^3 (R^2 = 0.97252, p = 0.1060, n = 3), and between mean grain size and average total number of organisms per cm^3 (R^2 = 0.59763, p = 0.04374, n = 3). These results demonstrate that the density of nematodes, non-nematode organisms, and total organisms in the endobenthos do not have a significant correlation with the mean grain size in that location.

Discussion

The results of this research do not support the hypothesis that areas with finer sediment have a greater abundance of nematodes; however, other conclusions may be drawn, especially since this paper provides the first published data on any marine nematode community in Bonaire.

Nematode density may be not correlated with mean grain size because the range of grain sizes among the stations was simply not large enough, and only three stations were used which limits the ability to detect trends in the data. Due to time constraints, only one sample from each station was analyzed to determine sediment composition, which may have resulted in a less accurate representation of its composition. Future studies may consider selecting more stations with sediment compositions that are more different, and obtaining more samples on which to perform granulometry.

It is possible that analysis of more subsamples from the endobenthos samples could have resulted in average nematode density values that were more different between the stations, or these averages may have had smaller standard deviations, thus making it possible to detect subtle trends. Non-nematode endobenthic organisms were not the focus of this research, but implementation of the suggested changes in future studies may yield more interesting results for other organisms as well as nematodes.

The overall average value for nematode density found in this research, 1.71 ± 0.32 nematodes per cm^3, is less than other comparable values found in the literature. Armenteros et al. (2009), from whom some of the methods for this paper are adapted, studied nematodes in Cienfuegos Bay, Cuba, which has a mean depth of 14 m, and found an average density of 76.5 individuals per cm^3. Around the island of Guadeloupe in the French West Indies, Boucher and Gourbault (1990) found an average nematode density of 1,794.44 individuals per cm^3 in benthos samples they collected at depths of less than 4.5 meters. Although this is by no means a perfect comparison, it is clear nonetheless that the average nematode density found in this study is different from those found in these two other studies done at similar depths in the same region. These studies may have been better

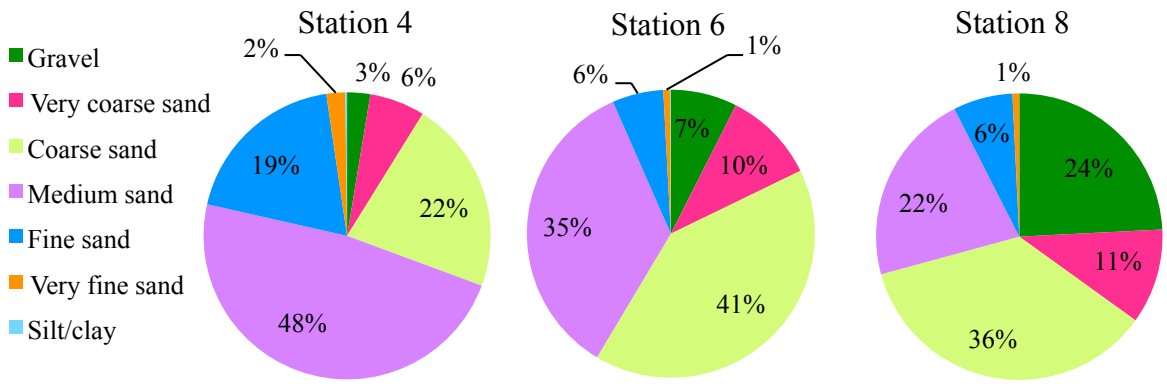

Fig. 3 Sediment composition of samples taken from three stations in the study site. The silt/clay component in all three samples was less than 1%

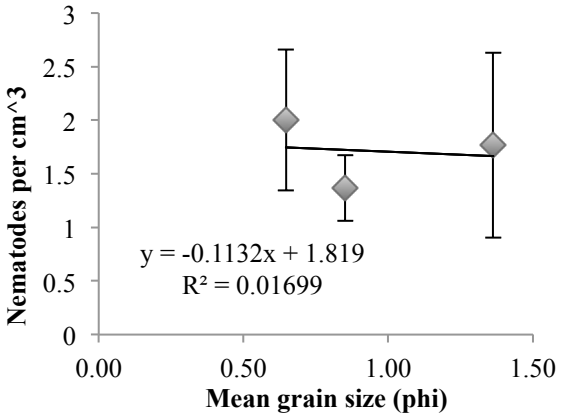

Fig. 4 Comparison of average nematodes per cm^3 (n=3 subsamples per station) to mean grain size across three stations, with a linear trendline. Error bars represent standard deviation

able to detect very small nematodes than the research described here, due to limitations in the methodology used for this study, which could account for some of the difference in average nematode density.

The difference in average nematode densities found in this research compared to these other studies could also have a more biologically relevant cause. Pollutants introduced into the environment could be affecting the density of nematodes in the endobenthos (Lambshead 1986), especially due to the proximity of the study site to a highly populated area. Another potential explanation is rising temperatures, which can be caused by climate change, and have been shown to impact different aspects of many ecosystems (Bongers and Ferris 1999; Danovaro et al. 2001). Bongers and Ferris (1999) found that nematodes respond rapidly to changes in their environment, including pollution and temperature changes; depending on the nematode groups and species originally found in the area, this response can manifest as an overall lower abundance of nematodes. Danovaro et al. (2001) suggested that temperature changes might reduce microbial biomass and activity, thus shrinking an important food source for some nematode groups, and limiting those nematodes' abundance. A lower abundance of all nematode groups was documented by Lambshead (1986) in an area affected by high levels of sewage and industrial waste contamination compared to an unaffected area. Global climate change may not have a detectable impact on Bonaire, but temperatures in the endobenthos may change for other reasons, such as low turbidity and less water mixing from the surface to the depths. Temperature change and pollution may not yet have significant impacts on Bonaire, so the probability of either of these being the explanation for the data obtained is uncertain. More research would need to be conducted measuring the impacts of each of these factors in the area.

Qualitative observations made during the study detected a variety of morphologies among nematode individuals, including differences in cuticular ornamentation. There was not sufficient time to fully record and quantify these differences, but several distinct morphological features were noted. Some nematode individuals had longitudinal rows of large bumpy protrusions in their cuticle near the anterior end. Others had many small, almost undetectable, circumferential ridges concentrated near the mouth. Some had cuticles appearing completely smooth. Cuticular ornamentation may be associated with sediment type; species with more highly ornamented cuticles may be more protected in unstable, shifting sediments with larger grain size, while smoother-cuticled species may be better suited to fine-grained sediment (Ward 1975). The sediment samples analyzed were heterogeneous and poorly sorted in terms of particle size, with no single grain size category comprising the majority of the sample (Fig. 3), which could be related to the variety of cuticular types seen across all samples. Further study of nematodes on Bonaire could quantify the variety of cuticle types seen to determine if level of ornamentation is correlated with sediment composition.

Free-living marine nematodes are extremely abundant and diverse, and may be an indicator of changes happening on our planet. Doing research on them now is key to provide baselines so we can see how nematode communities change over time. Although this research yielded no statistically significant

results, other scientists may learn from and expand upon the results of this study. The methods described here can be used to extract and analyze endobenthic fauna with a limited budget and resources, which may make it possible for analysis of nematodes and other meiofauna to be done more easily in the future.

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REPORT

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Effect of population density and aggressive behavior of Three Spot Damselfish (*Stegastes planifrons*) on macroalgae cover and parrotfish grazing activity

Abstract When overly abundant, macroalgae can be a major threat to the health of a coral reef ecosystem due to its capability to smother live coral and reduce the rates of recruitment. Several factors can contribute to macroalgal growth, one of the controlling elements being a lack of herbivorous grazing. When grazing pressure is high the ecosystem remains balanced, but when grazing pressure is low reefs can experience macroalgal blooms that have a lasting negative effect. This study examined the indirect causes of macroalgal cover change through assessing damselfish aggression. *Stegastes planifrons*, also known as the Three Spot Damselfish, are highly aggressive and territorial fish that will defend their territories against a number of intruding species. This study looked at the relationship between damselfish abundance and aggression and the grazing behavior of parrotfish, as well as the relationship between damselfish abundance and macroalgal cover on Bonaire, Dutch Caribbean. Video transects were implemented over the chosen study stations and then analyzed with Coral Point Count (CPCe) software to attain the percentages of macroalgae cover at each station. Aggressive behavior of the three spot damselfish as well as the grazing behavior of parrotfish were observed and recorded using SCUBA diving. It was found that damselfish aggression and parrotfish grazing were negatively correlated, and that parrotfish grazing followed the same trend line as the macroalgae cover. Based on the findings of this study it was concluded that *S. planifrons* aggression has no considerable effect on the grazing behavior of parrotfish, and it can be assumed that it does not contribute to increased macroalgal cover.

Keywords Bonaire • Caribbean • videotransect

Introduction

When assessing the rate of recovery in tropical reefs, one of the main factors to consider is the percent macroalgae cover. When present in large amounts, macroalgae has the ability to smother coral colonies by blocking their exposure to sunlight, which eventually results in their death (Hughes 1994). Having a high algal biomass also lowers coral recruitment rates because the new polyps cannot find a hard substrate to settle on (Hughes 1994). Keeping macroalgal abundance below these suffocating levels is critical for coral survival, and this is achieved through the many different species of herbivorous fish that inhabit tropical coral reefs, most importantly parrotfish. A study by Lewis (1986) found that parrotfish grazing considerably influences the distributions and abundances of algal species on tropical reefs.

An important factor in this relationship that has been less studied is the effect territorial damselfish behavior has on herbivores. Parrotfish are the most common herbivores encroaching on damselfish territories (Klumpp and Polunin 1989). Damselfish cultivate areas on the reef referred to as their “algal gardens”, which can contain several species of algae but are predominately characterized by filamentous turf algae (Ruyter van Steveninck 1984). A study by Lassuy (1980) showed that damselfish remove undesirable species of algae from their

gardens, but they actively maintain species that they intend to feed upon. Damselfish, especially *Stegastes planifrons*, are highly territorial of their algal gardens, and they often attack other herbivorous fish in attempts to expel them from their claimed area of the reef (Brawley and Adey 1977; Russ 1987). Because of their aggressive nature it has been hypothesized that damselfish may be classified as a keystone species for coral reefs, as they reduce the overall amount of grazing on surrounding algae surrounding their territory (Hixon and Brostoff 1983).

The latest in the series of reports by Steneck et al. (2015) showed that in recent years the density of three spot damselfish on Bonaire has increased significantly due to a decrease in predator abundance. This report also lists *S. planifrons* as one of the most territorial species on Bonaire. A few studies have been conducted on Bonaire to examine the effect *S. planifrons* has on the surrounding benthic community (Arnold et. al 2010; Vermeij et. al 2015), concluding mainly that their presence negatively effected coral recruitment. Vermeij et. al (2015) suggested that by deterring the grazing behavior of other herbivorous fishes, *S. planifrons* abundance may also have an effect on lower trophic levels such as primary producers. I hypothesize that the recent increase in *S. planifrons* may have an effect on the amount of grazing parrotfish nearby, and could result in an increase in macroalgae cover in areas where the damselfish are abundant.

This study builds on past research that has been done in this area, specifically highlighting the connections between *S. planifrons* abundance, the amount of grazing parrotfish, and the level of macroalgal cover. It also provides new data about damselfish in Caribbean coral reefs, since most of the previous studies on *Stegastes* spp. have been conducted in other areas of the world (Hixon and Brostoff 1983; Russ 1987; Jones 1992).

H_1: Areas with higher densities of *Stegastes planifrons* will have higher densities of macroalgae

H_2: Areas with higher densities of *Stegastes planifrons* will have less grazing parrotfish

Materials and methods

Study site

The study was conducted on the small island of Bonaire, Dutch Caribbean. Bonaire is roughly 288km^2 and is about 80 km north of Venezuela (Fig 1). The study site was a local dive site, south of Something Special, on the western side of the island known as Yellow Submarine (12°09’36.5’’N, 68°16’55.2’’ W). The reef at this location is a fringing reef that begins with 50 m of sand flat starting at a depth of 3 m and gradually sloping until the reef crest, which is approximately 6 m below the surface. The reef continues to slope downwards at a 45˚ angle until approximately 33 m, where there is another sand flat. This location has high biodiversity with an abundance of fish species, hard and soft corals, sponges and algae.

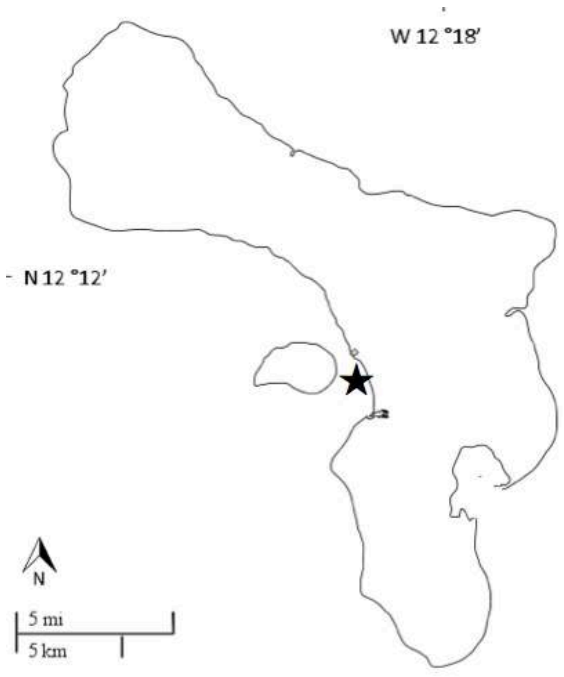

Fig. 1 Map of Bonaire, Dutch Caribbean. The black star indicates the studied site, Yellow Submarine (12˚09’36.5’’ N, 68˚16’55.2’’ W)

Damselfish density and station selection

Using SCUBA, potential stations were objectively chosen and surveyed to determine the abundance of *S. planifrons*. At each station 10 m transects were laid out along the reef using SCUBA and the number of *S. planifrons* at each station was counted and recorded manually using a T-bar to gauge the 2 m width (Russ 1987). Two stations were picked to represent areas with high *S. planifrons* abundance, and two were picked to represent areas with low abundance. Station A had five damselfish present, station B had seven damselfish, and stations C and D had 15.

Macroalgae cover

To estimate the percent macroalgal cover 1 m wide video transects were conducted on each side of the 10 m transect tape at every station. Sequentially each transect was analyzed using Coral Point Count software (CPCe).

Damselfish behavior

In order to assess the aggressive behavioral patterns of *S. planifrons*, one SCUBA dive was conducted at each of the four stations. Following a 5 min acclimation period, a 30 min observation period was used to record the number of parrotfish that swam through the survey area; every parrotfish was labeled as either grazing or non-grazing. *Stegastes planifrons* aggression was also measured by recording the number of bites and charges (i.e. quick and sudden accelerations towards invading fish) exhibited by each damselfish during the 30 min observation period. All dives were completed at a uniform depth of 10.5m.

Data handling

At each of the four study stations chosen, one transect was analyzed to determine the overall substrate composition. A total of 10 frames were pulled from each video transect and each frame was examined in the Coral Point Count computer program using 40 randomly selected points per frame. Each point was then categorized into one of four categorizes (i.e. sand, macroalgae, hard substrate or live cover).

Percent of parrotfish actively grazing was calculated for each station. Likewise, the number of damselfish bites and charges were combined to represent the total aggressive displays. These values were then standardized by the number of *S. planifrons* at each station to get an aggression index, which represent the number of aggressive displays per damselfish at each station. The aggression indexes were compared to the number of grazing and non-grazing parrotfish at each station. In order to find the underlying trend, macroalgae cover was also included in the comparison.

Results

Macroalgae cover

Coral Point Count software was used to calculate the percentage of macroalgae, hard substrate, live cover, and sand at stations A through D. The percentage of macroalgae was different across all study stations. Station A had 18.54% cover, Station B had 10.29%, Station C had 8.99%, and Station D had 7.25%. Furthermore, the number of *S. planifrons* at each station negatively correlated with macroalgae cover (Fig. 2). Besides macroalgae the overall composition of substrate was relatively constant at each station, with the exception of two values. At station D the percentage of live cover jumped to 50.72, compared to the minimum value of 34.83 at station C (Table 1). At station C the percentage of sand was 16.01, which was double the amount of sand found at the other three stations (Table 1). The amount of hard substrate did not vary from station to station.

Table 1 Percentage of the different substrate covers at stations A through D, calculated using CPCe computer software

Substrate Category	Station A	Station B	Station C	Station D
Sand	8.71	7.72	16.01	7.25
Macroalgae	18.54	10.29	8.99	7.25
Hard Substrate	33.43	36.33	40.17	34.78
Live Cover	39.33	45.66	34.83	50.72

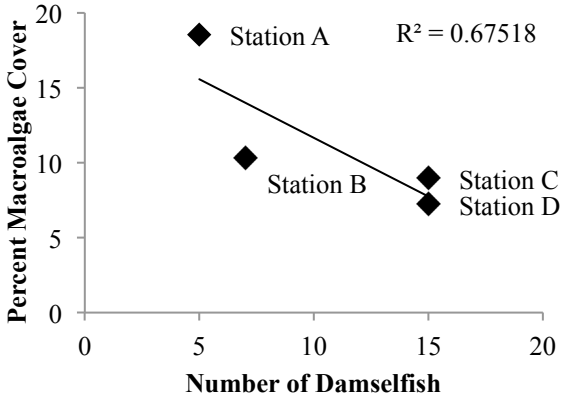

Fig. 2 Percentage of macroalgae cover in correlation to the amount of *Stegastes planifrons* at each location

Behavior analysis

Parrotfish

Stations C and D had a lower percentage of grazing parrotfish than stations A and B (Fig. 3). As the number of *S. planifrons* on site decreased, there was an increase in the amount of grazing parrotfish. Considering each station individually, there were no substantial differences between grazing and non-grazing behavior at stations B and D. However, strong contrasts are apparent at stations A and D. At station A there was double the amount of grazing parrotfish compared to not grazing (Fig. 3). At station C, there were half as many grazing parrotfish as non-grazing parrotfish. Non-grazing parrotfish abundance stayed uniform across all four stations.

Damselfish

The lowest aggression index observed was 1.14 and the highest was 2.20. There was no clear trend for the amount of aggressive displays compared the amount of grazing parrotfish observed. The number of non-grazing parrotfish remained constant when compared to aggression, while the number of grazing parrotfish started high then was followed by a decline and then a steady increase (Fig. 4). Additional inspection of the macroalgae cover at each site showed that it followed the same trend line as the grazing parrotfish when compared to aggressive displays (Fig. 4).

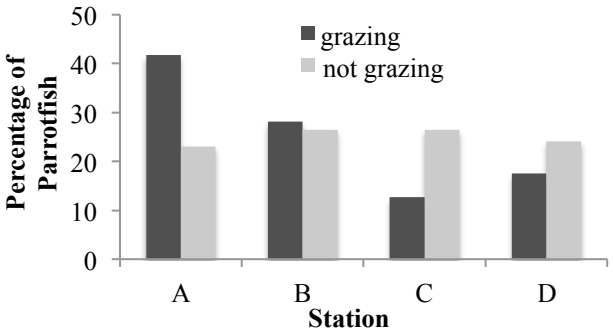

Fig. 3 Percentage of grazing and non-grazing parrotfish observed at each individual station. Stations A and B had low densities of damselfish (5 and 7), where stations C and D had high densities of damselfish (both 15)

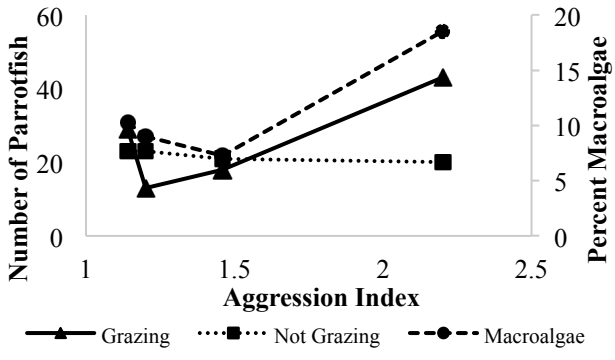

Fig. 4 Aggression index, represented as the number of aggressive displays (i.e. charges and bites) per *Stegastes planifrons* at each station, in relationship with overall parrotfish abundance and macroalgae cover. The primary y-axis indicates the number of parrotfish observed, while the secondary y-axis indicates the percent macroalgae cover at each station.

Discussion

The hypothesis that areas with a higher density of *S. planifrons* have higher densities of macroalgae was not supported. The data showed that the opposite trend was occurring, as the number of damselfish in the area increased when the percent macroalgae decreased. There are a number of plausible explanations that could be influencing this trend. The high sand and live cover at stations C and D provide less space for macroalgae to settle on, and that could be a reason why less macroalgae was found there. Another likely explanation is that at the stations with higher *S. planifrons* density more area is dedicated to algal gardens. The algal gardens of *S. planifrons* are made up of predominately filamentous turf algae (Ruyter van Steveninck 1984), which was not included under the category macroalgae during data analysis. Being that damselfish "farm" their gardens to get rid of undesirable macroalgal growth, the only place we would have seen macroalgae was outside their territories (Lassuy 1980). It makes sense that in areas with fewer damselfish there was more macroalgae, simply because there was more space outside of damselfish territories for it to grow.

Fewer parrotfish were seen feeding at stations C and D than at stations A and B, which supports the hypothesis that areas with a higher abundance of *S. planifrons* have fewer grazing parrotfish. In this case however the statistical correlation does not represent causation. The aggressive behavior of *S. planifrons* was studied to determine if their characteristic aggression was the cause for less grazing parrotfish. After standardizing the aggression index, the amount of parrotfish grazing seemingly increased, with an increase in the amount of aggression, despite an initial high value for grazing. The initial high value for grazing occurred at a station that, due to a scheduling error, was studied at 09:00 hrs where as the rest of the stations were studied around 13:00 hrs. A study done by Bruggeman et. al (1994) showed that over a 24 h period parrotfish feeding habits increased in the morning and were highest in the mid-afternoon. The high value seen at that station could be attributed to that feeding schedule. Being that the rest of the stations were studied at the same time though, time of day cannot account for the steady increase in grazers after the initial high value. Instead the progression can be attributed to the presence of macroalgae. The percent macroalgae cover followed the same trend line as the grazing parrotfish did when compared to aggression. It can be inferred that the increased aggression in *S. planifrons* is a reaction to the increased number of grazing parrotfish, who seemingly are not affected by damselfish abundance but rather choose to graze where there is the highest percentage of macroalgae.

The results of this study found that parrotfish are not affected by territoriality, which differs from the findings of Hixon and Brostoff (1983) and Jones (1992) that suggested herbivorous fish are affected by damselfish aggression, although their study did not look at parrotfish specifically. These two studies measured grazing behavior by the amount of bites, or density of the bites that herbivores took, and both revealed a statistical relationship between aggression and lack of herbivorous grazing. My study focused not on the bites taken but rather on the amount of parrotfish that were grazing. Parrotfish may stop feeding when attacked, which would lead to less recorded bites and therefore show a negative relationship between aggression and grazing. However, my research suggests that no biological relationship was present regarding *S. planifrons* aggression and grazing behavior. Since parrotfish did not alter their grazing patterns to avoid areas with more damselfish, their behavior appeared to be driven by macroalgae abundance.

Based on these findings, damselfish abundance cannot be used as a proxy to evaluate macroalgal growth on Bonaire. It appears that their abundance and territorial behavior did not affect parrotfish herbivory, and therefore has no behavioral effect on macroalgal cover. However, this study only sampled four sites, with only one sample at

each. Additional studies on *S. planifrons* aggression should be conducted to further support this assertion, perhaps expanding to other areas of the Caribbean and including other families of herbivorous fish. Long-term behavioral studies should also be implemented to determine if over a longer time span macroalgae levels increase, and if parrotfish alter their grazing in ways that could not be determined through the methods used in this study.

Acknowledgements I would like to thank the staff at CIEE Bonaire Research Station for their help in planning this study and for providing the materials necessary to complete my research. I would also like to thank Dr. Enrique Arboleda and Amy Gosney for the many hours spent proofreading and providing feedback on my research, as well as my dive buddy Saffron Data for her support in the field. None of this would have been possible without you all.

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REPORT

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Effects of structural complexity on Sharpnose Pufferfish, *Canthigaster rostrata* (Tetraodontidae), abundance and size in the shallows of Bonaire, Dutch Caribbean

Abstract The Sharpnose Pufferfish, *Canthigaster rostrata*, as well as other species within the Tetraodontidae family are common reef fish found in tropical and subtropical waters. Past studies on some of the 36 species within the same genus share consistent observations on their haremic structure, territorial nature, and spawning patterns. Although research has been done specifically on *C. rostrata* in Panama, little is known about *C. rostrata* in the Lesser Antilles. This study provides ecological data on the correlation between territory size and complexity, as well as the size and abundance of individual *C. rostrata*. Over the course of five weeks, the size, abundance, and behavior of *C. rostrata* were recorded. Data was collected at five specific structures at a local dive site known as Yellow Submarine. A custom-designed complexity chart was made to rank these structures in order of complexity. Results indicated that structure volume is a criterion that affects complexity, but that it is not the main one. Results also showed that more complex structures hosted more individuals on average from highest to lowest (mean ± SD), was 5.62 ± 1.18, 4.12 ± 1.80, 2.14 ± 1.06, 1.20 ± 1 and 1.62 ± 0.91. More complex structures also hosted larger individuals of *C. rostrata* on average from highest to lowest (mean ± SD), was 4.81 cm ± 0.6, 3.06 cm ± 0, 2.9 ± 0.35, 2.55 ± 0.44, and 2.43 ± 0.5.

Keywords Tropical marine ecology • reef fish • harem

Introduction

Canthigaster rostrata is one of the 36 species belonging to the *Canthigaster* genus (Matsuura 2014). As their common name indicates, they have a sharp beak at the end of their snouts, which they use to feed on small crustaceans, invertebrates, tunicates, and algae (Randall 1996). Species from this genus are found in tropical and subtropical waters and tend to live in habitats marginal to coral reefs. According to Bloch (1786), *C. rostrata*'s geographical distribution ranges from North Carolina, to Bermuda, Tobago, and the Lesser Antilles.

Like other members of the Tetraodontidae family, *C. rostrata* is capable of inflating as a means of defense when attacked, and contain tetrodotoxins in their skin. The lack of parental care succeeding fertilization, observed in at least one species (*Canthigaster valentini),* has been linked to the fact that tetrodotoxin is also found in the ovaries of members of the *Canthigaster* genus, making their eggs unpalatable to other reef fishes (Gladstone 1987). Published reports of ingested Sharpnose Puffers are rare, but known predators include Barracuda and peacock flounders (Randall 1967, Gochfeld and Olson 2008).

Past studies on *C. valentini* (Gladstone 1987)*, C. punctatissima* (Kobayashi 1986)*,* and *C. rostrata* (Sikkel 1990) share consistent observations on social structure, territorial behavior, and spawning. According to these studies, members of these species live in a haremic community where one large male guards multiple smaller territories, which are in turn defended by approximately one to four

smaller females. Benthic spawning occurs over an algal nest daily between 08:00 hrs and 16:00 hrs, and continues year-round (Gladstone and Westoby 1988). In Hawaii, a pair of *C. amboinensis* was observed spawning, which lasted for 20 to 30 seconds, versus the five second spawning bouts recorded of *C. rostrata* in the Caribbean (Sikkel and Sikkel 2012).

Despite the various data confirming the territorial behavior, social structure, and spawning of species of the genus *Canthigaster,* the existing literature does not thoroughly explore the impact of territory types on *C. rostrata* communities. This study aimed to determine if soft coral hosted more individuals than stony corals, or if structures with tangled ropes or anemones provided more shelter and thus hosted more *C. rostrata*. For this reason, this study focused on how structural complexity (e.g. size, holes, other living substrate) affects *C. rostrata* abundance and size in the Bonaire National Marine Park, Dutch Caribbean. This study is important because global coral reef degradation is causing habitat loss and declining populations of coral reef fishes (Feary 2007).

H_1: Larger physical structures will serve as the territory for more *Canthigaster rostrata* than smaller structures

H_2: More complex physical structures will serve as the territory for more *Canthigaster rostrata* than simpler structures

Materials and methods

Study site

The site used to carry out this study is a fringing reef located on the leeward side of Bonaire, Dutch Caribbean. It is locally known as Yellow Submarine (12° 09'36.43"N, 68°16'55.03"W) and is part of the Bonaire National Marine Park. The reef crest is at approximately six meters deep and slopes dramatically (~ 60°) from there on. The sand flat, which extends from the shore to the crest of the coral reef, contains mooring blocks and distinct coral heads, which are ideal habitats for *C. rostrata* who tend to inhabit areas marginal to the reef according to Fish Net.

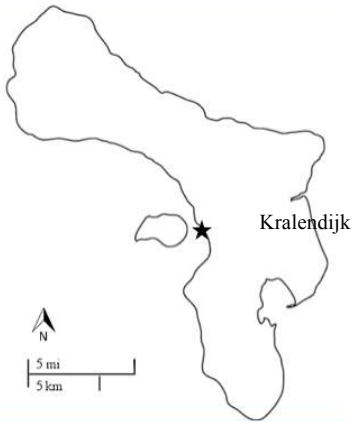

Fig 1 Map of Bonaire, Dutch Caribbean with the study site, Yellow Submarine (12° 9'36.43"N 68°16'55.03"W) marked with a star

Designated structures

For this specific study, five separate structures located at approximately the same depth in the sand flat (1.8 to 2.4 meters) were designated for data collection. They were selected because each one was relatively isolated (nearest structure was six meters away) and completely surrounded by sand, with the exception of structure B and E (which were half a meter apart); making the communities of *C. rostrata* at these structures very distinct. Structure A was a large *Orbicella annularis* with an anemone, structure B was a mooring block with entangled ropes, structure C was a *Siderastrea siderea* coral head adjacent to a large gorgonian, structure D was a tire with a few *Diploria strigosa* and *Diploria labyrinthiformis* growing on top of it, and structure E consisted of two metal barrels laying side by side. Structures A, B, C, D, and E were ranked from most complex to least complex. Complexity indexes were created based on the presence or absence of gorgonians, holes, ropes, overhangs, *Millepora complanata,* and anemones, which are all structural variables that *C. rostrata* seemed to seek for shelter according to personal observation. The structure with the highest value of a certain variable was given one point.

The values that preceded it were given points proportionally to one. This process was repeated for each complexity variable used. One complexity index included structure size and the other did not. In order to include structure size in the complexity index, the same adjustments had to be made. Therefore, the structure with the largest volume was given one point and the volumes that preceded it were given points proportionally to one.

Data collection

Fish counts and measurements were conducted bi-weekly for five weeks at 14:30 hrs (AST), and biweekly for one week at dawn (05:45) using SCUBA (Dives five and six) for a total of five data collection points. Because the amount of *C. rostrata* relative to the type of structure is a more relevant variable for this study, rather than the amount of *C. rostrata* relative to the time of day, the amount of individuals counted at dawn were averaged together with counts obtained from all the other day-time dives. Each structure was visited in the same order on each dive, and individual *C. rostrata* were counted and measured with a 10 cm T-bar made out of PVC pipes.

Data analysis

In order to determine the average abundance of *C. rostrata* per structure (Fig. 2), the quantity of *C. rostrata* was recorded at each of the five structures on eight separate dives. Structures D and C are missing some data and therefore have a different number of replicates (n = 5 and n = 7).

Average size of *C. rostrata* per structure (Fig. 3) was calculated by multiplying the amount of individuals of a certain size with their size in centimeters for each structure. Each structure (except E) obtained five totals, which were then averaged. Due to the amount of times structure E was visited, the number of replicates for E was three.

In order to obtain the average abundance of *C. rostrata* size class per structure (Fig. 4), the amount of *C. rostrata* counted over the course of five dives was averaged according to their size and per structure. All of the averages were then consolidated into 1 cm size ranges.

Results

The complexity indexes were derived from the custom-designed complexity chart. The first is the total not including structure size as a complexity factor with A, B, C, D, and E, ranked as 7, 7, 5, 5, and 3, and the second is the total including structure size as a complexity factor with A, B, C, D, and E, ranked as 8, 7.34, 6.21, 6.19, and 3.24 respectively (Fig. 2). Both complexity indexes express the same overall orders in rankings, with the main difference being that structures A and B are equally as complex (with seven points) and C and D are also equally complex (with six points). These results indicate that volume is a criterion that affects complexity, but shows that it is not the main one. For this reason, the all-inclusive complexity index was used as the standard index.

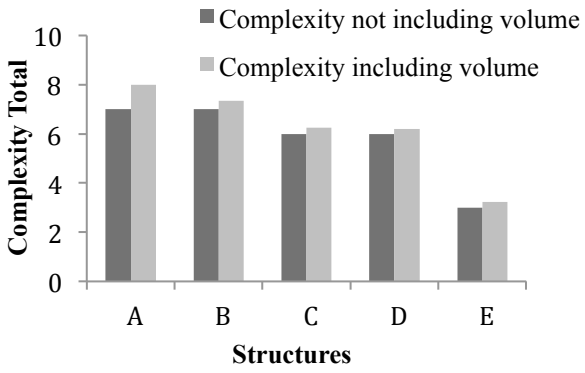

Fig 2 Darker columns show the complexity total of each structure before their volume is included. Lighter columns show the complexity total of each structure after their volume is included

Average abundance

Over the course of eight dives, the average abundance of *C. rostrata* present at each structure, from highest to lowest (mean ± SD), was 5.6 ± 1.2, 4 ± 1.8, 2 ± 1.1 , 1.6 ± 0.9, and 1.2 ± 1 for structures A, B, C, E, and D respectively (Fig. 3). There was not a

significant difference in the average abundance of *C. rostrata* found at structure A and B (n = 8, p = 2.53), structure B (n = 8) and C (n = 7, p = 3.65), structure C (n = 7) and D (n = 5, p = 7.54) and structure D (n = 5) and E (n = 8, p = 6.30). According to the custom-designed complexity chart, these means demonstrate that more complex structures host more *C. rostrata* with the exception of structure D which is more complex than E, yet had fewer *C. rostrata* on average.

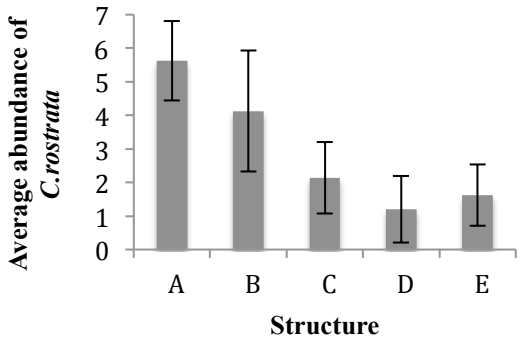

Fig. 3 Average abundance of *Canthigaster rostrata* per structure. Individual *C. rostrata* were counted at each structure on five separate dives. Structures are organized by order of their complexity from most complex to least complex. Structure A (n=8), structure B (n=8), structure C (n=7), structure D (n=5), and structure E (n=8)

Average size

The average size of *C. rostrata* for each structure from highest to lowest (mean ± SD), was 4.81 cm ± 0.6, 3.06 cm ± 0, 2.9 ± 0.35, 2.55 ± 0.44, and 2.43 ± 0.5, for structures A, B, E, D, and C respectively (Fig. 4).

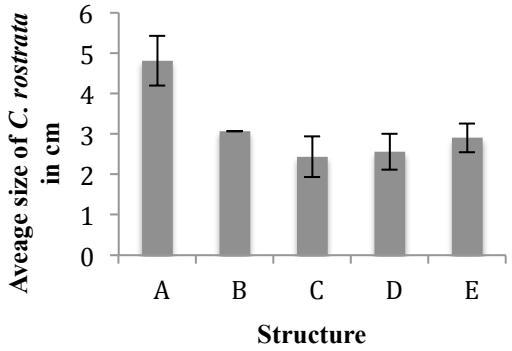

Fig. 4 Average size of *Canthigaster rostrata* in centimeters per structure. Each *C. rostrata* present at each structure was measured over the course of five dives. For structures A, B, C, and E, (n = 5) and for structure D (n = 3)

According to the custom-designed complexity chart, these means demonstrate that the most complex structures A and B have the biggest *C. rostrata* on average. Structures C and E have the second biggest *C. rostrata* on average, and structure D has the smallest *C. rostrata* on average, even though it is more complex than E.

Average abundance of size class

A closer look at *C. rostrata*'s size distribution per structure (Fig. 5) shows that structure A is composed of the highest size classes (3 cm - 4 cm to 6 cm -7 cm), structure B has the widest range of size classes from the lowest possible to the highest possible (1 cm - 2 cm to 6 cm - 7 cm), structures C and E are composed of the smallest size classes (1 cm - 2 cm to 4 cm - 5 cm), and structure D is only composed of the middle size classes (2 cm - 3 cm to 5 cm - 6 cm). This breakdown of the data depicted in Fig. 4, indicates average size of *C. rostrata* per structure, shows that more complex structures hosted higher size classes with the exception of structure D, which is the second least complex structure, yet had one higher size class than structure C.

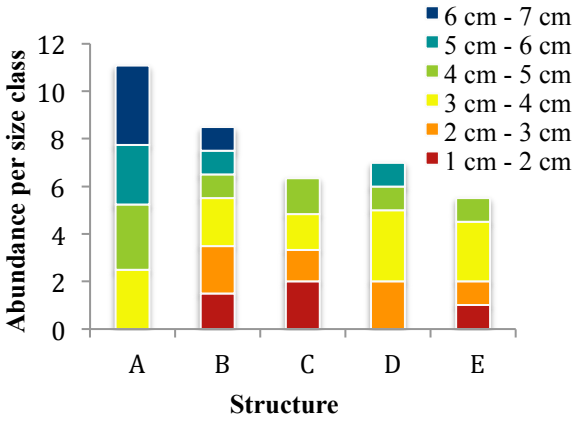

Fig. 5 Average abundance of *Canthigaster rostrata* size class per structure. This graph breaks down the data of figure 4 by showing the composition of the different size classes at each structure that make up the size averages displayed in figure 4

Discussion

Based on the data collected from the five structures over the course of five weeks, the first hypothesis and the second hypothesis were supported although the results were not statistically significant. Overall, *C. rostrata* average abundance and size increased with structural complexity. The inconsistency observed between structure D and E offers interesting additional information that might reinforce the hypotheses even more. Structure E, a pair of barrels, has a larger volume than structure D, a tire, yet ranks last in the complexity chart both before and after including size as a criterion. Even though it is the least complex structure, it still has a higher average abundance of *C. rostrata* and a higher average size of *C. rostrata* than structure D. This inconsistency might actually be linked to the fact that structure E is located in very close proximity to structure B, which is one of the most complex structures. For this reason, it is highly possible that structure B helped attract more individuals, and larger individuals, to structure E further reinforcing the influence of structure volume on of *C. rostrata* size and abundance.

Previous studies done in the Pacific Ocean as well as the Caribbean Sea, shared consistent observations on *C. rostrata*'s haremic structure, yet it was not seen at the Yellow Submarine study site. No Sharpnose Pufferfish were seen patrolling territories the way males were described doing so in the literature (Sikkel 1990). Amongst the challenges of determining social structure was the difficulty of identifying male and female *C. rostrata*. Despite regular observation, sexual dimorphism did not seem to be related to coloration or size. Two dawn dives were done in an attempt to see *C. rostrata* spawning and thus gain a better understanding of sexual dimorphism in this species, but no interactions were observed. Difficulty to identify males from females as well as any social structure has many implications. Perhaps *C. rostrata*'s social structure is unique in the Dutch Caribbean, or perhaps *C. rostrata* communities in the shallows are not fully mature. In fact, Sharpnose Pufferfish observed on the reef seemed more abundant and larger. This could explain why haremic behavior and spawning were not observed. If this study were to be continued, it is suggested that data be collected on the coral reef at Yellow Submarine or elsewhere in Bonaire in order to compare results.

However, some of the behaviors mentioned in another source (Reef Net 2003) were seen at the study site. On four separate occasions, Sharpnose Pufferfish were seen changing to a speckled and pale coloration from a brighter coloration. The first time this phenomenon was observed was at dusk. The bright blue lines of *C. rostrata* visible during the day practically disappeared in order to help conceal them as they sleep (Reef Net 2003). The three other times this phenomenon was observed were likely an intruding or submissive male camouflaging in a territory that was not his. Conspecific aggressive territorial behavior was observed frequently, yet interspecific aggressive territorial behavior was not observed. Individuals were almost always seen in the exact same spots, and sometimes it could be confirmed that it was the same exact individual due to size and markings like scars, but interestingly, even when it was a different individual it still defended the same territory. In future studies it would be worthwhile to search when *C. rostrata* change territories and if it has to do with individual mortality or overpopulation of a structure. Furthermore, it would be interesting to find out how they communicate to each other to switch if that is the case. It was observed during the dawn dives that individuals do not retreat to the reef at night and return to structures in the shallows in the morning because as soon as day broke, individuals were observed swimming out from the structures in the shallows. This observation makes it even harder to understand when individuals would switch territories if they were "residents" to their structures to a certain extent.

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REPORT

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Pelagic plankton diel vertical movement, diversity, and density in relation to nitrate concentration in Bonaire, Dutch Caribbean

Abstract Plankton are the base of the marine food web and are studied for a broad range of research relating to diversity and ocean health. These organisms have not been well studied in Bonaire and this study provided a preliminary assessment for the pelagic net plankton movement and diversity. Water samples and plankton tows were collected using a Niskin bottle and 20-micrometer closable plankton net respectively at four depths: 90 m, 60 m, 30 m, and 10 m. The water samples were processed for nitrate concentration and the 5-meter vertical plankton tows were analyzed for plankton abundance using the following categories: diatoms, dinoflagellates, copepods, and other zooplankton. Dinoflagellates displayed diel vertical migration with higher density at 10 m and 30 m during the day and lower density at 10 m and 30 m at night. Simpson's Diversity Index (SDI) did not show a significant difference in the diversity at 90 m and 10 m during the day or night. Nitrate concentration and plankton density were not found to be correlated. This study created a preliminary assessment for further research into the effects of the lunar cycle, nitrate, and movement of the pelagic net plankton of Bonaire.

Keywords Dinoflagellates • Zooplankton • Water nutrients

Introduction

Plankton provide an important trophic food web link for fish recruitment and have been used to study ecological issues from biodiversity loss to fisheries management (Sherman 2015). These studies include global biogeochemical cycles and indications of climate change impacts (Barton et al. 2013; Voss et al. 2013). This regulates the nutrient availability to plankton. Of the many aspects examined in these studies, plankton movement and diversity are commonly studied when forming preliminary assessments.

Plankton classification falls into several categories based on size and function. Phytoplankton are primary producers and zooplankton are primary consumers. Within the broad category of phytoplankton, two groups are larger than 20-micrometers: diatoms and dinoflagellates (Nybakken and Bertness 2004). Net plankton is a term used to describe all plankton that fit into this size category (Nybakken and Bertness 2004). Within net plankton, copepods, a subclass of zooplankton, are important as primary herbivores and carnivores and dominate net plankton (Nybakken and Bertness 2004). The rest of the net zooplankton community is diverse and non-dominant (Nybakken and Bertness 2004). These divisions were used in this study to identify important plankton groups.

A well-known phenomenon of the pelagic plankton ecosystem is diel vertical migration (DVM), which describes the diurnal vertical movement of mobile plankton through the water column (Jephson 2012). Zooplankton movement occurs from deep waters during the daylight hours to shallow waters at night and back again (Bollens and Frost 1989; Bianchi and Mislan 2016). These movements occur when the zooplankton feed in the shallows at night to avoid predators during the day (Bianchi and Mislan 2016). The zooplankton movement and predation makes it beneficial

for dinoflagellates to perform the opposite movement (Jephson 2012). In addition, as primary producers, phytoplankton photosynthesize making them surface dwellers during the day (Jephson 2012). Unlike other phytoplankton, dinoflagellates have two flagella allowing them to influence their movement (Jephson 2012). This motility allows them to move to deeper water at night to avoid predation by zooplankton (Jephson 2012); the movement is also related to nutrient uptake because there is a higher nutrient supply in deeper water (Jephson and Carlsson 2009).

The main barriers preventing plankton from moving through the water column are the thermocline and halocline (Jephson and Carlsson 2009; Souza et al. 2014). The response and tolerance of plankton to salinity barriers can be species-specific based on the individual ability to tolerate more extreme conditions (Jephson and Carlsson 2009). The influence of temperature stratification on the DVM of plankton varies with the gradient in temperature (Souza et al. 2014). Larger thermal differences were found to reduce the number of dinoflagellates capable of crossing the thermocline (Souza et al. 2014).

This study examined the DVM of plankton groups off the leeward side of Bonaire and the effects of the time, depth, and nitrate concentration on their density. There has not been much research on pelagic plankton off the coast of Bonaire. The data collected in this study created a preliminary assessment of the pelagic plankton of Bonaire. Therefore, the hypotheses are as follows:

H_1: Daytime density of dinoflagellates will be higher at shallower depths than at deeper depths. Nighttime density of dinoflagellates will be lower at shallower depths than at deeper depths

H_2: Daytime density of zooplankton will be lower at shallower depths than at deeper depths. Nighttime density of zooplankton will be higher at shallower depths than at deeper depths

H_3: The diversity of plankton will be different between 10 m and 90 m indicating a thermocline or halocline

H_4: Higher nitrogen level in water samples will correlate to higher density of plankton

Materials and methods

Study organisms

Four classifications of plankton were used in this study: diatoms, dinoflagellates, copepods, and other zooplankton. Of the phytoplankton, diatoms are identified by their glass box and lack of means for locomotion (Nybakken and Bertness 2004). Dinoflagellates have two flagella and either lack a skeleton or produce a protective layer of carbohydrate cellulose (Hackett et al. 2004). Of the zooplankton, copepods have distinctly large antennae and characteristic body shape (Nybakken and Bertness 2004). Non-copepod zooplankton were classified as other zooplankton. These characteristics were used to identify and categorize the plankton.

Study site

Bonaire is part of the Dutch Caribbean located in the Southern Caribbean approximately 100km north of Venezuela. This study was conducted in the ~225 m ocean channel between Klein Bonaire and Kranlendjk (12°08'9"N 68°17'221"W) (Fig.1). A GPS unit was used to return to the same location for every collection. This site is an unobstructed water column with generally mild currents driven mostly by wind.

Data collection

Water samples and vertical plankton tows were collected at 90 m, 60 m, 30 m, and 10 m during the day (11:00 to 13:00 hrs) and at night (20:00 to 22:00 hrs). A Niskin bottle was lowered to each depth and closed using a messenger weight to collect water samples. The closable

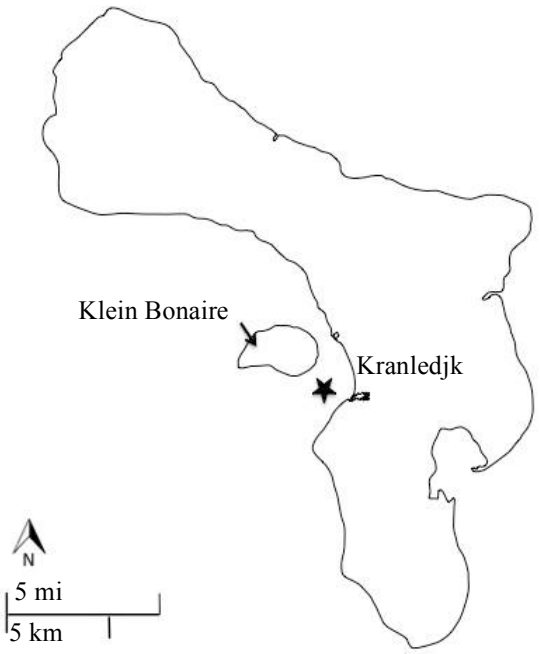

Fig 1. Map of study site between Klein Bonaire and Kranledjk in the southern Caribbean. Star indicates data collection site (12°08'9"N 68°17'221"W)

plankton net was lowered to each depth for five-meter vertical tows and closed using a messenger weight at the end of each tow. After each tow the plankton collected in the cod end jar were transferred into separate 500 mL containers using filtered seawater.

Data collection

Water samples and vertical plankton tows were collected at 90 m, 60 m, 30 m, and 10 m during the day (11:00 to 13:00 hrs) and at night (20:00 to 22:00 hrs). A Niskin bottle was lowered to each depth and closed using a messenger weight to collect water samples. The closable plankton net was lowered to each depth for five-meter vertical tows and closed using a messenger weight at the end of each tow. After each tow the plankton collected in the cod end jar were transferred into separate 500 mL containers using filtered seawater.

Sample processing

Water samples were collected into 100 mL containers and immediately stored in a freezer in to the laboratory. At the dock, 5-6 drops of $MgCl_2$ (10%) were added to each of the plankton samples. Ten minutes later the containers were filled with formalin (~4% formaldehyde). The plankton samples were fixed in the laboratory for 24-48 hours before being poured through a 20-micrometer sieve and rinsed into 100 mL containers using 70% ethanol.

Laboratory analysis

Plankton abundance was counted using a Sedgewick Rafter Counting Cell, which use exactly 1 mL of sample in each count. Five 1-mL subsamples (5% of reduced volume sample) were taken from each of the 100 mL plankton samples. Assuming the plankton were uniformly distributed throughout the plankton tow, the plankton abundance counted from the reduced volume sample was used to calculate the actual density by dividing the abundance by 5% (17... L) of the total plankton tow volume (353.88 L). Plankton were counted and classified into the following categories: dinoflagellates and diatoms for phytoplankton and copepods and other zooplankton.

The water samples were processed using a Trilogy Laboratory Fluorometer following CIEE nitrate protocol. Every sample was analyzed three times and averaged together to increase accuracy.

Data analysis

The mean density was calculated for diatoms, dinoflagellates and zooplankton, both copepods and other zooplankton combined. A t-test was conducted for each depth comparing day and night mean density for dinoflagellates and zooplankton to see if the difference was significant. The Simpson's Diversity Index of all the 90 m and 10 m samples were calculated and a t-test was performed for both day and night samples to look for a significant difference by depth during the day or night. The concentration of nitrate was tested for correlation with density of diatoms, dinoflagellates, and zooplankton (copepods and other zooplankton combined).

Results

The data analyzed included samples from four days of collection with 32 five-meter vertical plankton tows and 31 water samples. None of the hypotheses in this study were supported based on the statistical tests of the data; however, the data indicated certain trends for density, diversity, and density in relation to nitrate concentration.

Mean density for time and depth

The density of dinoflagellates did not change significantly over time and depth: 90 m (t-test, $t_{2,3} = 0.81$, $p = 0.48$), 60 m (t-test, $t_{2,3} = 0.58$, $p = 0.60$), 30 m (t-test, $t_{2,3} = 2.03$, $p = 0.14$), and 10 m (t-test, $t_{2,3} = 1.81$, $p = 0.17$)(Fig. 2B). Mean dinoflagellate density during the day was highest near the surface with a value of 0.78 individuals per L (ind per L) (SD ± 0.80) at 10 m and 0.61 ind per L (± 0.58) at 30 m (Fig. 2B). The lowest mean densities during the day were 0.13 ind per L (± 0.07) at 90 m and 0.20 ind per L (± 0.07) at 60 m. At night the dinoflagellate density trend was relatively constant across depth with more dinoflagellates found at 60 m (0.35 ind per L ± 0.52) and 90 m (0.21 ind per L ± 0.21) than at 30 m (0.10 ind per L ± 0.10) and 10 m (0.14 ind per L ± 0.10) (Fig. 2B).

Zooplankton densities did not show significant differences for depth and time: 90 m (t-test, $t_{2,3} = 0.22$, $p = 0.84$), 60 m (t-test, $t_{2,3} = 0$, $p = 1$), 30 m (t-test, $t_{2,3} = 0.81$, $p = 0.48$), 10 m (t-test, $t_{2,3} = 1.81$, $p = 0.17$)(Fig. 2A). Overall, the highest mean density was shallow with 7.12 ind per L (± 5.21) at 10 m during the day, which does not support the established DVM of zooplankton (Fig. 2A). Other values during the day were 1.88 ind per L (± 1.01) at 30 m, 1.99 ind per L (± 1.16) at 60 m, and 1.19 ind per L (± 1.24) at 90 m help to demonstrate that there was no difference (Fig. 2A). At night there was a slight decrease in density towards deeper samples. The densities dropped from 2.78 ind per L (± 1.83) at 10 m to 2.60 ind per L (± 1.60) at 30 m and 1.99 ind per L (± 1.16) at 60 m to 1.34 ind per L (± 0.79) at 90 m (Fig. 2A).

Simpson's Diversity Index

Plankton Simpson's Diversity Index was not significantly different between 90 m and 10 m during the day (t-test, $t_{2,3} = 0.12$, $p = 0.91$) or night (t-test, $t_{2,3} = 0.88$, $p = 0.44$) (Table 1). These values were calculated using diatoms, dinoflagellates, copepods, and other zooplankton as separate groups. No discernable trends were found (Table 1).

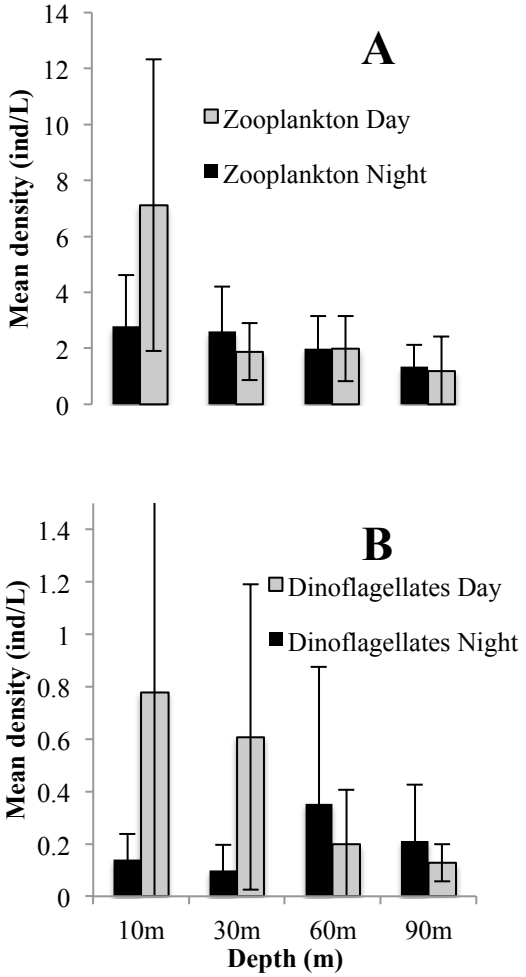

Fig. 2 Mean density of zooplankton (A) and dinoflagellates (B) (individual per liter) by depth and time of day (n = 4). The error bars represent standard deviation

Table 1 Average Simpsons Diversity Index values (±standard deviation) for day (t-test, $T_{2,3}$=0.12, p=0.91) and night (t-test, $T_{2,3}$=0.88, p=0.44) at 10 m and 90 m

Depth	Day	Night
10 m	0.21 (± 0.30)	0.11 (± 0.14)
90 m	0.24 (± 0.27)	0.05 (± 0.03)

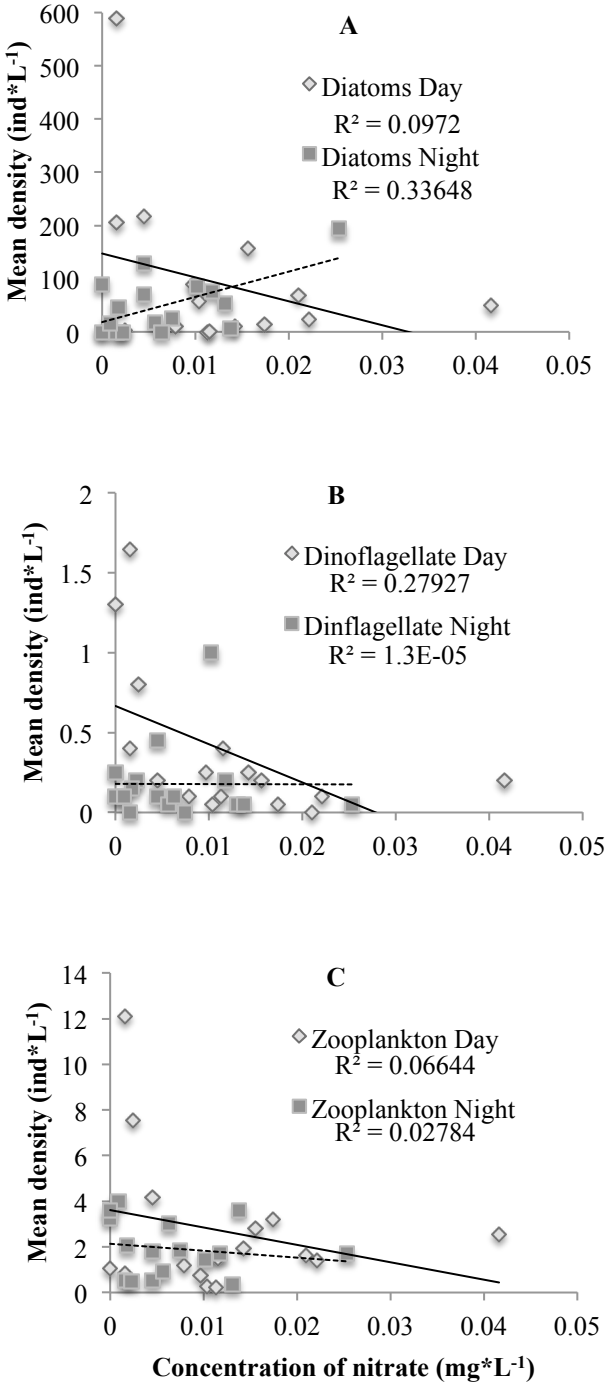

Fig. 3 Mean plankton density (individual per L) for diatoms (A), dinoflagellates (B), and zooplankton (C) correllated with nitrate concentration in mg/L. The linear trendlines represent the correlation between the concentration of nitrate and mean density

Nitrate and plankton density correlation

Nitrate concentration did not correlate with mean plankton density for diatoms, dinoflagellates, and zooplankton, combining copepods and other zooplankton, during the day or night (Fig. 3). There was a slight negative correlation seen during the day between dinoflagellates density and nitrate concentration (R^2 = 0.28) (Fig. 3B). A slight positive correlation between diatoms density and nitrate concentration was seen at night (R^2 = 0.34)(Fig. 3A). No correlation was found between plankton density and nitrate concentration for diatoms (R^2 = 0.10) and zooplankton (R^2 = 0.07) during the day or for zooplankton (R^2 = 0.03) and dinoflagellates (R^2 = 0) at night (Fig. 3). All correlations between nitrate concentration and density were weak.

Discussion

The data set for this study was small and therefore influenced the statistical test of the hypotheses. However, the data do show some trends, which are useful in creating a preliminary assessment, upon which to develop future studies. Overall the trends supported the predictions of this study with the exception of zooplankton DVM.

Mean density for time and depth

This study looked at the mean density of different plankton groups over time and depth to test if they performed DVM movements by analyzing plankton samples collected at 10 m, 30 m, 60 m, and 90 m depths. Time was factored as a comparison between day (11:00 to 13:00 hrs) and night (20:00 to 22:00 hrs).

The biological process of dinoflagellate DVM would predict higher surface density during the day and lower surface density at night (Jephson 2012). This hypothesis was not supported based on the statistical tests of the data; however, dinoflagellate mean density during the day was higher at 10 m and 30 m than 60 m or 90 m (Fig. 2B). At night the

highest mean density was found at 60 m (Fig. 2B). These observations indicate that the dinoflagellates are shown to perform DVM. The lack of significance in the densities of dinoflagellates over time is likely due to the small sample size and high standard deviation. The data may have been more strongly supported if more sub-samples were counted.

The biological process of zooplankton DVM predicts higher surface density at night and lower surface density during the day (Bollens and Frost 1989; Bianchi and Mislan 2016). No statistically significant difference was found between day and night densities at each depth. The highest mean zooplankton density was at 10 m during the day, the opposite of the predicted distribution (Fig. 2A). During the night, however, mean zooplankton density increased towards the surface, with the highest value found at 10 m (Fig. 2A). This indicates the zooplankton may not have moved very far between the sample times.

The highest mean density values were found during the full moon (March 23) with a gradual decrease as the moon waned. This study was not conducted over a long enough period of time to show a full lunar cycle. However, a study of plankton vertical distribution off the coast of Hawaii showed mean plankton densities at depths above 100 m are significantly affected by the lunar cycle (Benoit-Bird et al. 2009). Benoit-Bird et al. (2009) found that mean density was highest at the full moon and lowest at the new moon. This study follows the same pattern as the data collected in this study; therefore, the unexpected mean densities could be explained by the lunar effect.

Simpson's Diversity Index

The diversity of the plankton composition at 90 m and 10 m was compared to determine if there was a difference in the planktonic community above and below the thermocline or halocline, if present. This study was unable to provide sufficient data to indicate the presence or absence of a thermocline or halocline. The Simpson's Diversity Index (SDI) takes into account species richness and evenness. This study did not identify plankton to the species taxonomic level but the index was calculated using representative groups. One of these groups, diatoms occurred most commonly in long chains and their abundance was very high. This was likely the most influential factor in the diversity index. Due to dinoflagellates and zooplankton moving in opposite directions in similar abundance with their DVM, the diversity index is not a representation of their movement. It indicated that there is not a significant difference in the composition of the net plankton community due to depth. This could also indicate a lack of a thermocline or halocline because movement does not appear to be restricted between depths (Souza et al. 2014).

Nitrate and density correlation

Nitrate and plankton density were predicted to be positively correlated. There was no correlation between mean plankton density and nitrate concentration. Many of the samples were at too low of nitrate concentrations to be measured by the fluorometer. The lack of correlation is likely due to confounding factors related to the nitrogen cycle and fluxes (Capone et al. 2005; Voss et al. 2013). The nitrogen cycle is a complex system driven by many factors beyond plankton (Capone et al. 2005). For example, certain types of cyanobacteria could be more influential in nitrogen fixation and integration into the ocean than net plankton (Voss et al. 2013). This study did not consider that high plankton concentration could cause more nitrate consumption making this form of measurement ineffective for identifying limiting nutrients. With consideration for biogeochemical cycles and nitrogen flux, it is logical that there are many more influences and factors that impact the nitrate concentration of the ocean (Capone et al. 2005; Voss et al. 2013). These results indicate that nitrate is not correlated with plankton density.

Conclusion

This study found trends, although not statistically significant, that support dinoflagellate DVM but not zooplankton DVM. Furthermore, nitrate was shown not to correlate with plankton density. It would have been useful to have a full set of data for several lunar cycles to better understand the variation in zooplankton density. This preliminary assessment research provides data for future studies investigating the effects of the lunar cycle, limiting nutrients on the pelagic plankton community, pelagic plankton diversity, and DVM.

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REPORT

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Habitat preferences, behavior, and inter-species associations of the yellowline arrow crab *(Stenorhynchus seticornis)* in Bonaire, Dutch Caribbean

Abstract *Stenorhynchus seticornis* (Yellowline arrow crab) is a decapod crustacean native to the Western Atlantic in tropical and subtropical climates. *Stenorhynchus seticornis* is abundant in the Caribbean and has been studied associating with many different species from different phyla. Bonaire is a small island in the Southern Caribbean where *S. seticornis* is common, however no research on *S. seticornis* has been published on Bonaire. This study provides new information on *S. seticornis* and its ecological role on the reefs of Bonaire. This study examined the habitat type, habitat substrates, behaviors, and inter-species associations of *S. seticornis* by surveying at two depths, 8 and 14 m. *Stenorhynchus seticornis* was observed more frequently at 14 m (n = 53) compared to 8 m (n = 27). There was a higher frequency of inter-species associations and more total species observed associating at 14 m compared to 8 m. The majority of *S. seticornis* at 8 m were observed on sand under ledges, while at 14 m *S. seticornis* were recorded primarily on turf algae in crevices. The predominant behavior of *S. seticornis* at 8 and 14 m were eating and hiding respectively. The data collected contributes new information about *S. seticornis*, which is an abundant crustacean in Bonaire and is not fully understood. The results suggest that *S. seticornis* associates across many phyla and could serve an important role in the larger coral reef ecosystem.

Keywords Decapods • *S. seticornis* density • diversity of associations

Introduction

Coral reefs are complex ecosystem rich with different species interactions and biodiversity (Abelson 2006). Diversity and interactions within the coral reefs add stability to the ecosystem, which allows it to function at a high level and provide humans with vital ecological services (Alvarez-Filip et al. 2013). Associations between organisms in this ecosystem are one part of the complex web of interactions that keeps the diversity on coral reefs functioning. There are documented studies of symbiotic relationships and associations among many different phyla in marine ecosystem, particularly on coral reefs (Wirtz et al. 2009).

A common interaction is cleaning symbiosis between the "cleaner" or "cleaners" which remove dead tissue or ectoparasites from the "client" organism (McCammon et al. 2010). McCammon et al. (2010) found that cleaning interactions can be observed in terrestrial habitats but are more widely documented in Indo-Pacific cleaner fishes, specifically with wrasses and gobies. Cleaning fish are the most well documented cleaners in the scientific community, but there are studies that examine the role of invertebrates as symbiotic cleaners (Becker et al. 2005).

Cleaner shrimp have been observed performing a "rocking-dance" to signal to client fish that they are hungry and looking to clean (Becker et al. 2005). There is currently no scientific evidence that *Stenorhynchus seticornis* use posture or dancing to signal client species, but *S. seticornis* has been observed as a cleaner among different species

of reef fish (Medeiros et al. 2011). Another study classified *S. seticornis* as a detritivore which can account for cleaning of decaying matter on client species (Netchy et al. 2015). Medeiros et al. (2011) reported that *S. seticornis* were commonly associated with anemones, but the study also recorded the arrow crab cleaning 4 species of reef fish including *Gymnothorax funebris,G. vicinus, G. moringa, and Holocentrus adscencionis*. These four client species have diets high in crustaceans, but no aggressive behavior toward *S. seticornis* was observed in this study which could be evidence of a strong mutualism (Medeiros et al. 2011).

A study conducted in the Caribbean found that decapod crustaceans, such as *S. seticornis*, have a variety of symbiotic relationships among different host species (Hayes et al. 1998). *Stenorhynchus seticornis* has been observed associating with echinoids such as *Diadema antillarum* for protection amongst the long spines of the urchin (Hayes et al. 1998). Another study found *S. seticornis* uses the base of gorgonians as a habitat (Wicksten and Cox 2011), which could be seen as a commensal symbiotic relationship. *Stenorhynchus seticornis* is a symbiotic generalist that has been observed on a variety of substrates and habitat types interacting with species from a number different of phyla (Joseph et al. 1998). The purpose of this study was to explore the habitat preferences of *S. seticornis* and any associations with other species to determine the ecological role of *S. seticornis* on the reefs of Bonaire. This study hypothesizes that:

- H_1: Higher density of *S. seticornis* will result in a high frequency of inter-species associations
- H_2: Higher density of *S. seticornis* will result in a high diversity of associating species

This study will use the previously discussed studies as reference and as a basis of information when observing and recording habitat, abundance, and interactions of *S. seticornis* in Bonaire. There is currently no published scientific data of *S. seticornis* on Bonaire, so this study will provide new information about *S. seticornis*, which is an abundant species in the Caribbean and western Atlantic.

Materials and methods

Study Site

Research was conducted in the southern Caribbean on the island of Bonaire in the Dutch Caribbean. The study site was on the western (leeward) side of Bonaire in the town of Kralendijk, just south of the Something Special dive site (12°09'33"N 68°16'55"W) (Fig. 1). The study site started at the dock of the Yellow Submarine dive shop and extended northwest following the crest of the reef for approximately 100 m. From the shore there is a sand flat which extends approximately 60 m out and gets gradually deeper before reaching the reef crest which is roughly at 8 m of depth. From there the reef crest has a fairly steep slope until it hits a shelf at about 30 m.

The study site was divided into two varying depths at 8 m (shallow) and 14 m (deep) for surveying. Two research divers swam out in line with the Yellow Submarine dock, descended and went north laying a transect for 30 m then turned and began the survey. The divers followed the transect, one on each side of the tape, creating a 2 m wide surveying width. If the divers ran low on air then the transect was finished on the next dive. The stoppage point was marked and landmarks were used to remember where the transect was laid.

The researchers entered the water at approximately 18:00 each dive and immediately recorded general weather conditions, water temperature, and water visibility. The researchers also attempted to note factors that might have influenced the study such as other scuba divers in the study area, excessive boat traffic on a particular day, etc.

Together, the divers used underwater slates to record the number, size, and where *S. seticornis* were seen along the transect as well as species surrounding or associating with *S. seticornis* (within 50 cm). Within the 50 cm the approximate distance of the organism from *S. seticornis* was measured as well as what type of association took place. The divers recorded the observed habitat substrate, characterized the structure of the habitat, and described the behavior of *S. seticornis* (Table 1).

Using a digital camera to photograph *S. seticornis* in its environment allowed for more detailed analysis of the habitat substrate, habitat structure, and surrounding species. The divers used t-bars, measuring tapes, or rulers to estimate sizes of *S. seticornis*. These methods allowed for the further study of *S. seticornis* and any interactions or associations displayed. These study depths were chosen because *S. seticornis* was previously observed at both sampling depths by the researchers.

Data analysis

To analyze the data a statistical t-test was used to compare the mean density of *S. seticornis* at the two varying depths as well as the mean size at the different depths. A comparison of crab size vs number of associations was also performed to find a correlation between the different variables. At the two depths, the different preferences of habitat substrate, habitat structure, and crab behavior was compared and the frequency of associations and number of species observed was examined.

Table 1 The different possible descriptions used to classify substrate, habitat structure, and behavior

Classification	Description
Substrate	Rubble, sand, sponge, coral, turf algae
Habitat	Crevice, cave, flat, under ledge, vertical flat
Behavior	Eating, hiding, cleaning

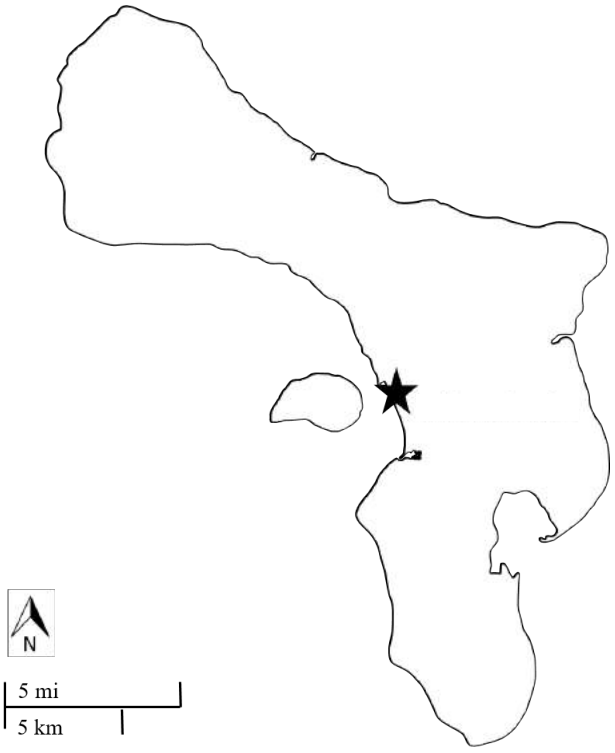

Fig 1 A map of the island of Bonaire. The black star indicates the location of the study site south of Something Special (12°09'33"N, 68°16'55"W)

Results

Over the course of the study, 429 m² of reef and benthos were surveyed while scuba-diving. There were 27 *S. seticornis* individuals observed at a shallow 8 m depth and 53 individuals at the 14 m depth. A total of 89 associations were observed within 50 cm of *S. seticornis* encompassing 20 species associations. *Stenorhynchus seticornis* was observed at five different types of habitat structures and five different types of substrates (Table 1), which varied in frequency at the two depths (Fig. 2 and 3). Estimated water visibility while conducting surveys ranged from 9 to 18 m, with a mean visibility of 15 m.

Mean density and size

The mean (± SD) *S. seticornis* density, and size per 100 m² was calculated and recorded in this study. Density at the 8 m depth was 9 (± 8.7), which was significantly lower than the density

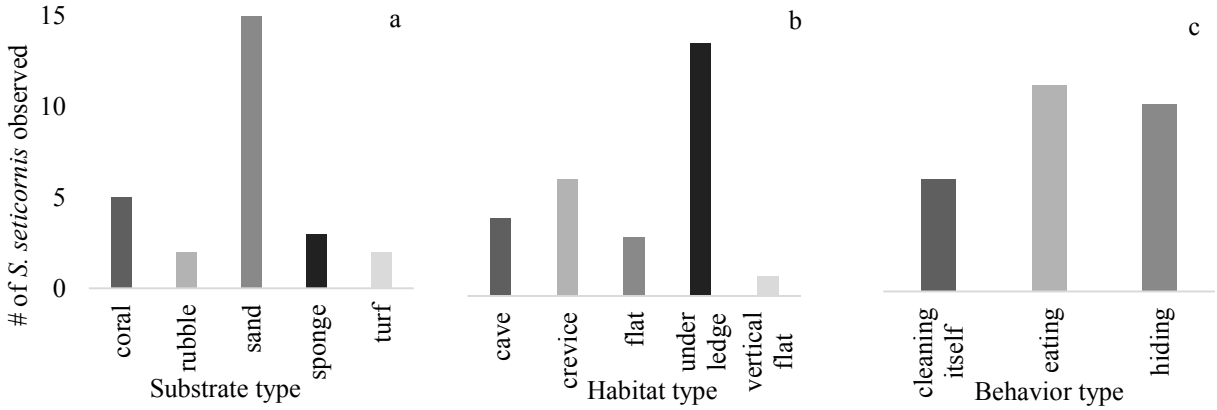

Fig. 2 Number of *S. seticornis* observed at 8 m at (a) varying substrates, (b) habitat types, (c) and behaviors

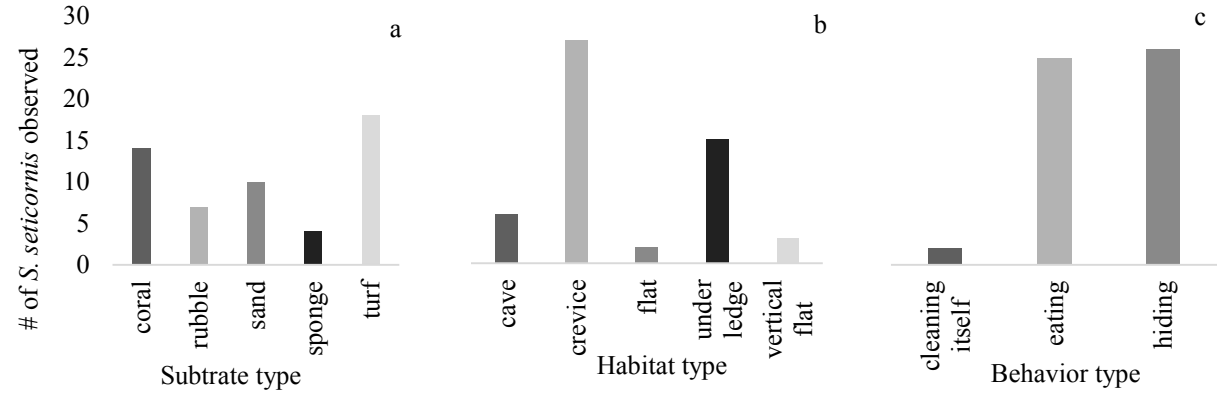

Fig. 3 Number of *S. seticornis* observed at 14 m at (a) varying substrates, (b) habitat types, (c) and behaviors

at the 14 m depth 68 (± 42.2) (t-test; n = 10; p = 0.05). The mean *S. seticornis* size (cm) at 8 m was higher 7.6 (± 3.2) compared to 14 m 6.9 (± 3.7), but this difference was not significant (t-test; n = 80; p = 0.86).

Observed habitat type, substrate and behavior

The majority of *S. seticornis* observed at 8 m were on a sandy substrate (n = 15) (Fig. 3 a). The other four observed substrates at 8 m had lower frequencies of: coral (n = 5), rubble (n = 2), sponge (n = 3), and turf algae (n = 2) (Fig. 2 a). At 14 m *S. seticornis* was observed primarily on turf algae (n = 18), coral (n = 14), or sand (n = 10) (Fig. 3 a).

At 8 m *S. seticornis* were most commonly found under ledges (n = 13) (Fig. 2 b). At 14 m the most commonly observed habitat structure for *S. seticornis* was in a crevice (n = 27) (Fig. 3 b).

The most dominant behavior of *S. seticornis* at both depths were eating and hiding while the least cleaning itself (Fig. 2 c; Fig. 3 c). Eating (n = 11) was observed more than hiding (n = 10) at 8 m (Fig. 2 c), but at 14 m hiding (n = 26) was slightly more common than eating (n = 25) (Fig 3 c).

Frequency of species associations

There were a total of 18 different species or families observed associating with *S. seticornis* (Fig. 4). These included *Coryphopterus glaucofraenum* (Bridled goby), *Elacatinus horsti* (Yellowline goby), *Coryphopterus personatus* (Masked goby), *Chromis multilineata* (Brown chromis), *Myripristis jacobus* (Black bar soldier fish), *Sabellidae* (Tube worm), *Canthigaster rostrata* (Sharpnose puffer), *Stegastes partitus* (Bicolor damsel), *Stegastes diencaeus* (Longfin damsel), *Ophiuroidea* (Brittle stars), *Cephalopholis cruentata* (Graysby), *Tripneustes ventricosus* (Sea egg urchin), *Mulloidichthys martinicus* (Yellow goatfish), *Holacanthus tricolor* (Rockbeauty), *Ancylomenes pedersoni* (Pederson cleaner

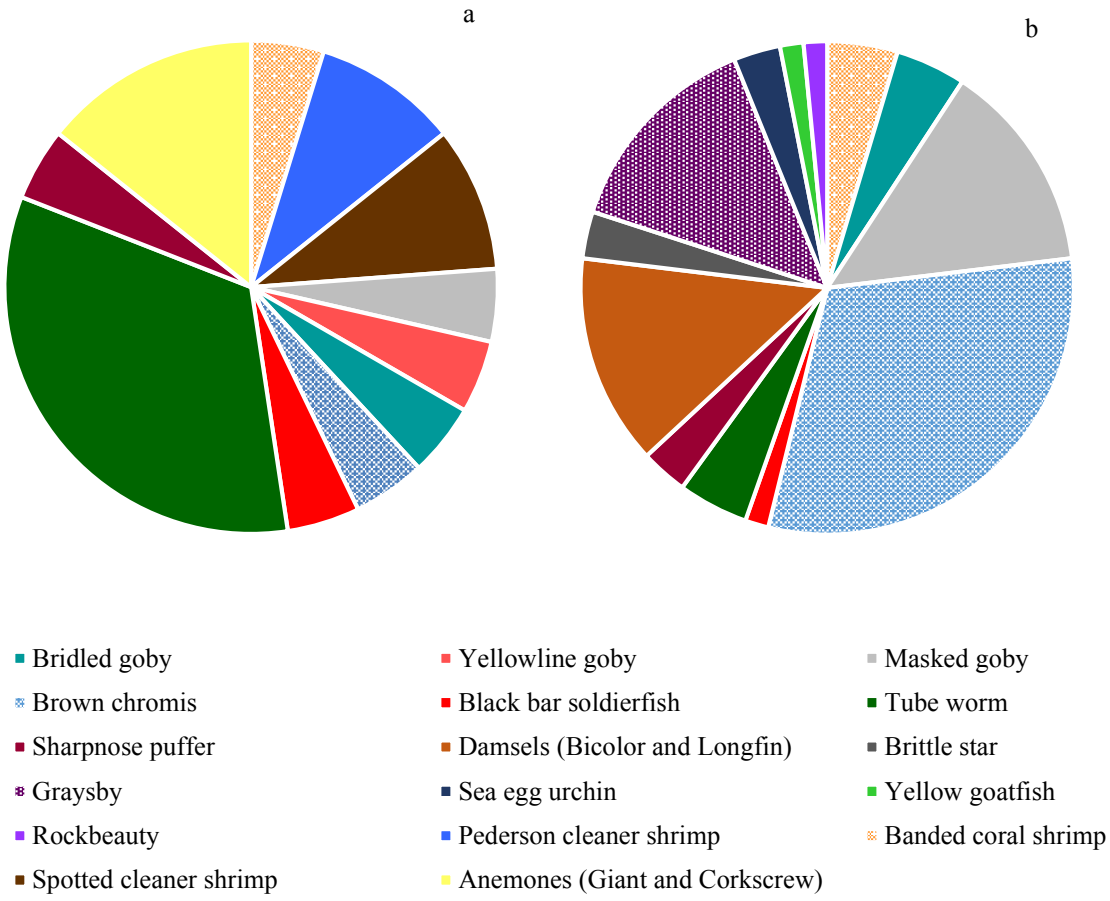

Fig. 4 Frequency of species that associated with *S. seticornis* at (a) 8 m and (b) 14 m

shrimp), *Stenopus hispidus* (Banded coral shrimp), *Periclimenes yucatanicus* (Spotted cleaner shrimp), *Condylactis gigantean* (Giant anemone), and *Bartholomea annulata* (Corkscrew anemone). Overall, the 8 m depth had less species associations observed compared to the 14 m depth (Fig. 4).

At the 8 m depth the most common association with *S. seticornis* were *Sabellidae* (n = 7) (Fig. 4 a). The least common observed associations at 8 m were with the *Myripristis jacobus* (n = 1), *Chromis multilineata* (n = 1), and *Canthigaster rostrata* (n = 1) (Fig. 4 a). The most common organism found with *S. seticornis* was the *Chromis multilineata* (n = 20) at the 14 m depth (Fig. 4 b). At the 14 m depth *S. seticornis* were observed least often with *Myripristis jacobus* (n = 1), *Mulloidichthys martinicus* (n = 1), and *Holacanthus tricolor* (n = 1) (Fig. 4 b).

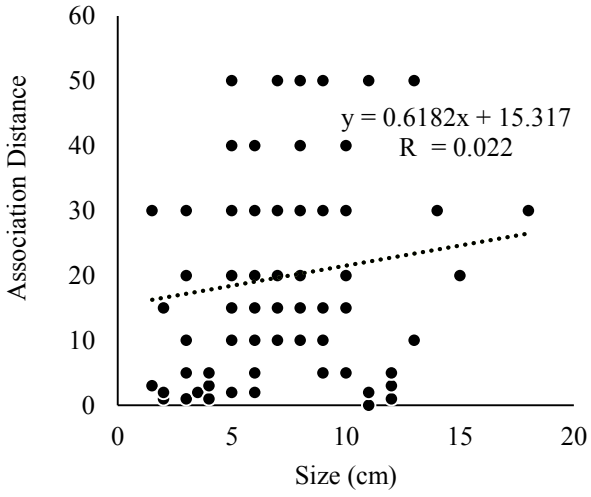

Fig. 5 Relationship between the sizes of *S. seticornis* and the associations with organism of other species. The trend line shows a positive correlation

Distance of association compared to size

The observed sizes of *S. seticornis* were compared to the distance of the observed association (Fig. 5). A positive correlation was found between increasing size and increasing distance of the association (linear regression, $n = 89$, $r^2 = 0.022$) (Fig. 5). However the majority of *S. seticornis* were between 5 - 10 cm in size and displayed associations from a wide range of association distances 2 - 50 cm.

Discussion

Within this study *S. seticornis* were observed associating with different species in many different phyla similar to Hayes et al. (1998). Additionally, like Hayes et al. (1998), small *S. seticornis* were observed associating with urchins and retreating near the spines when approached. This study also observed *S. seticornis* in close association with two types of anemone (*Condylactis gigantean* and *Bartholomea annulata*) and three different cleaner shrimp species (*Ancylomenes pedersoni*, *Stenopus hispidus*, and *Periclimenes yucatanicus*). Medeiros et al. (2011) reported the associations of *S. seticornis* and anemones, and recorded cleaning interactions between *S. seticornis* and 4 differernt client species; the literature did not state any known associations between *S. seticornis* and other cleaning crustacean species. No explicit cleaning interactions were observed in this study, but an ecological role of *S. seticornis* on Bonaire could include the cleaning of client species. The two hypotheses presented for this study were supported; hypothesis one and two suggested a higher density of *S. seticornis* would result in a higher frequency of association and a higher diversity of species associating respectively. This can be supported by the fact that the higher density of *S. seticornis* at 14 m had more total associations and more associating species.

The mean density was found to be significantly higher at 14 m which could be due to a preference of depth, or it could be attributed to the difference in the availability of habitat at the two depths. At 8 m the dominant substrate was sand and rubble, compared to the 14 m depth which had more corals and complex structures. The mean size of *S. seticornis* was smaller at 14 m, which could be because the habitat was more varied and had more structures for juvenile *S. seticornis* to inhabit.

A positive correlation was found between increased size and an increased distance of association, but it is important to highlight the fact that *S. seticornis* of the medium size class (5 - 10 cm) were found near other species across a wide range of association distances. More data could provide a more accurate relationship between size and distance of associations.

More species were observed associating at 14 m of depth, which could be due to the higher frequency of *S. seticornis* at 14 m ($n = 53$), compared to 8 m ($n = 27$). However, it is more likely that the greater variety and abundance of suitable habitat was the main reason that more species were found in association with *S. seticornis* at the deeper depth. This finding supports the second hypothesis that a higher density of *S. seticornis* would be found with a higher variety of associating species at 14 m.

While recording the behavior of *S. seticornis* it was noted that there were times when differentiating between eating and cleaning was difficult due to the fact that some *S. seticornis* were observed eating and cleaning themelves simultaneously. A more detail distinction of eating and cleaning behavior may be useful if this study were to be repeated. Additionally, larger *S. seticornis* may have been more likely to continue performing a behavior rather than retreating and hiding compared to smaller *S. seticornis*, due to the higher perceived threat of the approaching researchers. This behavior is difficult to control for when conducting field research so an *ex situ* study may be useful when making observations on varying sizes of *S. seticornis.*

Another factor that could have affected the study were other divers in the study area in 2

out of the 10 dives conducted for gathering data. The dives were always conducted between 17:45 and 19:00 hours, which is just before sunset. It was noted that more crabs were observed toward the end of the dives, which could mean that *S. seticornis* are more nocturnal and a future study could be conducted similarly but after sunset.

In conclusion, the study observed *S. seticornis* at 8 and 14 m in a variety of habitat types, substrates, and performing different behaviors. The variety of habitat types and substrates are likely due to differences in the habitat available at the different depths. The different frequencies of associations and number of species associating at the different depths is also likely due to the differences in habitat at the two depths. Studies such as Hayes et al (1998) and Medeiros et al. (2011) observed associations between *S. seticornis* and species of different phyla, which was similarly observed in this study. This study increases knowledge of the ecological role of *S. seticornis* on Bonaire, however more controlled research in the lab or night studies in the field can be done to gain a broader understanding of the roles and functions performed by *S. seticornis* in the coral reef ecosystem.

Acknowledgements I would like to thank my research partner, Victoria Cassar, for her help preparing for dives, conducting data collection surveys, taking photos, analyzing data, and always giving good vibes during our time in the water. Thank you to Fadilah Ali and Sara Buckley for your continued assistance and critical advising which helped structure, organize, and properly perform my study; as well as all the help you gave in revising this paper to be ready for publishing. I would also like to thank the entire staff of CIEE Bonaire for their support in any way possible, with special thanks to Astird de Jager and Mark Tsagaris for helping me become a trained and confident scientific diver.

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REPORT

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Interactions among sponges, algae, and coral in Bonaire, Dutch Caribbean: an analysis of sponge and algae prevalence in relation to coral abundance and health

Abstract Stressors causing coral reef degradation are making reefs susceptible to domination by other organisms. One documented phase-shift is increased macroalgal cover of deteriorated reefs. Sponges also have the potential to overtake reefs because of their tolerance for rising temperatures and ocean acidification, ability to outcompete corals for space, and tendency to grow on available substrate created by coral mortality. This study aimed to address gaps in the literature on sponge/coral relationships as well as simultaneously study the interactions between coral and both of its potential competitors. Percentage encrusting sponge cover, percentage algae cover, and encrusting sponge density were compared to percentage live, damaged, and dead coral cover to examine the interactions among coral, sponges, and algae. Sponge/coral interactions were also classified to assess sponge aggressiveness. Data was collected at Yellow Submarine dive site using belt transects and photoquadrats. Although no correlations were significant, most comparisons found that sponges and algae decreased with more live coral cover and increased with more dead coral cover. No significant differences among the abundance of sponge/coral interaction types were found on the reef slope, but there were significant differences present on the reef crest. In both locations, most interactions were not aggressive overgrowth interactions. The relationships among sponges, algae, and coral suggest that both sponges and algae tend to grow on substrate made available by coral death. By examining the interactions of both sponges and algae with coral, comparison of these relationships was possible, potentially prompting future work that also assesses multiple ecologically important interactions.

Keywords Non-aggressive • percent cover • reef degradation

Introduction

Pollution and overfishing have been identified as causes of long-term reductions in coral reef diversity and structural complexity (Pandolfi et al. 2003). While these stresses continue to threaten coral reef health, global warming and ocean acidification are also predicted to have detrimental effects on reef systems in the future (Pandolfi et al. 2011). Consistently elevated temperatures can cause corals to expel their symbiotic zooxanthellae in a process known as coral bleaching, and bleaching events are capable of causing high mortality and reduced reproduction of affected corals (Baird and Marshall 2002). Rising atmospheric CO_2 levels and declining seawater carbonate ion concentrations are projected to reduce rates of coral carbonate accretion, causing further reef degradation (Hoegh-Guldberg et al. 2007). Research collectively suggests that deterioration of reefs due to these processes, along with overfishing of herbivorous species and nutrient runoff, has caused some reefs to shift from coral-dominated to macroalgae-dominated states (Hughes 1994; Hughes et al. 2010).

Although the primary literature has focused

on coral-macroalgal phase shifts, there is also documentation of coral reefs shifting to states dominated by other organisms, including sponges (Norström et al. 2009). Sponges are present from the poles to the tropics and perform a variety of important tasks in the reef community, including binding corals to the reef framework, nutrient cycling, and contribution to primary and secondary production (Bell 2008). Despite their importance to coral reef ecosystems, sponges have been shown to be capable of successfully competing with corals for space (Diaz and Rützler 2001) as well as taking over substrate made vacant by coral mortality (Aerts 2000; Bell et al. 2013). Combined with their tolerance for rising temperatures and ocean acidification, these abilities give sponges the potential to dominate reef systems in the coming years (Bell et al. 2013). More acidic oceans may even enhance sponge ability to outcompete corals for space; in some sponge species, elevated seawater CO_2 levels have corresponded to increased rates of chemical bioerosion (Enochs et al. 2015). Heightened erosive abilities of sponges, coupled with decreased calcification rates of corals due to ocean acidification, would accelerate coral mortality and give sponges a competitive advantage in reef communities (Enochs et al. 2015).

While they are destructive, aggressive interactions are not the only form of spatial competition between corals and sponges. Although Caribbean bioeroding sponges have increased along with coral mortality, this is due mostly to their growth on dead coral rather than aggression towards live colonies (Bell et al. 2013). It is also possible for non-bioeroding sponges to dominate reef ecosystems, and such shifts have been documented in recent decades (Bell et al. 2013).

Although competition between corals and macroalgae has been analyzed and sponge/coral interactions have also been researched, the relationships among organisms from all three groups have not been widely discussed in the primary literature (González-Rivero et al. 2011). This study seeks to address this gap through analysis of algae and sponge abundance in relation to coral prevalence and health, as well as by assessment of the aggressiveness of sponge/coral interactions. As encrusting sponges have the most easily quantifiable surface area, they were the focus of data collection. Because percentage sponge cover and sponge density have been shown to each correlate differently with percentage hard coral cover, encrusting sponge density was assessed in addition to percentage sponge cover (Powell et al. 2010). Encrusting sponge density was defined as the number of individual encrusting sponge patches per square meter. Two hypotheses were tested:

H_1: Percentage encrusting sponge cover, percentage algae cover, and encrusting sponge density will decrease with more live coral cover and increase with more dead and damaged coral cover

H_2: The majority of encrusting sponge/coral interactions at the study site will be non-aggressive – that is, sponges will not be overgrowing living coral tissue (Aerts and van Soest 1997)

Because a potential consequence of coral reef deterioration is macroalgae and sponge domination, the interactions among corals, sponges, and algae are an important focus of research. The purpose of this experiment was to analyze both algae and sponge abundance in the same study, allowing their interactions with coral to be directly compared. The experiment also aimed to analyze types of individual sponge/coral interactions. This allowed sponge aggressiveness, in addition to cover and density, to be assessed. The results of this project can prompt future research involving larger numbers of interactions among ecologically important organisms, which could potentially impact strategies for managing changing reef systems.

Materials and methods

Study site

Research was conducted in the waters off the western (leeward) coast of Bonaire, Caribbean Netherlands, an island approximately 80 km north of Venezuela. Data was collected at Yellow Submarine (Yellow Sub; 12°09'36.2"N, 68°16'55.2"W; Fig. 1), a local dive site south of Something Special dive site and just north of Kralendijk, Bonaire's largest city. The fringing reef at Yellow Sub is separated from shore by a sand flat 50 m wide, with the reef crest located at a depth of approximately 8 m. Yellow Sub is heavily trafficked by divers and boats, and fishing is permitted.

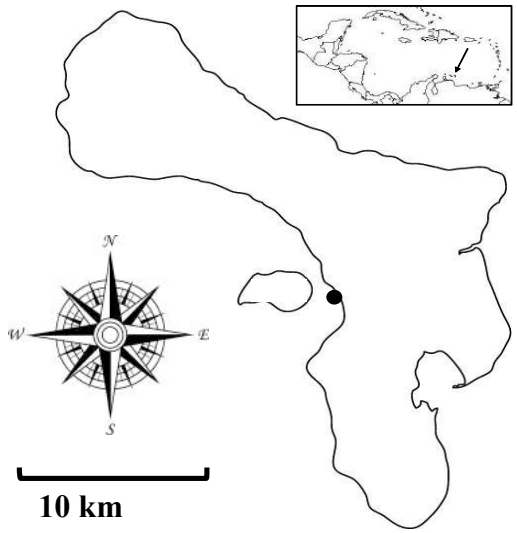

Fig. 1 Map showing the location of Bonaire, Dutch Caribbean, in the Caribbean Sea. The black dot marks Yellow Sub dive site (12°09'36.2"N, 68°16'55.2"W)

Data Collection

Seven sampling areas of Yellow Sub were selected prior to data collection. Surveys were conducted directly in front of Yellow Sub dock as well as 50 m, 100 m, and 150 m to the north of the dock and 50 m, 100 m, and 150 m to the south of the dock (Fig. 2). Because sponge species composition (Alcolado 1994) and the frequency of sponge overgrowth (Aerts 1998) have been found to vary with depth, three 10 m by 2 m transects at two different depths were laid per sampling area. Data was collected by laying two transects at 8 m and one transect at 15 m on the first day of data collection. On the next day of data collection, one transect was laid at 8 m and two transects were laid at 15 m. Alternation between these two sets of transect depths continued throughout the data collection period. For each transect, 10 photographs were taken on alternating sides of the transect, starting on the shoreside, using a 1-m quadrat (Fig. 3).

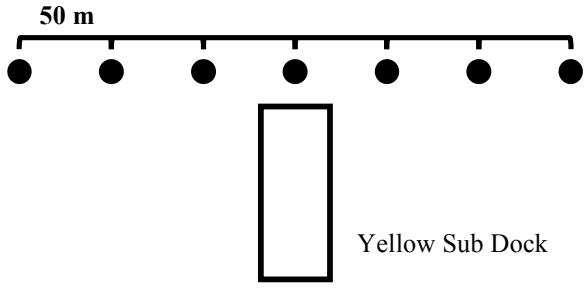

Fig. 2 Illustration showing the location of data collection sites. Each dot represents a different collection site, and each bracket represents a distance of 50 m

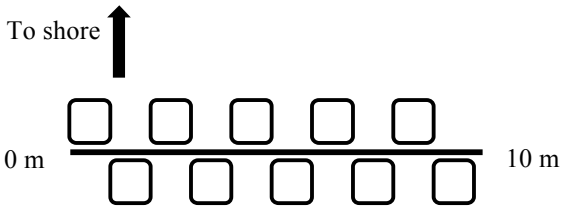

Fig. 3 Illustration showing the position of photo quadrats and 10 m x 2 m transect during data collection

Data analysis

For each day of data collection, quadrat photographs from one transect per depth were analyzed using Coral Point Count with Excel extensions (CPCe). Fifty random points were overlaid on each quadrat image, and the substrate under each point was categorized (Powell et al. 2010). Coral tissue was classified according to its condition (Table 1). For each transect, the percentages of the transect covered by encrusting sponges, live coral, damaged coral (classified as diseased coral, DCOR) dead coral with algae, and dead coral without algae (classified as old dead coral, ODC) were deter-

Table 1 Criteria for assessing the condition of hard coral tissue. Modified from Lang et al. (2010)

Tissue Condition	Description
Live	Polyp tissue and zooxanthellae uniformly present, growth anomalies absent
Damaged	Conspicuous tissue loss, discoloration, anomalies in polyp size and/or growth patterns, bleached tissue (coral polyps present, zooxanthellae absent)
Dead	Only skeleton remaining, no soft tissues present

Table 2 Types of sponge/coral interactions. Modified from Aerts and van Soest (1997)

Interaction	Description
Overgrowth	Sponge overgrowth of living coral tissue
Peripheral Contact	Parallel contact of sponge and living coral tissue for ≥ 3.000 cm
Tissue Contact	Parallel contact of sponge and living coral tissue for ≤ 2.999 cm
Non Contact	Sponge growth ≤ 4.999 cm away from living coral tissue
None	Sponge growth ≥ 5.000 cm away from living coral tissue

mined. Other sponges, coralline algae, sand, rubble, and miscellaneous materials (classified as other, O) were also identified but not used in data analysis. Points overlaid on or outside the photoquadrat structure were classified as tape, TAPE. While live coral was identified to species level, only the total percentage of live coral cover was used in analyses in order to assess the relationship among encrusting sponges, algae, and overall coral health. Measuring tools in the computer program Image J were also used on photoquadrat images. For sponges not in contact with live coral tissue, their distance from the nearest live coral tissue was measured to determine if the sponge/coral interactions were "non-contact" or "none" (Aerts and van Soest 1997; Table 2). For sponges that were growing along the edge of live coral tissue, the distance of growth was measured to classify the interactions as "peripheral contact" or "tissue contact" (Aerts and van Soest 1997; Table 2). Interactions were classified as "overgrowth" if live coral tissue was present underneath the sponge (Aerts and van Soest 1997; Table 2). Sponge density was determined through visual inspection of each photoquadrat.

At each depth, percentage encrusting sponge cover, percentage dead coral covered by algae, and encrusting sponge density were compared to percentages of live, damaged, and total dead coral cover. For clarity, these variables will hereafter be referred to as sponge cover, sponge density, algae cover, live coral cover, damaged coral cover, and dead coral cover. A scatterplot of each variable was created to check for a normal distribution of data, as indicated by a bell-shaped curve. No variables were normally distributed, so Spearman's rank correlation (r_s) was used to assess the relationships between each set of variables. An Internet p-value calculator was used to obtain all p-values. The mean abundance of each type of sponge/coral interaction per transect was also calculated at each depth, and a one-way ANOVA was performed to compare these values. Abundance was defined as the total number of a specific type of interaction recorded per transect.

Results

Percentage encrusting sponge cover is not dependent on percentage live, damaged, or dead coral cover on the reef slope or reef crest

To determine the relationship that sponge cover had to coral cover and health, sponge cover was compared to live, damaged, and dead coral cover for each transect. These relationships were evaluated at depths of 15 m (Fig. 4a, b, c) and 8 m (Fig. 4d, e, f) and analyzed using Spearman's rank correlation (r_s). Sponge cover decreased as coral cover increased at both 15 m (r_s = -0.321, df = 5, p = 0.482) and 8 m (r_s = -0.072, df = 5, p = 0.878), but these correlations were not significant. At a depth of 15 m, sponge cover decreased non-significantly as damaged coral cover increased (r_s = -0.393, df = 5, p = 0.383), while sponge cover increased non-significantly with damaged coral cover at

8 m (r_s = 0.101, df = 5, p = 0.830). Sponge cover also increased non-significantly with percentage dead coral cover at 15 m (r_s = 0.321, df = 5, p = 0.482) and 8 m (r_s = 0.306, df = 5, p = 0.504). These findings demonstrated that sponge cover was independent of live, damaged, and dead coral cover on the reef slope and the reef crest.

Percentage algae cover is not dependent on percentage live or damaged coral cover on the reef slope or reef crest

To determine the relationship that algae cover had to coral cover and health, algae cover was compared to live and damaged coral cover for each transect. These relationships were eval-

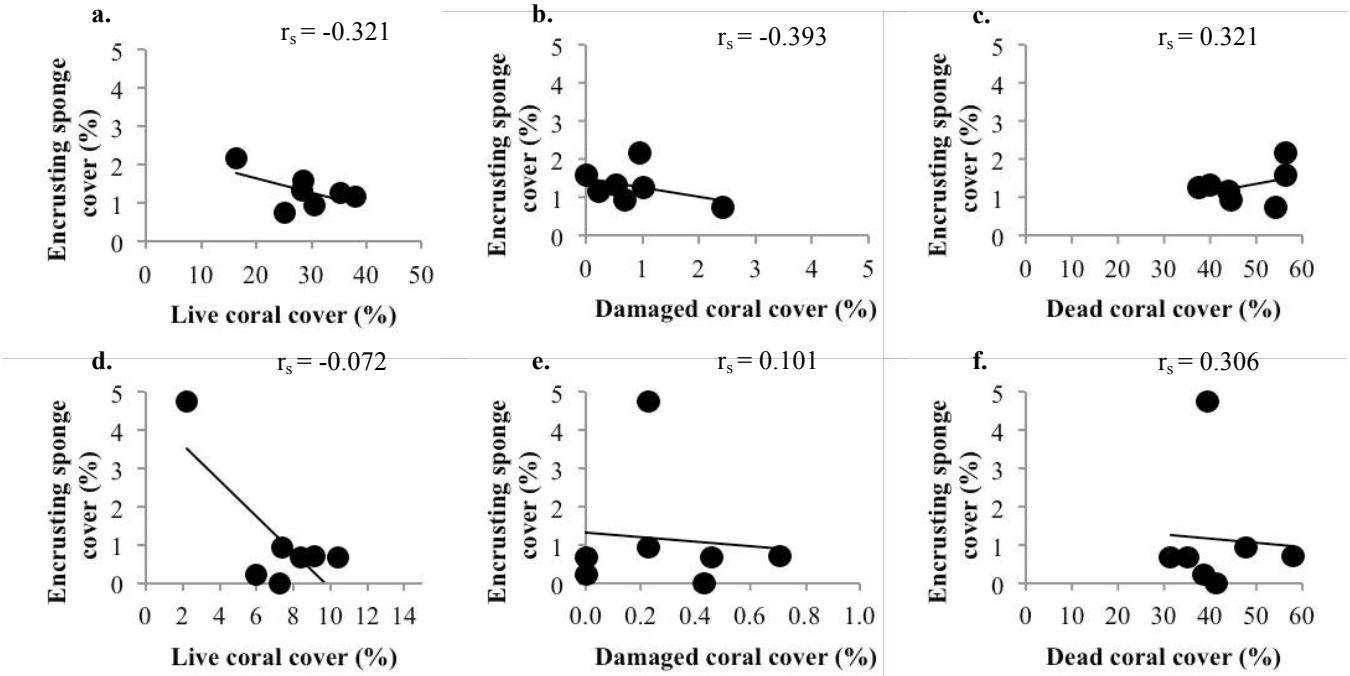

Fig. 4 Relationship between encrusting sponge cover (%) and live, damaged, and dead hard coral cover (%) per transect at depths of **a, b, c** 15 m (n = 7) and **d, e, f** 8 m (n = 7); r_s = Spearman's rank correlation

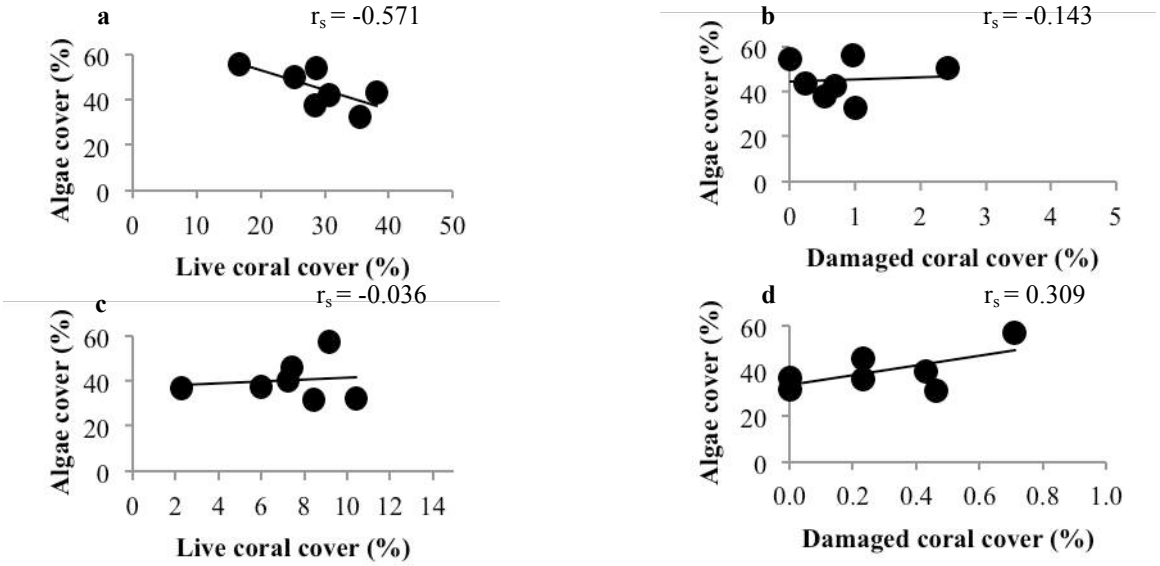

Fig. 5 Relationship between algae cover (%) and live and damaged hard coral cover (%) per transect at depths of **a, b** 15 m (n = 7) and **c, d** 8 m (n = 7); r_s = Spearman's rank correlation

uated at depths of 15 m (Fig. 5a, b) and 8 m (Fig. 5c, d) and analyzed using Spearman's rank correlation (r_s). Algae cover decreased as live coral cover increased at 15 m (r_s = -0.571, df = 5, p = 0.180) and at 8 m (r_s = -0.036, df = 5, p = 0.939), but these correlations were not significant. At a depth of 15 m, algae cover decreased non-significantly as damaged coral cover increased (r_s = -0.143, df = 5, p = 0.760), while algae cover increased non-significantly with damaged coral cover at 8 m (r_s = 0.309, df = 5, p = 0.500). These results demonstrated that algae cover was independent of live and damaged coral cover on the reef slope and the reef crest.

Mean encrusting sponge density is not dependent on percentage live, damaged, or dead coral cover on the reef slope or reef crest

To determine the relationship that sponge density had to coral cover and health, sponge density was compared to live, damaged, and dead coral cover for each transect. These relationships were evaluated at depths of 15 m (Fig. 6a, b, c) and 8 m (Fig. 6d, e, f) and analyzed using Spearman's rank correlation (r_s). Sponge density decreased as live coral cover increased at both 15 m (r_s = -0.107, df = 5, p = 0.819) and 8 m (r_s = -0.214, df = 5, p = 0.645), but these correlations were not significant. At a depth of 15 m, sponge density decreased non-significantly as damaged coral cover increased (r_s = -0.107, df = 5, p = 0.819), while sponge density increased non-significantly with damaged coral cover at 8 m (r_s = 0.163, df = 5, p = 0.726). Sponge density increased non-significantly with dead coral cover at 15 m (r_s = 0.536, df = 5, p = 0.215) but decreased non-significantly as dead coral cover increased at 8 m (r_s = -0.571, df = 5, p = 0.180). These findings showed that sponge density was independent of live, damaged, and dead coral cover.

There are differences in the abundance of sponge/coral interaction types on the reef crest but not on the reef slope

To assess the differences in abundance of sponge/coral interaction types, mean numbers of overgrowth, peripheral, tissue, non-contact, and nonexistent interactions per transect were calculated. Interactions were recorded at depths of 15 m (Fig 7a) and 8 m (Fig 7b). At 15 m, the mean (± SD) number of overgrowth interactions was 2.3 ± 1.70; peripheral, 4.6 ± 2.07; tissue, 3.3 ± 4.19; and non-contact, 5.4 ±

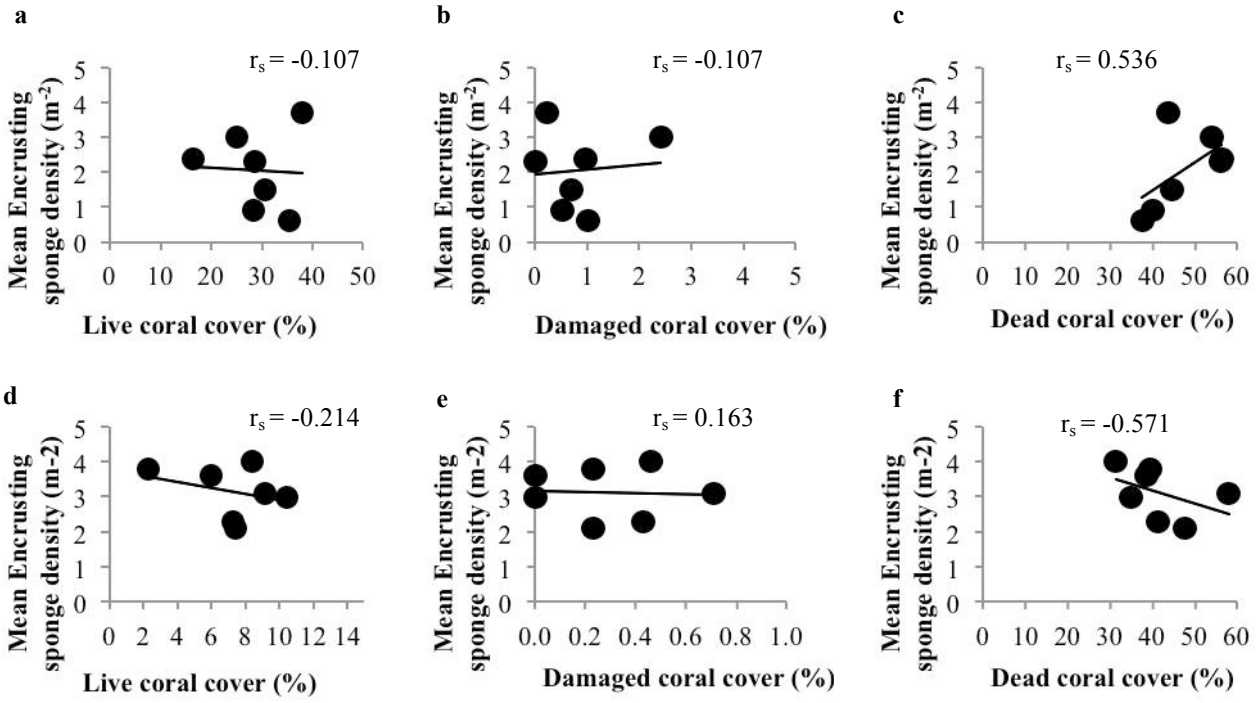

Fig. 6 Relationship between mean encrusting sponge density (m^{-2}) and live, damaged, and dead hard coral cover (%) per transect at depths of **a, b, c** 15 m (n = 7) and **d, e, f** 8 m (n = 7); r_s = Spearman's rank correlation

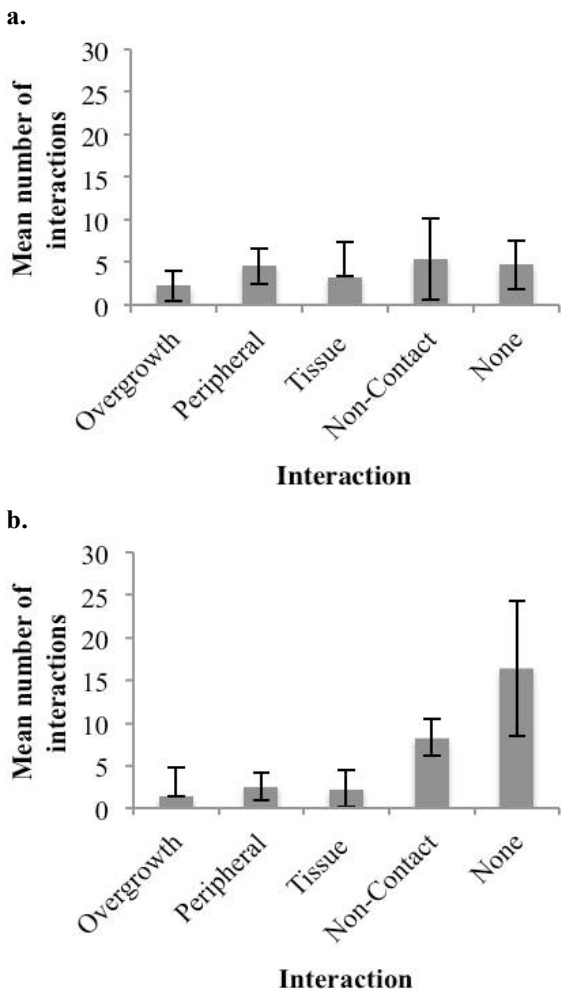

Fig. 7 Mean number of different types of sponge/coral interactions per transect (± SD) at depths of **a** 15 m (n = 7) and **b** 8 m (n = 7)

4.76. The mean (± SD) number of sponges with no interactions was 4.7 ± 2.81. At 15 m, there was no significant difference among the abundance of interaction types as determined by one-way ANOVA ($F_{4, 25}$ = 0.941, p = 0.457).

At 8 m, the mean (± SD) number of overgrowth interactions was 1.4 ± 3.36; peripheral, 2.6 ± 1.62; tissue, 2.3 ± 2.14; and non-contact, 8.3 ± 2.14. The mean (± SD) number of sponges with no interactions was 16.4 ± 7.91. At 8 m, there was a significant difference among the abundance of interaction types ($F_{4, 25}$ = 14.518, p = 2.959×10^{-6}). At both depths, the least amount of sponges was engaged in overgrowth interactions. At 8 m, the most sponges were engaged in no interactions, and this was the largest mean number of interactions found at either depth. These results demonstrate that the abundance of sponge/coral interaction types varies significantly on the reef crest but not on the reef slope.

Discussion

Percentage encrusting sponge cover is not dependent on percentage live, damaged, or dead coral cover on the reef slope or reef crest

The relationships between sponge cover and live, damaged, and dead coral cover did not support the first hypothesis that sponge cover would decrease with more live coral cover and increase with damaged and dead coral cover. No significant correlations were found between these variables.

Despite the lack of significant correlations, some non-significant trends were found to be consistent with the predictions of the hypothesis. Sponge cover decreased as live coral cover increased at depths of 15 m and 8 m. This decrease affirms the results of previous research that also found a decrease in percentage sponge cover with greater hard coral cover (Powell et al. 2010). The fact that less sponge cover is present with greater coral cover also suggests that sponges tend to take advantage of dead coral as opposed to actively overgrowing live coral colonies. This is supported by records of both bioeroding and non-bioeroding sponges dominating Caribbean reefs by settling on available substrate, even though bioeroding sponges have the capability to aggressively compete with corals (Bell et al. 2013).

Sponge cover increased with damaged coral cover at a depth of 8 m, a result also consistent with the predictions of the hypothesis. However, sponge cover decreased with damaged coral cover at 15 m. A possible explanation for this result is the difference in live coral cover on the reef slope and the reef crest. At Yellow Sub, there is less live coral cover on the reef crest than on the reef slope. Most photoquadrats at 8 m contained

predominantly sand, rubble, and dead coral with algae. At 8 m, dead coral and rubble usually surrounded damaged coral, while at 15 m live coral more often surrounded damaged coral. The small amount of overgrowth interactions observed suggests that the sponges at the study site engage in mostly non-aggressive interactions. This enables sponge cover to increase with the damaged coral on the reef crest (when surrounded by available substrate) and decrease with the damaged coral on the reef slope (when surrounded by live coral). Thus, these trends probably suggest more about the substrate surrounding the damaged coral at these two depths than the growth patterns of sponges on damaged coral substrate.

The relationship between sponge cover and dead coral cover was also consistent with the predictions of the hypothesis. Sponge cover increased with dead coral cover at depths of both 8 m and 15 m, reinforcing the correlation between sponge cover and live coral cover. These results are similar to the findings of Powell et al. (2010). They also further support the idea that sponges of all types tend to dominate reefs by growing on available substrate rather than live coral (Bell et. al 2013).

Percentage algae cover is not dependent on percentage live, damaged, or dead coral cover on the reef slope or reef crest

The relationships between algae cover and live and damaged coral cover did not support the first hypothesis that algae cover would decrease with more live coral cover and increase with damaged coral cover. No significant correlations were found between these variables.

Although the hypothesis was not supported, the decrease in algae cover with rising live coral cover was consistent with its predictions. Lower algae cover in the presence of more live coral is consistent with widespread observations of macroalgae-dominated reefs. In these ecosystems, less coral is associated with copious amounts of macroalgae (Hughes 1994; Hughes et al. 2010).

Algae cover increased with damaged coral cover at a depth of 8 m, a result also consistent with the predictions of the hypothesis. However, it decreased with damaged coral cover at 15 m. As with the correlations between damaged coral and sponge cover, these results may suggest more about the substrate at 15 m and 8 m than about the relationship between algae cover and damaged coral cover. The reduction of live coral and corresponding increase in macroalgae that has been documented in the literature (Hughes 1994; Hughes et al. 2010) suggests that algae cover would increase with the damaged coral on the reef crest, which is dominated by dead coral and rubble. Algae cover would thus decrease with rising damaged coral cover on the reef slope, where live coral is more prevalent and less substrate is available.

Mean encrusting sponge density is not dependent on percentage live, damaged, or dead coral cover on the reef slope or reef crest

The relationships between sponge density and live, damaged, and dead coral cover did not support the first hypothesis that sponge density would decrease with more live coral cover and increase with damaged and dead coral cover. No significant correlations were found between these variables.

Despite the lack of support for the hypothesis, sponge density decreased with increasing live coral cover at depths of 15 m and 8 m. These trends correspond with the predictions of the hypothesis. They also suggest that more live coral corresponds to fewer individual sponges in addition to less sponge cover. Previous research found that while sponge cover decreases with more coral cover, sponge density increases due to the greater number of micro-habitats available (Powell et al. 2010). An explanation for the discrepancy between the results of past research and the results of this study could be that differences in sponge predation and sedimentation are present between the study

sites (Powell et al. 2010). Both of these factors are known to inhibit sponge growth (Powell et al. 2010), and if high sedimentation and sponge predation levels are present at Yellow Sub, fewer sponges may also be present. An alternative explanation could be differences in structural complexity between the two study sites. Less structural complexity at Yellow Sub than at the site surveyed in Powell et al. (2010) would result in fewer available micro-habitats, reducing the number of areas available for individual sponges to grow.

Sponge density increased with damaged coral cover at a depth of 8 m, a result also consistent with the predictions of the hypothesis. However, sponge density decreased with more damaged coral cover at 15 m. As with sponge and algae cover, these results may be reflecting differences in the substrate at 15 m and 8 m. Because few sponge/coral overgrowth interactions were observed, it is possible that individual sponges would be better able to grow on the reef crest (where there is an abundance of available substrate) than on the reef slope (where overgrowing live coral would be necessary).

Sponge density increased with dead coral cover at 15 m, supporting the hypothesis. However, there were fewer sponges with more dead coral cover at 8 m. The latter trend aligns with the finding of Powell et al. (2010) that fewer sponges were present with less hard coral cover. Powell et al. (2010) attribute their findings to a reduced number of micro-habitats for sponges. At 8 m, low structural complexity and few micro-habitats may account for the reduced number of sponges with increased dead coral cover. At 15 m, the correlation can be explained by the possibility that more dead coral is not associated with less structural complexity. Coral heads are more abundant at 15 m than 8 m at Yellow Sub, resulting in higher structural complexity than at the reef crest, which has more sand and rubble. If dead coral at 15 m retains its original structure and maintains the rugosity of the reef, it would combine a complex structure with available substrate and provide more habitats for sponges. This would allow sponge density to increase with dead coral cover.

There are differences in the abundance of sponge/coral interaction types on the reef crest but not on the reef slope

The abundance of different sponge/coral interaction types on the reef crest and reef slope supported the hypothesis that the majority of encrusting sponge/coral interactions at the study site would be non-aggressive (i.e., not overgrowth interactions). Although no difference in mean abundance of interaction types was found on the reef slope, the least amount of sponges was engaged in overgrowth interactions. Because there were greater numbers of sponges involved in each of the four non-aggressive categories, the hypothesis that most interactions would be non-aggressive was supported. On the reef crest, a difference in mean abundance of interaction types was found, with most sponges engaging in no sponge/coral interactions at all. The least amount of sponges was involved in overgrowth interactions on the reef crest as well. These results are supported by research suggesting that bioeroding sponges, as well as less aggressive sponges, usually grow on available substrate rather than living coral (Bell et al. 2013). The significantly higher number of sponges with no interactions at 8 m is most likely due to the difference in substrate between the reef slope and the reef crest. With lower coral cover on the reef crest, it is more likely that a sponge will not be in close proximity to coral.

There are several limitations of this study that could be eliminated in future research. During data collection, many sponges were observed in crevices or under coral heads where they could not be seen in photoquadrat data. Future work would benefit from employing a methodology allowing more angles of the substrate to be viewed. For example, placing photoquadrats at varying, predetermined angles to the substrate might allow for an increased view of hidden sponges

(Powell et al. 2010). The low sample size (n = 7) was also a limitation of this experiment. Future studies should increase the number of replicates through additional sampling or by concentrating sampling efforts at a single depth.

Despite the fact that the majority of results obtained were non-significant, the trends found in this study suggest that both sponges and algae increase if less live coral is present. These findings align with research documenting both coral-macroalgal phase shifts (Hughes 1994; Hughes et al. 2010) and the potential for sponges to dominate coral reefs in the future (Aerts 2000; Diaz and Rützler 2001; Bell et al. 2013). Although more substantial research is needed regarding interactions among coral, sponges, and algae, specifically in the areas of future work suggested above, these findings indicate that preservation of healthy coral reefs is necessary to prevent increases of sponges and algae.

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REPORT

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Structure and substratum preference of a schooling fish: observing the habitat use and nutrient input of smallmouth grunts (*Haemulon chrysargyreum*) in Bonaire

Abstract Various marine organisms are known to consciously select specific types of habitat that provide maximum shelter from potential predators. Reef fish such as *Haemulon chrysargyreum* (smallmouth grunts) are commonly seen congregating in groups around the coral structures in Bonaire. Observing schooling fish can provide pertinent information on the refuge provided by structurally complex and diverse ecosystems. This study assessed the habitat preference of *H. chrysargyreum* based on species of coral, complexity of sites, and substrate type. Levels of phosphate, nitrate, and ammonia were also analyzed in the areas occupied by shoaling *H. chrysargyreum* to see if they provide a significant input of nutrients into the coral reef ecosystem. The results of this study demonstrate that *H. chrysargyreum* prefer areas of medium to high complexity accompanied with a soft substrate (sand, rubble) and an overhanging structure. Nutrient level analysis was inconclusive and, therefore, requires further studies. This research sought to identify certain species of coral and structures that are used by *H. chrysargyreum* for habitation. Such knowledge can aid conservation efforts by honing in on specific areas that schooling fish utilize for shoaling and feeding. Additionally, data from this study provided preliminary assessment for future studies on the potential nutrient input of *H. chrysargyreum* to the marine ecosystem.

Keywords Shelter • excretion • predation risk

Introduction

In virtually any marine ecosystem, various species of fish and invertebrates can be found inhabiting the protective structures formed by rocks, corals, and underwater foliage. The variations in shape and complexity of these habitats have been found to affect which species are occupying these areas (Gardiner and Jones 2010). This phenomenon, known as habitat preference, is known to play a significant role in the relative fitness of organisms that display this behavior (Werner et al. 1983). This is supported by Messmer et al. (2011) who observed that fish discern their habitat of choice not just by shape and structure of coral, but also by species of coral (Messmer et al. 2011). In many animals, this behavior allows them to find a habitat best suited to avoiding predation, therefore improving their relative fitness and chances of survival (Lamouroux et al. 1999). Some species of perch (*Perca fluviatilis*) demonstrated the ability to evaluate a habitat by weighing certain factors such as predation risk and foraging profitability depending on their size and maturity (Werner et al. 1983). In fact, this behavior is so prevalent that preference models based on biotic and abiotic factors of habitats can be formulated to predict which structures organisms would choose to inhabit (Lamouroux et al. 1999).

These studies provide valuable and pertinent data on species' distribution and abundance (Brooker et al. 2013), aspects that could possibly have an effect on nutrient availability in the coral reef habitat. Studying

this relationship is important to marine ecosystems because there is a strong correlation between fish abundance and concentration of nutrients (Layman et al. 2013). This relationship is due in part to the excretion from reef fish which releases nutrients such as ammonia into the aqueous environment (Roopin et al. 2008). A better understanding of these factors could benefit coral preservation and restoration efforts by identifying species that are heavily utilized by reef fish. Further, more effective management of these fish's habitats could potentially sustain the input of nutrients they provide for the coral reef ecosystem.

Corals are particularly important for the marine ecosystem of Bonaire because they provide complex structures that fish can use for protection from predators (Morrissey and Gruber 1993). The smallmouth grunt (*Haemulon chrysargyreum*) is a common species of schooling fish seen congregating around the structures formed by corals (Krajewski et al. 2004). Therefore, this study aims to determine whether *H. chrysargyreum* display habitat preference and provide an input of nutrients into the coral reef ecosystem. Not many studies have been conducted on the habitat preference of *H. chrysargyreum* and how their preference and behavior may affect the health of coral reefs. Thus, based on past studies and observations, the study's hypotheses are as follows:

- H_1: Coral species and structures with medium to high complexity will tend to be inhabited by more and larger schools of *H. chrysargyreum*
- H_2: *H. chrysargyreum* will have a preference for large, vertically-growing species of coral that provide substantial cover
- H_3: Areas with a high abundance of *H. chrysargyreum* will have significantly higher levels of nutrients than areas of low to no abundance

This study will provide insight into habitat preference and nutrient input that can help expand and improve coral reef preservation by identifying specific species of corals that are inhabited by *H. chrysargyreum*. Historic degradation of corals and eventual flattening of reef structure pose a serious threat to the health and overall framework of these marine ecosystems (Alvarez-Filip et al. 2009). Protection of the coral reef is important because low structural complexity in a habitat is linked to lower biodiversity and recruitment of marine organisms (Bodmer et al. 2015). Even degraded and diseased coral have been found to affect the abundance of reef fish that settle in these ecosystems, sometimes causing a shift in the community structure (Feary et al. 2007). However, increased habitat diversity usually promotes the number of available niches (Dustan et al. 2013), thereby enriching the ecological community with a higher biodiversity (Messmer et al. 2011). Data from this study will also establish whether *H. chrysargyreum* is an important contributor of nutrients to the island's coral reefs. Overall, this will benefit Bonaire because the island relies on its pristine and productive coral reefs for eco-tourism, food, and overall aesthetics of its environment.

Materials and methods

Study site

Data collection was conducted in the area between 'Yellow Submarine' dive site (N 12°09'36.2", W 68°16'55.2") and 'Something Special' dive site (N 12°16'22.3", W 68°28'46.7") on Bonaire, Dutch Caribbean, an island (~288 km^2) approximately 80 km north of the coast of Venezuela (Fig. 1). The total distance covered between these sites is approximately 380 m. These two sites, located on the western side of the island in northern Kralendijk, have a large area of sand flat that extends about 50 m from shore to a reef crest 9 m below the surface. The reef crest transitions into a reef slope that extends downward to a depth of about 30 m (Foxman 2015). This site was selected for my research because of the

high abundance of *H. chrysargyreum* found on the coral reef community along the reef slope.

Study organism

This study focused on the habitat preference of *H. chrysargyreum* (smallmouth grunts), a species of small schooling fish found in the coral reefs of Bonaire. The grunt (*Haemulidae*) family is an abundant species of nocturnal predators that primarily feed on benthic invertebrates in mangroves and seagrass beds (Burke 1995). During the day, however, they school in large groups on the reef slope of Bonaire at various depths, often closely congregated around the complex structure of the reef.

Data collection

Data was collected by SCUBA diving northward starting from 'Yellow Submarine' dive site towards 'Something Special' dive site. Data collection was conducted at a maximum depth of 12 m for 50 min on the reef slope. Schools of *H. chrysargyreum* were observed by counting and recording the total number of individual *H. chrysargyreum* in the school. The common names and abundance of other species within the school were also recorded. The depth and temperature of the area where the *H. chrysargyreum* were congregating were documented as well. Data collected included the species of coral along with their length and height (cm), sponges, and algae within a one-meter radius from the center of the school. Health of the coral was also observed by noting whether a disease was present or absent on the coral.

The structure of the area of schooling *H. chrysargyreum* was classified with a complexity scale: (0) No complexity – no overhead or lateral cover, (1) Low complexity –some lateral cover, (2) Medium Complexity – either overhang or lateral cover or both, (3) High Complexity – either overhang or much cover within and around overhang or both (Fig. 2). Substrate type(s) were also recorded as rubble, sand, algae, or dead coral. If there was an overhang within the one-meter radius from the center school, its height and length (cm) were measured with a 10-m measuring tape. Photos were taken with a Canon Powershot S110 point-and-shoot camera of areas where the schools of *H. chrysargyreum* were congregating for reference and analysis.

Water samples were collected at every area of high abundance along with one sample collected every dive of an area where *H. chrysargyreum* were absent. No abundance areas were observed approximately at the same depth of areas with high abundance of

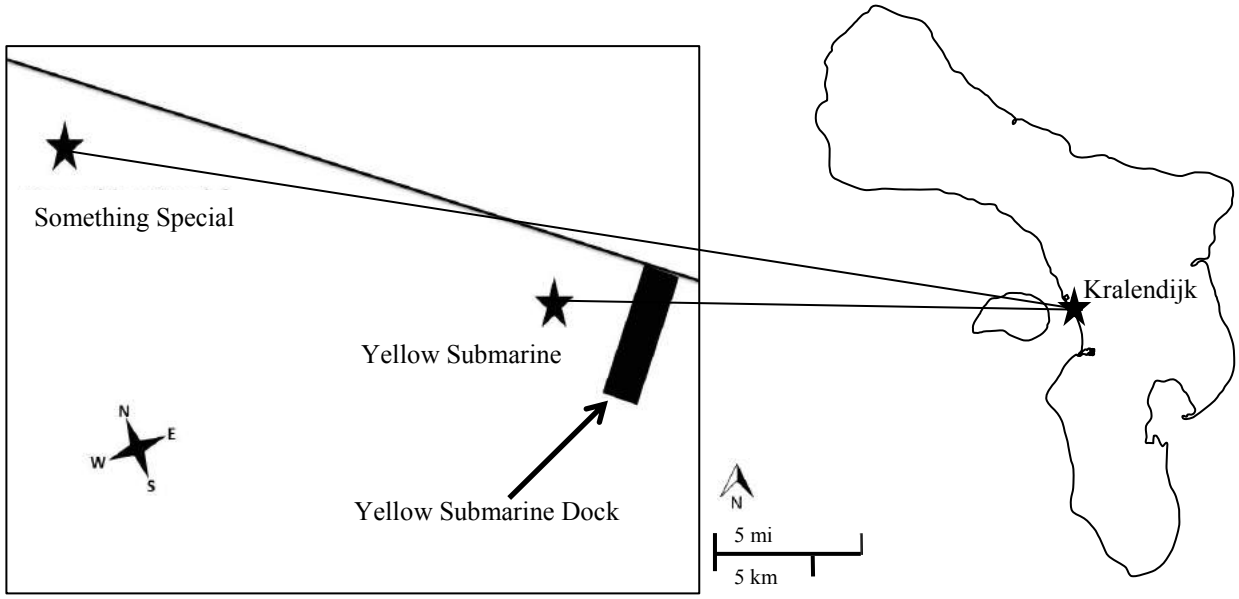

Fig. 1 Map of the Kralendijk waterfront from dive site 'Yellow Submarine' to the dive site 'Something Special'

H.chrysargyreum. Samples were collected with 50-mL plastic syringes treated with 10% HCl and Mili-Q distilled water before the dive. Water samples were classified based on the school size of *H. chrysargyreum*. Sizes included small (less than 15 individuals), medium (between 15 and 30 individuals), and large (more than 30 individuals). The actual number of individual *H. chrysargyreum* was recorded on the syringes. All collected water samples were frozen in 100-mL plastic bottles and stored in a freezer after collection. The starting point for each dive was the point where data collection previously ended.

Data analysis

Habitat preference was assessed by observing number and size of *H. chrysargyreum* schools and their association with coral species, presence and area (m^2) of overhang, substrate type, and the level of complexity of the habitat. Linear regression tests were ran to assess the significance of these relationships. School composition was also observed by comparing the total number of *H. chrysargyreum* with the abundance of other species of fish that were within the school.

During the last week of data collection, water analysis was conducted on the collected samples with a fluorometer to observe differences in nutrient levels among areas from high to no abundance of *H. chrysargyreum*. For this study, the levels of ammonia, phosphate, and nitrate were tested with a sub-sample of the water samples. Four samples of no abundance, three samples of low abundance, five samples of medium abundance, and five samples of high abundance were tested for phosphate. Four samples of no abundance, two samples of low abundance, four samples of medium abundance, and four samples of high abundance were tested for nitrate and ammonia. Linear regression tests were used to assess the association between the concentration of these nutrients and the school

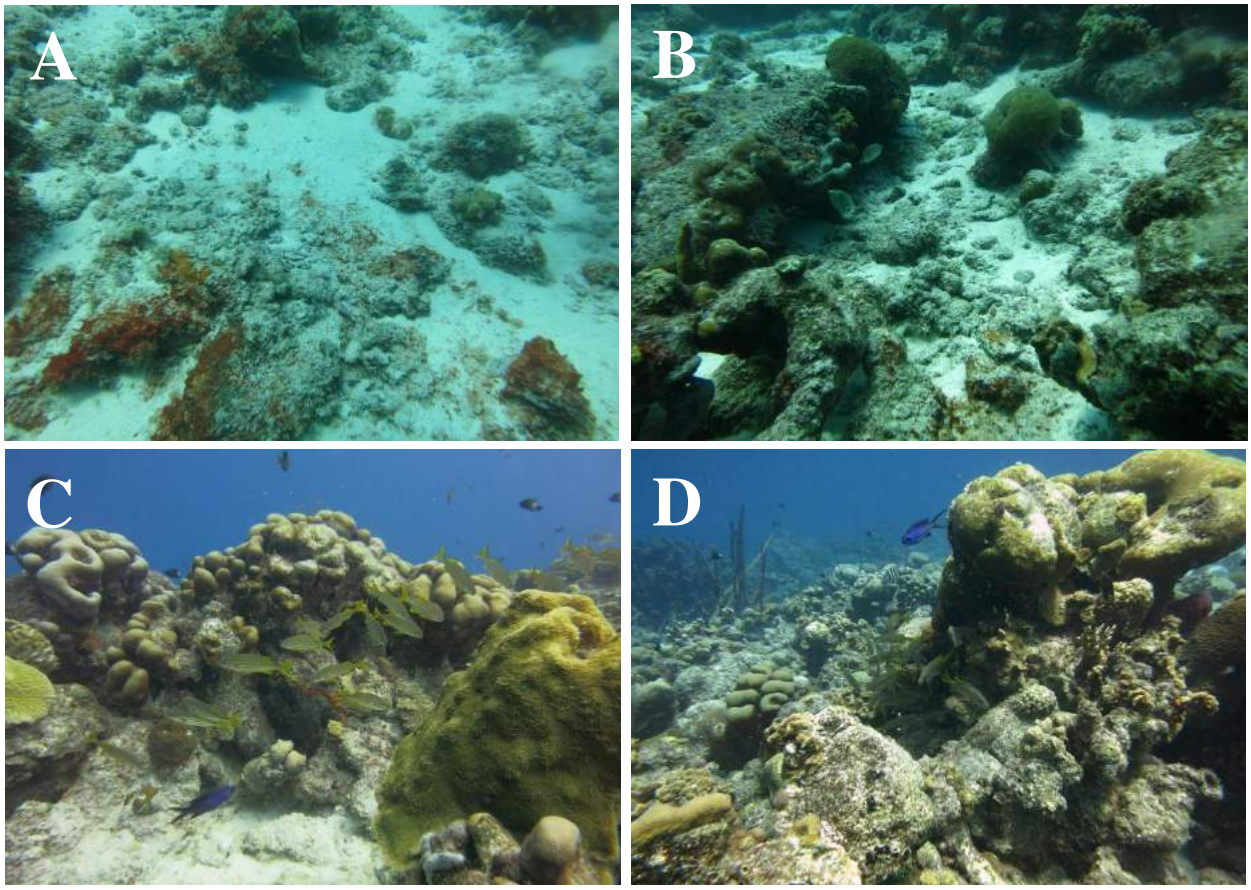

Fig. 2 Areas classified with complexity scale (starting top left, clockwise): no complexity (A), low complexity (B), medium complexity (C), high complexity (D)

size. Differences in nutrient levels between schools would indicate some relationship between school size and nutrient levels in the water.

Results

Coral species

Over the course of data collection, 22 schools of *H. chrysargyreum* were observed and analyzed. All schools were in areas that had a combination of different species of coral. Of the 22 recorded areas, 20 had *Undaria agaricites* within a 1-m radius of the center of the school (Fig 3). The massive coral *Orbicella annularis* was observed in 19 of the 22 areas and *Montastraea cavernosa* was observed in 14 of the 22 areas (Fig 3).

The relationship between school size and the number of different coral species within the area had no significant association (Linear Regression; n = 22; $R^2 = 0.0144$). The smallest schools (six individuals) were in areas with three and four different coral species, while the largest (70 individuals) was in an area with six coral species (Fig 4). The highest number of coral species recorded was eight different species, occupied by 32 *H. chrysargyreum*. All schools were in areas that had at least three different coral species and an average (± SD) of five different coral species were found at each site with *H. chrysargyreum*.

Overhang

Analysis of preference for overhang was performed only when it was present. The relationship between school size and the area (m^2) of overhang had no significant association (Linear Regression; n = 22; $R^2 = 0.0096$). The smallest school (six individuals) was congregating next to an overhang with an area of 0.4 m^2, while the biggest school (70 individuals) was next to an overhang with an area 0.3 m^2 (Fig 5). The largest overhang had an area of 1.12 m^2 and was occupied by a school of 26 individuals (Fig 5). The majority (18 schools) were observed within an area with

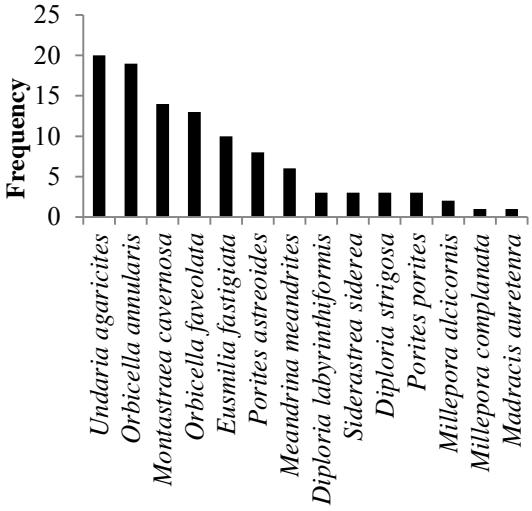

Fig 3. Frequency of coral species observed within a 1-m radius from the center of schooling *H. chrysargyreum* (n=22)

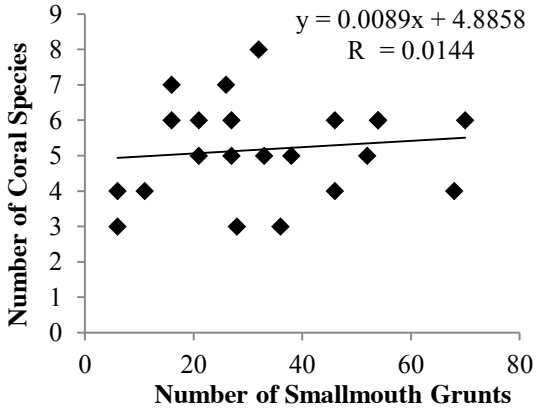

Fig 4. Association between size of school (number of *H. chrysargyreum*) and the number of different coral species in the area where the school is congregated

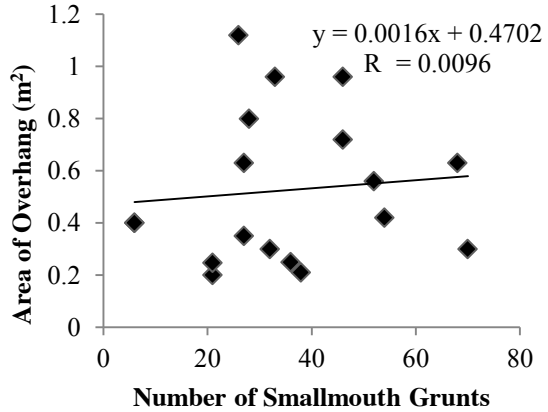

Fig 5. Association between size of school (number of *H. chrysargyreum*) and the area of overhang (m^2) they were congregating within or around

overhang while the remaining four had none (Fig 6).

Substrate

Most substrates of the schooling fish were a combination of four different types (rubble, sand, algae, and dead coral), with the majority of them containing rubble and sand. Of the 22 schools, rubble had the highest frequency of occurrence, observed 20 times (Fig 7). Other common substrates were sand, seen 17 times, and algae, seen 13 times (Fig 7).

Complexity level

Schools of *H. chrysargyreum* displayed some preference for more complex habitats. All observed schools of *H. chrysargyreum* were found in areas of either medium or high complexity while none were observed in areas of no or low complexity. On average, larger schools tended to inhabit areas of high complexity. An average (± SD) of 35 (± 19.71) individuals were found in areas of high complexity while an average school size of 30 (± 17.88) was found in areas of medium complexity (Fig 8).

School composition

Most schools of *H. chrysargyreum* consisted of other species of fish. Small schools (≤15 individuals) were largely made up of other species, with one school composed of 11 *H. chrysargyreum* and 24 other species. *Haemulon flavolineatum* (French grunt) were found in the highest abundance (25 individuals) in a school of 46 *H. chrysargyreum*. *Pomacentridae* or damselfish, specifically *Microspathodon chrysurus* (yellowtail damselfish) and *Stegastes partitus* (bicolor damselfish), had the highest frequency of occurrence, 15 of the 22, in schools of *H. chrysargyreum* (Fig 9). *Holocentridae* or squirrelfish, specifically *Myripristis jacobus* (blackbar soldierfish) and *Neoniphon marianus* (longjaw squirrelfish), had the second highest frequency of occurrence, 14 of the 22 total (Fig 9).

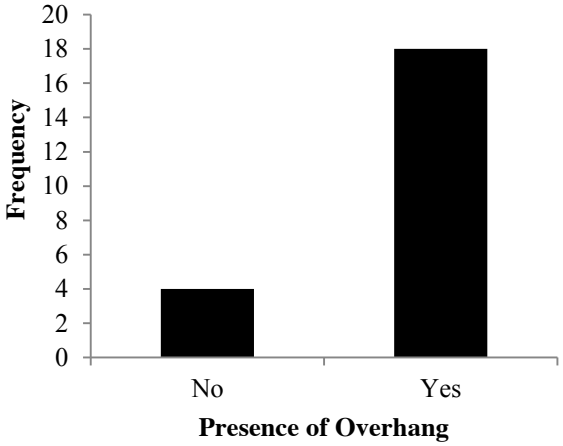

Fig 6. Frequency of the presence of overhang within a 1 meter radius from the center of schooling *H. chrysargyreum* (n=22)

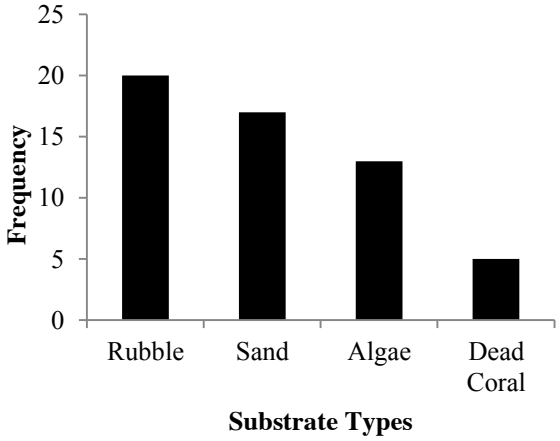

Fig 7. Frequency of different types of substrate of the schooling *H. chrysargyreum* (n=22)

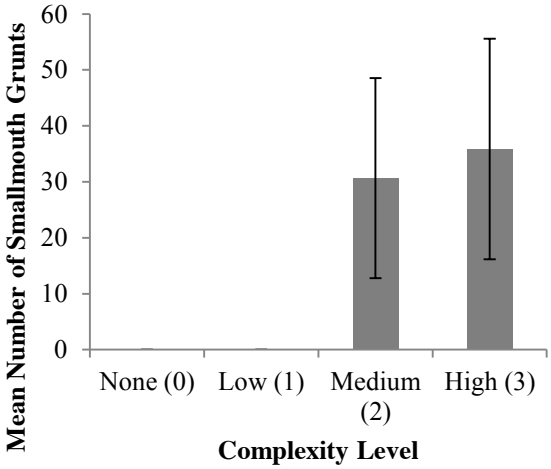

Fig 8. The mean number of *H. chrysargyreum* (± SD) found at the four different levels of complexity

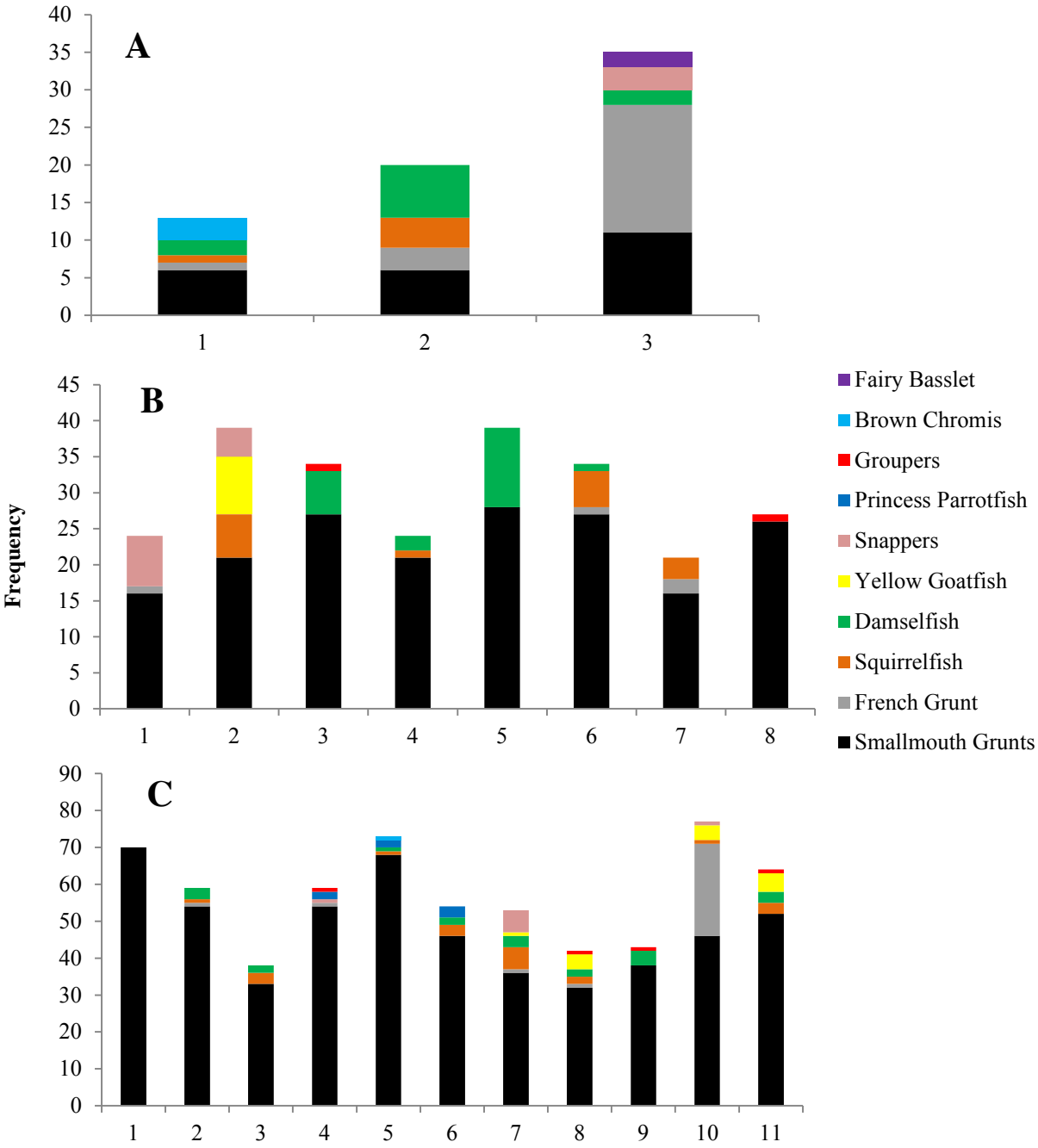

Fig 9. Frequency of different species of fish found within small (A), medium (B), and large (C) schools of *H. chrysargyreum* (n=22). Groupers: red hind and graysby, Snappers: mahogany and schoolmaster, Damselfish: yellowtail and bicolor, Squirrelfish: blackbar soldierfish and longjaw squirrelfish

Nutrient levels

Out of the 22 schools, 14 samples were tested for the concentrations (ug*L^{-1}) of phosphate (PO_4), nitrate (NO_3), and ammonia (NH_4). An extra three samples were tested for the concentration of phosphate. Regardless of presence or abundance of *H. chrysargyreum*, almost all areas (13 out of 14) had some levels of nitrate. The school of 21 individuals had the highest reading of 0.042 ug*L^{-1} while the largest school of 70 had a reading of 0.013 ug*L^{-1}. There was no significant association between school size and the concentration of nitrate (Linear Regression; n=14; $R^2 = 0.0529$).

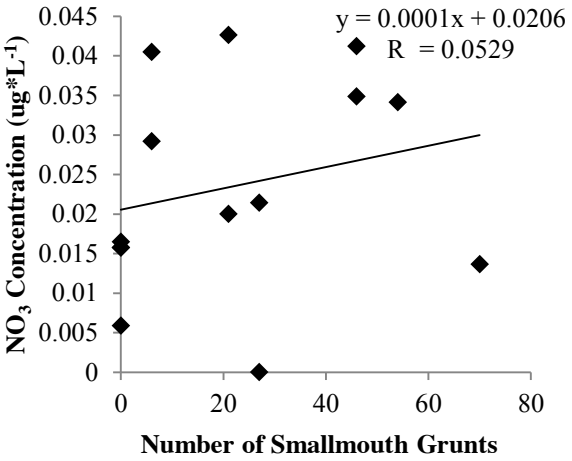

Fig 10. Association between size of school (number of *H. chrysargyreum*) and the concentration (ug*L^{-1}) of nitrate (NO_3) in the area where they were congregating

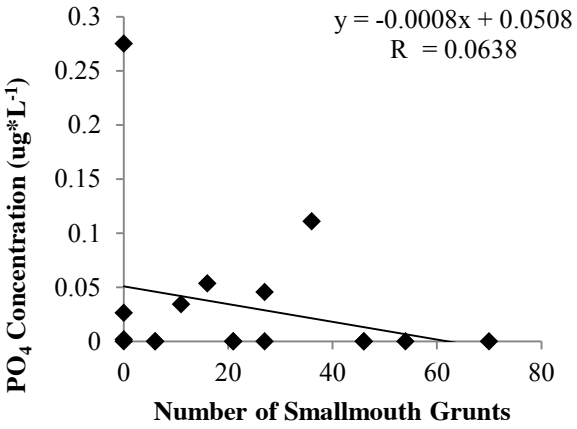

Fig 11. Association between size of school (number of *H. chrysargyreum*) and the concentration (ug*L^{-1}) of phosphate (PO_4) in the area where they were congregating

The results for the phosphate readings reported no significant correlation between school size and levels of PO_4 (Linear Regression; n = 17; R^2 = 0.0638). An area of no abundance had the highest reading of 0.27 ug*L^{-1}. 9 out of the 13 schools with *H. chrysargyreum*, including the largest school, had no readable concentrations of phosphate.

The results for ammonia levels also had no significant correlation with school size (Linear Regression; n = 14; R^2 = 0.0139). A school of 46 individuals had the highest reading of NH_4 concentration of 190.84 ug*L^{-1}. Out of the 10 schools with *H. chrysargyreum*, six of them including the largest had no readable concentration of ammonia.

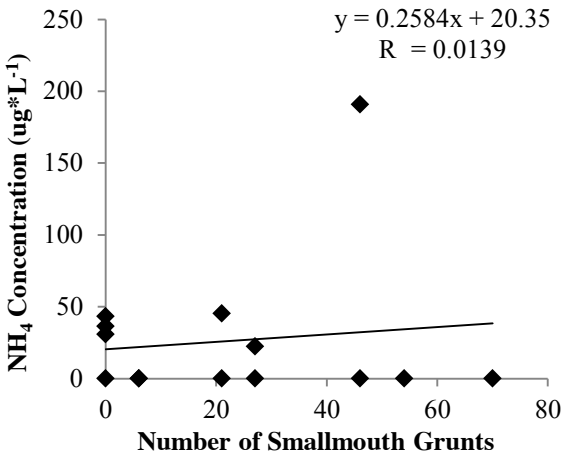

Fig 12. Association between size of school (number of *H. chrysargyreum*) and the concentration (ug*L^{-1}) of ammonia (NH_4) in the area where they were congregating

Discussion

All schools of *H. chrysargyreum* were found congregating in areas of medium to high complexity. The first hypothesis of this study, which stated that coral species and structures with medium to high complexity will have more and larger schools of *H. chrysargyreum*, is partially supported by this result. This preference for habitats with complex structure can be explained by the reduced risk of predation associated with the increased cover. Survivorship of reef fishes is strongly affected by the availability of a preferred microhabitat and substrate, ideally one that provides ample shelter from larger predators (Beukers and Jones 1997). Besides taking cover in the complex habitat of the coral structure, *H. chrysargyreum* also found protection by shoaling in large numbers, further supporting the first hypothesis of this study. All schools observed had an average of 33 individuals, with the largest containing 70 grunts. Fish that actively shoal in large groups decrease their risk of predation through increased surveillance (Goulart and Young 2013) and rapid transmission of information about predator position from one individual to another (Herbert-Read et al. 2011). No solitary *H.*

chrysargyreum were observed, indicating that they prefer to assemble in groups.

Additionally, schools preferred to congregate around areas with an overhanging structure. This result further supports the first hypothesis in regards to a preference for more complex habitats. Besides the added cover provided by overhang, it has also been found to reduce the effects of high water temperature and the extent of daily fluctuating water temperature (Orpwood et al. 2010). With average global temperature expected to rise between 1.4^{o}C and 5.8^{o}C over the course of the century (Nguyen et al. 2011), marine species will need to find ways to tolerate this warmer environment. With more than 80% of the schools found near overhang, this could be their method of coping with increased water temperature. Future studies can observe whether this is, or is becoming, a significant factor in the habitat preference of *H. chrysargyreum*.

All areas where the schools were observed had a combination of various coral species. The three most common species recorded were *U. agaricites*, *O. annularis*, and *M. cavernosa*. All three are some of the most abundant species found in Bonaire, with *U. agaricites* having the highest frequency of occurrence (Steneck et al. 2011). Thus, it cannot be said for sure whether or not *H. chrysargyreum* prefer these corals for habitation. However, the massive coral *O. annularis* provides ample lateral and overhead cover due to its vertical growth. This partially supports the second hypothesis of this study which stated that *H. chrysargyreum* will have a preference for large, vertically-growing species of coral. Many of the areas with schools had most of its cover and structure provided by multiple stalks of *O. annularis*. But with global sea temperatures on the rise, many of the coral species observed are prone to various detrimental conditions induced by warm waters. A vast majority of the corals recorded were infected with some form of ailment such as bleaching or black band disease. This could possibly lead to shifts in coral-community composition, with areas becoming more dominated by thermally tolerant species (Hoegh-Guldberg et al. 2007). Thus, it would be interesting to see if there is also shift in coral species used by schools of *H. chrysargyreum* over time. If so, habitat preference may be more accurately measured by observing if the fish congregate around coral species that are similar in shape or structure as the other coral species prior to the community shift.

Further, all areas observed with schooling *H. chrysargyreum* had at least three different species of coral. This makes sense since most areas along the reef slope of Bonaire contain a diverse array of different corals. However, if the current trend of rising average sea temperature continues along with ocean acidification, then 60% of the coral reefs are expected to be lost by 2030 (Hughes et al. 2003). If this is the case, then groups of *H. chrysargyreum* will associate with fewer coral species, possibly compromising their refuge from predators. Thus, the disappearance of complex structure provided by corals may force *H. chrysargyreum* to shoal in larger groups to outweigh the cost of less cover from the coral reef. Though the results indicated, on average, larger schools were found in more complex sites, these habitats are being heavily reduced by various environmental factors, driving reef fish to seek protection elsewhere.

The substrate of the areas where schools were congregating was also a mixture of different types. Substrate is commonly an indicator of the foraging behavior and preferred prey items of predators. Though *H. chrysargyreum* are known to migrate to seagrass and mangroves to feed during the night, they have also been observed foraging for food during the daytime (Pereira and Ferreira 2013). Thus, when they are shoaling during the day on the reef slope, *H. chrysargyreum* could be feeding on microbenthic vertebrates found in the substrate. Since rubble and sand were the most frequent substrate types recorded, organisms found within the sediment could be potential prey for schooling *H. chrysargyreum*. In fact, a study found that a vast majority of the foraging activity of *H. chrysargyreum* was in soft

substrata such as sand and algae (Pereira and Ferreira 2013).

Within the groups of *H. chrysargyreum*, other species of fish were also found schooling with the grunts. The most common fishes other than *H. chrysargyreum* were *Haemulon flavolineatum* (French grunt), *Pomacentridae* (yellowtail damselfish, bicolor damselfish), and *Holocentridae* (blackbar soldierfish, longjaw squirrelfish). Taking refuge from predators in a large school is most likely the main reason why other species, particularly those that are commonly preyed on, are drawn to shoals of *H. chrysargyreum*. The high frequency of *Holocentridae* was an interesting observation because they were mostly seen hiding in the shaded cover of an overhang. This further supports the hypothesized preference of *H. chrysargyreum* for an overhead structure, which is often occupied by these nocturnal species. These two species may share a preference for overhang, therefore explaining why they are often found together in an area. Many of the schools also included *H. flavolineatum*, a close relative of the *H. chrysargyreum*, as well as *Mulloidichthys martinicus* (yellow goatfish). This mixed shoaling of species with similar shape, size, and color is a phenomenon known as social mimicry which has been known to visually confuse predators (Krajewski et al. 2004). Thus, groups of *H. chrysargyreum* provide an important source of refuge for small reef fish from predation. With more individuals in the school, the *H. chrysargyreum* also benefit from the increased surveillance for potential predators.

Nutrient levels in areas of schooling *H. chrysargyreum* showed no significant differences between sites of no to high abundance. However, analysis on nutrients was a snapshot study limited by a small sample size. Regardless, the third and final hypothesis is rejected. Past studies have found that certain marine species do provide a substantial input of nutrients into the aqueous environment through excretion. Certain zooplanktivorous damselfish produce large quantities of metabolic waste such as dissolved ammonia and particles of organic matter (Roopin et al. 2008). This input of natural waste into the coral reef ecosystem provides vital nutrients for various marine organisms. Reef fish such as *H. chrysargyreum* could be a possible source of these nutrients due to their daily migrations to seagrass beds and mangroves to feed (Pereira and Ferreira 2013). These ecosystems receive substantial terrestrial runoff, accumulating large volumes of nutrients such as nitrogen and phosphorus (Chen and Twilley 1999). Since other studies have observed *H. chrysargyreum* migrating to these areas to feed on microbenthic invertebrates, it is possible that they could transport nutrients from mangroves and seagrass beds to the coral reefs through excretion of organisms they consume. Further, since coral reefs are oligotrophic, a natural supply of nutrients provided by the excretion of *H. chrysargyreum* will be beneficial to the overall ecosystem. In fact, certain symbiotic relationships between sea anemones and damselfish are facilitated by metabolic byproducts excreted by the damselfish to promote growth in the anemone (Roopin et al. 2008). Further studies can be conducted on nutrient content from *H. chrysargyreum* excretions and how it is utilized by the coral reef ecosystem.

Overall, the results of this study demonstrate that schools of *H. chrysargyreum* do prefer to congregate in areas of medium to high complexity with a soft substrate and an overhanging structure. These schools are also relied on by other species of fish for refuge from predators and could possibly provide a supply of nutrients for the coral reef ecosystem. Thus, this species of grunt is very important to the overall health and balance of Bonaire's coral reefs. Proper management and restoration of the marine ecosystem of the island will be crucial in maintaining the population of *H. chrysargyreum* as well as other species that shoal with them. Future studies can further expand on these results by analyzing the habitat preference and nutrient input of other schooling fish seen in Bonaire such as *Lutjanus mahogoni* (mahogany snapper) or *H. flavolineatum*. Results from these studies could

extend our knowledge of nutrient input of shoaling species as well as improve management efforts by better identifying coral species and structures heavily relied on by reef fish.

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REPORT

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The impact of competition, predation and disease on fluorescence patterns of RFP and GFP across the surface of scleractinian corals

Abstract Globally, scleractinian coral populations are declining, and to fully understand this decline it is important to study potential coral stressors *in-situ*. One particularly interesting means of studying stressor effects is fluorescence in corals. Till now fluorescence research has focused primarily on laboratory studies. These experiments cannot fully account for real world effects of stressors such as disease, predation or competition on corals fluorescent patterns in nature. The purpose of this study was to develop a means of *in-situ* observation to study how coral are using fluorescent proteins in nature. Five sample organisms were used for each of the three categories of stress, and one group of healthy corals were used as control, UV photographs of each were then taken on a weekly basis. Visual trends across the photographs were analyzed for gradients in both red and green fluorescence using Photoshop. From this we detected patterns on predated and competing corals as well as significant gradients in both diseased and healthy corals. Healthy coral results indicated issues in light dispersal across coral colonies necessitating a reworking of the methodology for clearer results. However the presence of discernable trend lines across all other categories supports that this methodology could still be effective for future monitoring efforts. RFP and GFP associated proteins are good candidates for indicating the health of threatened coral reefs due to their ease of use and associations with important coral functions making the methodology discussed here significant in allowing their use.

Keywords Coral Stressors • UV protection • reactive oxygen species

Introduction

Scleractinian (stony/hard) corals are foundational species on coral reefs as they provide complex structures that in turn provide essential habitats and other necessary resources to dependent organisms (Rosenberg et al. 2007, Angelo et al. 2008). However, these habitats are increasingly being threatened with approximately 30% of the worlds' coral reefs currently seriously degraded (Munday 2004). Coral reefs are a relatively fragile ecosystem that rely heavily upon the health of hermatypic, or reef building corals, to exist. Owing to the inherently minimal levels of nutrients available in oligotrophic waters these corals have grown to rely heavily on a light-dependent symbiosis with the unicellular dinoflagellate, Symbiodinium microadriaticum, (zooxanthellae) (Angelo et al. 2008).

This symbiotic relationship is beneficial for each partner as the zooxanthellae provides oxygen and sugars through photosynthesis for the coral while the polyp gives the algae carbon dioxide as well as protection (Rosenberg et al. 2007). The relationship can also prove detrimental to corals however as when zooxanthellae are exposed to high temperature, disease and or direct attacks they may begin to produce Reactive Oxygen Species (ROS) which are toxic to the corals (Brown 1997; Coles and Brown 2003; Lesser et al. 2006; Palmer et al. 2009; Weis 2008). In this case the coral will sometimes forcibly expel the

zooxanthellae in a process known as bleaching (Brown 1997). Large scale mortality due to the loss of photosynthetically produced energy after bleaching events is becoming more frequent on modern reefs and contributes significantly to their decline (Hoegh-Guldberg and Smith 1989).

Coral species have developed ways to acclimate to situations which would normally increase ROS concentrations. Adaptations for ROS control include controlling the type and or number of algal cells and their pigment content, as well as adjusting the complement of Ultra Violet (UV) screening, and antioxidant molecules (Falkowski and Dubinsky 1981, Richier et al. 2005). In addition, to these regulatory methods some coral species are also able to collect the necessary antioxidants from their food sources as well as their symbionts (Franck et al. 2002). These processes can be overwhelmed in cases where disease, heat or other stressor have disturbed the symbiotic relationship of the coral (Weis 2008). Therefore, investigation into recovery of corals in response to stress is vital in understanding their potential for successful recovery in the increasingly stressful environments they inhabit.

Recent studies have shown varying relationships between differently pigmented proteins and their functional roles in coral health and recoverability. Within this study we will be focusing on the role of red and green fluorescent proteins (RFP and GFP) role in coral response to stress based upon their position within the coral. Current research into this subject has shown RFP to be the primary protein associated with chlorophyll as well as being indicative of H_2O_2 scavengers which help limit ROS's in corals (Franck et al. 2002; Palmer et al. 2009). Green fluorescent proteins have also been linked to photoprotective and more specifically UV light protection within corals (Dove et al. 2001; Angelo et al. 2008). The high expression levels of RFP and GFP-like proteins in coral tissues and their advantageous photophysical properties allow these pigments to be used as potential indicators of the health of threatened coral reefs (Franck et al. 2002; Oswald et al. 2007).

Both RFP and GFP fluorescence can be measured by UV light to induce fluorescence (Franck et al. 2002). Past research has focused primarily on RFP analysis within a laboratory setting. This lack of experimentation is presumably due to difficulties in developing and utilizing a sufficient photography setup to adequately capture RFP and GFP. This study aims to develop a field research method using UV light at night to photograph the fluorescence of corals. This research will help to determine if RFP are relocated within corals in response to different stressors.

Here we will focus upon yellow band disease (YBD) due to its direct effects on zooxanthellae and relatively quick rate of infection. Yellow band disease primarily affects *Orbicella annularis* and is characterized by a pale yellowish ring surrounding an area of deceased coral (Cervino et al. 2001). This disease spreads relatively quickly at approximately 0.6 cm $month^{-1}$ (Cervino et al. 2001). Yellow band disease is of specific interest because the bacteria involved directly attack the zooxanthellae within the coral's gastrodermal tissues (Cervino et al. 2008). Attacks cause the organelles to be compromised with degraded photosynthetic pigments both *in vitro* and *in situ* (Cervino et al. 2008). Degradation of the photosynthetic pigments weakens the coral polyp by limiting production of nutrients from zooxanthellae. An effect like this raises the question of how corals might defend against such a direct attacks against their zooxanthellae. This study hypothesizes that RFP, which depicts the chlorophyll within the coral polyp, will be located further from the area of demarcation as these organelles are shifted away in order to protect them from disease.

Disease is not alone in directly attacking sections of a coral head. Competition for space often causes corals to directly push against one another in an attempt to take over the others position. These competitive corals enter into an energetically costly fight in which each coral gains or loses ground in increments of only a

few millimeters from each attack (Chornesky 1989). These movements involve relatively taxing processes such as the use of digestive filaments or production of fighting tentacles (Chornesky 1983). These energetically demanding resistance and attack methods raise the question of how the organism is providing the excess energy needed to fight. If zooxanthellae are providing energy to corals then RFP will be increased near these competitive edges to help with fending of competing corals.

Another factor affecting corals that will be tested in this study is predation by herbivorous fish on corals. Direct attacks on the coral can weaken it which potentially limits its ability to cope with elevated levels of ROS. This weakened state may be partially offset by the presence of RFP within the remaining chlorophyll in the area. This idea is depicted by the non-normal and often pinkish color exhibited by the damaged tissue in corals affected by predation (Palmer et al. 2009). This study aims to determine whether or not corals *in-situ* localize RFP to areas of damage which would support the idea that this is an active stress response of the organism.

Overall this study will aide in filling in the current lack of field methods for observing RFP *in-situ* so that we may better determine how a coral responds to stressors. This information can then be used to determine the significance of RFP (i.e. chlorophyll) to coral health and more specifically with disease, competition and predation.

- H_1: Areas directly on the line of competition will have higher concentrations of RFP (i.e. chlorophyll) with concentrations decreasing across medial and into distal coral locations.
- H_2: Predated upon corals will display increased concentrations of RFP (i.e. chlorophyll) on the area of demarcation with a decrease in concentrations moving outward across medial and into distal coral areas.
- H_3: Diseased corals will exhibit lower concentrations of RFP nearer to the line of disease with increasing concentrations moving out across medial and into distal coral locations.

Materials and methods

Study Site

All research associated with this study occurred on the Caribbean island of Bonaire specifically on the fore reef near the Something Special dive site (12° 9' 34.652" N 68° 16' 56.236" W)(Fig. 1). This site was chosen because the coral colonies exhibit; yellow band disease, predation, and competition that provide the types of coral colonies needed for this study on RFP intensity. Corals were selected and marked with specific colors of flagging tape that indicated colonies with predation, YBD or competitive edges that would be used in this study during preliminary dives.

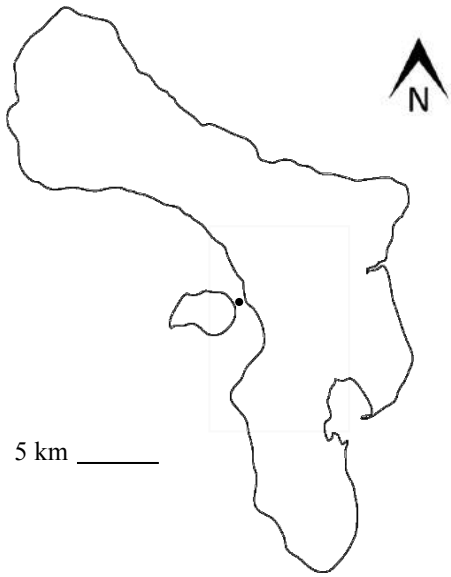

Fig. 1 Map of Bonaire, Dutch Caribbean. The *black dot* indicates the study that is located to the south of Something Special (12°09'36.3"N, 68°16'54.9"W)

Disease corals were selected only if diseased tissue covered less than 50% of the surface to ensure sufficient amounts of live tissue were available for analysis. In regard to competitive responses corals in direct contact with the opposing organism were marked, to

increase the likelihood that competition was occurring. To assess predation, corals that were facing upwards and relatively isolated were chosen so they could be easily photographed.

After corals were marked, the colonies were photographed during the daytime and again at night using UV light photography. The daylight photos were juxtaposed with the florescence photographs to determine areas of focus on the colonies to measure RFP intensity.

Data collection

Data collection for this experimentation occurred over a five week period (5th of March 2016 to the 2nd of April 2016). Research dives occurred at night from approximately 20:00 hrs to 21:00 hrs, during which time photographs were taken of the corals using UV flashlights. Photos were taken from uniform distances through the use of direct attachment of both camera and UV lights to an underwater camera mount approximately 15cm by 15cm in base diameter and approximately 25 cm in height. Diffusers were used to equilibrate the lights to ensure only UV light energy reached the coral surface.

Data analysis

Prior to actual analysis, photos were taken and cropped to include only coral tissues within a uniform box area (Fig. 2). This area was then divided into parts to allow sampling from three distinct coral areas; on demarcation, medial and distal (Fig. 2). Finally, randomized points were overlaid on to the uniform box areas. Points that were completely in the shadows or completely overexposed (determined by placement of pixels over Photoshop CC generated histogram) were omitted (Fig. 2). These randomized points were then analyzed using the histogram functionality of Photoshop CC (version 2015.1.2) to determine the relative intensity (mean number of each color pixel in each area) of RFP as you move further from the line of disease, competition or predation. The intensities for GFP were analyzed in the same manner as RFP. The mean number of pixels in each category were averaged together and the standard deviation was calculated for each. T-tests were also run to determine any difference in pixel counts from demarcation to distal locations. T-tests for demarcation to medial and medial to distal locations were only performed if demarcation to distal T-test results were positive.

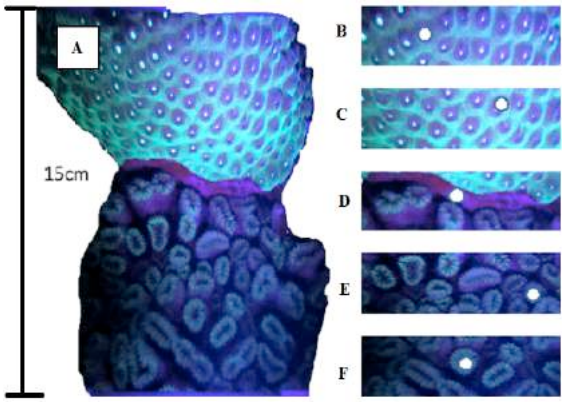

Fig. 2 Displays the process of finding sample locations for image processing in Photoshop 2015.1.2 to determine amount of red and green pixels in fluorescent images. Picture A represents the total area used as background fluorescence of the coral colonies. Samples for red and green fluorescence patterns were randomly selected from five sections of the image as demonstrated visually on the right side of the figure. Distal locations were sampled from sections B and F, Medial locations were sampled from sections C and E and the Line of Demarcation was sampled from section D. *Smaller white dots* within image sections represent randomly selected areas for fluorescence analysis

Results

Procedural competency

The underwater camera and UV flashlight set-up proved to be a viable field method for procuring images of sufficient resolution for subsequent red and green fluorescence analysis. Analysis protocols utilizing Photoshop's histogram functionality were successful in measuring the different levels and types of light across varying coral colonies. These protocols were also notable in their ability to adequately show both red and green fluorescence across coral colony surfaces (Fig. 3).

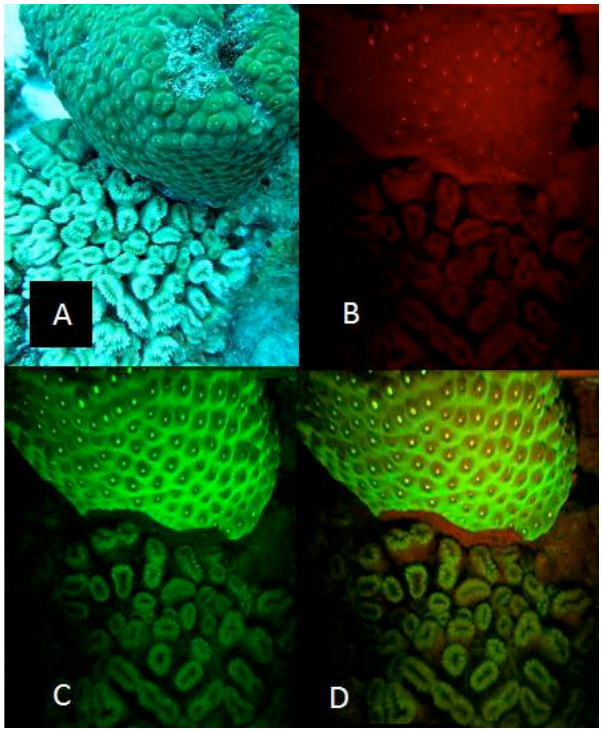

Fig. 3 Photographs (15 by 13cm area) of coral competition illustrating how the coral colonies appear in daylight (A), a night-time UV photograph analyzed in Photoshop to display red fluorescent pixels only (B), a night-time UV photograph analyzed in Photoshop to display green pixels only (C), and a night-time UV photograph analyzed in Photoshop to display green and red pixels (D)

Visual trends across coral colonies

Coral colonies involved in competition depicted clear lines of red fluorescence along the line of demarcation (Fig. 3). Yellow Band Diseased (YBD) corals displayed gradients of red to green tissue moving progressively away from the line of demarcation (Fig. 4). The YBD coral polyps also appeared to be retracting their tentacles in such a way as their oral plates were obscured from view. Predated upon colonies expressed slightly higher levels of red fluorescence on the area of predation with less noticeable radiation outwards than on Yellow Band Diseased Corals (Fig. 5).

Competition: patterns of fluorescence

The mean number of red pixels decreased from the line of demarcation (108.9 ± 28.8) to medial (105.1 ± 22.8) and distal (87.0 ± 17.7) locations (Fig. 6). There was no significant decrease in red pixels from demarcation to distal coral locations ($T_{5,5}$, p = 0.26, n = 5)(Table. 1) . The mean number of green pixels increased from demarcation (76.094 ± 46.022) to medial (88.991 ± 27.293) coral locations and decreased toward distal (74.056 ± 22.781) coral locations (Fig. 6). Mean values for green pixels showed no significant decrease across demarcation to far coral locations ($T_{5,5}$, p = 0.33, n = 5) (Table. 1).

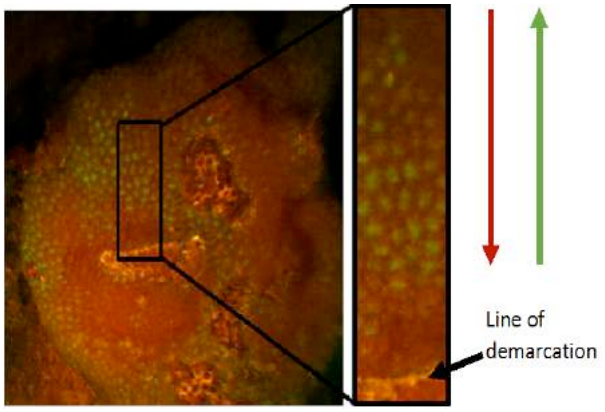

Fig. 4 Image shows fluorescing *Orbiecela annularis* coral colony with Yellow Band Disease. The photograph was taken at night on the reef using a UV flashlight and underwater digital camera. This image displays red and green pixels following image processing in Photoshop 2015.1.2. *Cut away box* highlights the visual gradient of increasing red fluorescence (*red arrow*) closer to the Line of Demarcation and increasing green fluorescence (*green arrow*) with increasing distance from the Line of Demarcation

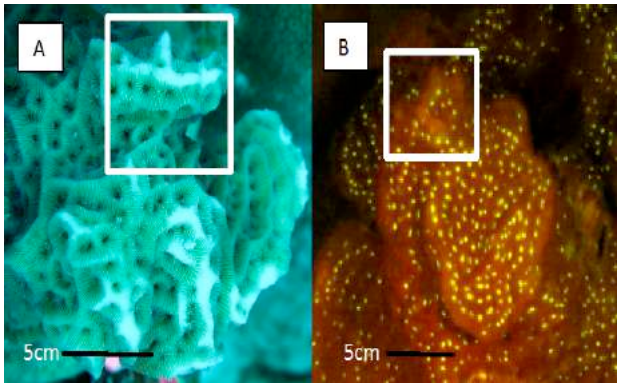

Fig. 5 Photographs depict predated coral in the daylight (A) compared with the image using UV photography with post-image processing in Photoshop 2015.1.2 to analyze red and green fluorescent channels (B). *White boxes* depict the incidence of predation on the coral.

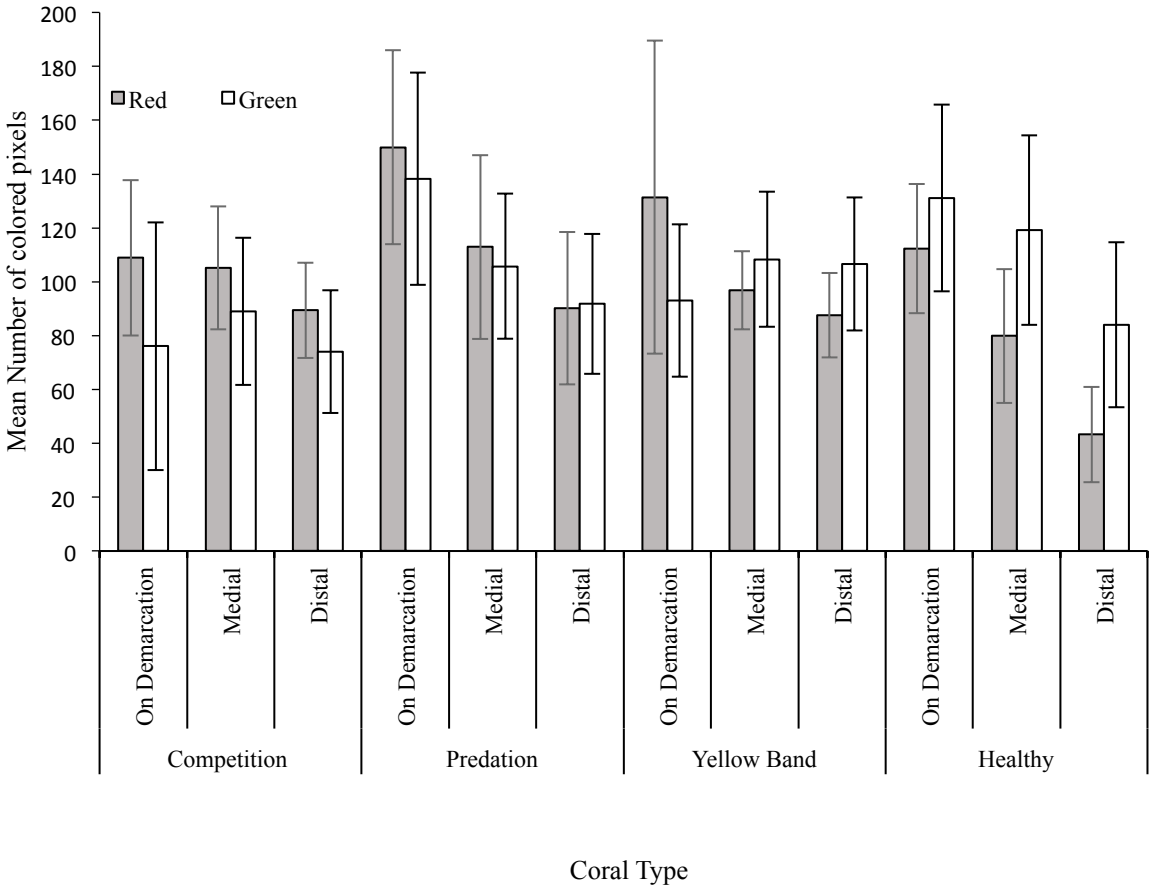

Fig. 6 Mean number of pixels displayed for both red and green channels within sample points across; overall image, on demarcation, medial and distal coral locations (n = 5 for Competition, Yellow Band and Healthy corals; n = 4 for Predation)

Predation: patterns of fluorescence

The mean number of red pixels progressively decreased from the point of demarcation (149.9 ± 36.1) to the medial (112.9 ± 34.1) coral areas into the distal (90.2 ± 28.3) edges of the coral (Fig. 6). Mean values for red pixels showed no significant decrease across demarcation to far coral locations ($T_{4,4}$, p = 0.15, n = 4) (Table. 1). The mean number of green pixels progressively decreased from demarcation (138.3 ± 39.5) to medial (104.7 ± 26.9) to distal (91.8 ± 25.9) coral locations (Fig. 6). Mean values for green pixels showed no significant decrease across demarcation to far coral locations ($T_{4,4}$, p = 0.09, n = 4) (Table. 1).

Yellow band disease: patterns of fluorescence

The mean number of red pixels decreased from the line of demarcation (131.4 ± 58.2) into medial (96.9 ± 14.5) and finally into distal (87.6 ± 15.7) coral locations (Fig. 6). Mean values for red pixels showed statistically significant decrease across demarcation into far ($T_{5,5}$, p = 0.0003, n = 5) and demarcation to medial ($T_{5,5}$, p = 0.0079, n = 5) coral locations(Table. 1). The mean number of green pixels increases from demarcation (92.9 ± 28.3) to medial (108.4 ± 25.1) and then decreases again into distal (106.6 ± 24.7) coral locations (Fig. 6). Mean values for green pixels showed no statistically significant decrease or increase from demarcation to distal coral locations ($T_{5,5}$, p = 0.24, n = 5) (Table. 1).

Healthy corals: patterns of fluorescence

The mean number of red pixels progressively decreased from center (112.3 ± 23.9) to medial (79.9 ± 24.9) to distal (43.2 ± 17.7) coral locations (Fig. 6). Mean values for red pixels

Table 1. Depicts the results of t-test to compare mean red fluorescence and mean green fluorescence across the coral surfaces. T. test were only run for demarcation to medial and medial to distal coral locations if a significant value was found in either red or green fluorescence from demarcation to distal locations (n = 5 for all but predation corals where n = 4)

	Competition		**Predation**		**Diseased**		**Healthy**	
	Red p-value	Green p-value	Red p-value	Green p-value	Red p-value	Green p-value	Red p-value	Green p-value
LoD - Distal	0.267	0.333	0.145	0.087	* 0.000	0.241	* 0.005	0.071
LoD - Medial	-	-	-	-	* 0.008	0.289	* 0.026	0.357
Medial- Distal	-	-	-	-	0.105	0.918	* 0.002	* 0.039

* used to indicate that this value is significant, LoD: Line of Demarcation, - data not found due to lack of significance in preliminary test

showed statistically significant decreases fordemarcation to far ($T_{5,5,}$ p = 0.005, n = 5), center to medial ($T_{5,5,}$ p = 0.025, n = 5) and medial to distal coral locations ($T_{5,5,}$ p = 0.0021, n = 5) (Table. 1). The mean number of green pixels progressively decreased from demarcation (131.1 ± 34.7) to medial (119.2 ± 35.3) to distal (83.9 ± 30.6) coral locations (Fig. 6). Mean values for green pixels showed significant decrease only between medial and distal segments ($T_{5,5,}$ p = 0.04, n = 5) and none between center and distal or medial and distal coral locations (Table. 1).

Discussion

The first major aim of this study was to determine how to study RFP and GFP distributions *in-situ* using digital photography and UV light to induce fluorescence of living coral colonies at night. Previous research conducted by Franck et al. (2002) measured RFP in corals within a controlled laboratory setting. This research, while useful in determining what could potentially occur in corals, was inherently limited in that it did not account for the full range of factors impacting life on coral reefs. Without these variables being taken into account laboratory research is largely unable to be extrapolated to natural ecosystems. This is why experimental methodologies, such as the one performed in this paper, are useful in understanding laboratory findings in natural ecosystems. This study showed that filtered UV lights can be used to capture high quality photos for analysis of GFP and RFP gradients. The experimental methodology in this research was shown to be successful in gathering fluorescence data for corals exhibiting a variety of stressors such as disease predation and competition.

Another aim of this study was to determine if there were patterns in fluorescence across corals experiencing competition, predation and yellow band disease. Patterns in RFP were analyzed beginning with corals experiencing competition. While no statistically significant gradients were found for RFP, observations of competing coral images depict high concentrations of RFP along the line of competition indicating an area of high energy production for the coral. Therefore a line of red fluorescence directly along the line of demarcation would support the first hypothesis which stated that GFP would be concentrated toward the line of competition. Green Fluorescent Protein levels measured across competing coral heads were shown to have the highest concentrations in the medial areas of the coral with nearly identical amounts for both the area of demarcation as well as distal coral locations. This sort of pattern is likely due to shadowing toward the edges of the coral colonies which was particularly prominent in this group due to the proclivity for odd angles within the coral subjects making clear photography difficult. According to Dove et al. (2001) GFP associated proteins are likely responsible for protecting the photosynthetic

machinery of the dinoflagellates. Therefore, low levels of GFP in the area of demarcation indicate deficiencies for these competing polyps in regard to light protection which would leave them susceptible to ROS overload. This negative effect could be negated by the antioxidant properties found in relation with RFP's giving another possible explanation for their abundance.

Corals displaying Predation were analyzed for RFP gradients for which there were no significant gradients however visual inspection of photographs indicated red fluorescence concentrated on the area of demarcation. Visual concentration gradients as well as general numerical trends indicate that areas actively being predated upon require more RFP (i.e. chlorophyll) than healthier portions. This goes along with the second hypothesis which states that predated upon corals will display increased concentrations of RFP along the area of demarcation. The general trend for GFP on predated upon corals showed a consistent decrease moving away from the line of demarcation. As GFP is associated with light protection for corals a lack may be detrimental in terms of polyp health or may simply indicate a lesser reliance of photosynthesis in healthier coral segments. However further experimentation which includes a higher sample size may serve to solidify this correlation.

Yellow Band Disease displayed trends in RFP which indicated a statistically significant decrease from the area of demarcation into distal coral locations as well as between demarcation and medial coral locations. High levels of RFP right along the area of demarcation indicate a higher energy demand as well as a need for antioxidants. This is necessary to combat the ROS that will inevitably appear with a lack of GFP related UV protection and increased photosynthesis from concentrated chlorophyll. In general this trend does not support the proposed hypothesis that RFP would be in higher concentrations away from the line of demarcation. Instead results seem to indicate an opposite response which is significant as it indicates a higher importance is placed on transfer of energy to the areas surrounding the demarcation than avoiding the potentially negative impacts it may have on nearby organelles. Visual inspections of Yellow Band disease corals also indicated polyps near to the area of demarcation have receded tentacles which block the oral plate. This morphological difference is significant as it indicates these polyps are likely not feeding properly. This deficiency would therefore require the polyp to secure more energy from other sources such as chlorophyll, which would explain the high concentration of RFP in this area.

Apart from these stressed coral subjects healthy corals were utilized as a control for light intensity across the coral head from the UV light source. In theory all areas of the healthy corals surface should have had uniform amounts of red and green florescence. However, there was a statistically significant difference from the center mark to distal coral locations indicating that light intensity was higher toward the center of coral images. From this we conclude that the results of healthy coral colonies were higher closest to the center of the image and need to be taken under consideration. However, distinct differences in RFP amounts and the variability in GFP trends (increasing at times from the center as opposed to the consistent decrease seen in healthy corals) across stressed coral surfaces as compared to healthy coral results indicate there are factors beyond light intensity that are at play. This means that should light intensity be made more standard the average number of pixels of each color may be slightly smaller in central areas but general trends would likely still persist. Therefore, it is assumed that not all results were due to this technical error. In order to prevent such pinpointed light it may be necessary to further diffuse the UV flashlights to spread out their beams more evenly over the coral head.

These results indicate that the methodology does result in meaningful estimates or relative concentrations of red and green fluorescence. They also indicate a general trend of RFP being concentrated toward or on the area of

demarcation. This trend may indicate that stressed corals place a higher significance on energy production than light protection. It may also mean that GFP related proteins are being produced in higher concentrations only as you move away from demarcation as these require protection from the potentially harmful stressor. This scenario would thereby necessitate the presence of excess RFP to compensate for ROS increases resulting from decreased UV protection. However, the presence of any kind of gradient be it significant or only visual represents the potential this methodology could have for monitoring in the future.

With adequate refinement this sort of methodology would allow scientist to better utilize fluorescence as an ecological monitoring tool. As GFP and RFP are already utilized so widely in other genetic and biological laboratory settings they are desirable candidates for coral monitoring due to familiarity and therefore ease of access. Work in this vein would allow for many new ways to monitor coral health both before and after a stress in a quick and relatively easy way. Photographic data could then be collected to view patterns of recovery or decline over time as well as noting differences in coral species in regard to fluorescent patterning. In this regard this papers methodology is a useful first step in giving us another means of monitoring how the corals respond to different stressors in the field.

The high expression levels of RFP and GFP in coral tissue and their advantageous anti-oxidant and UV protective properties also make these pigments promising candidates for indicating the health of threatened coral reefs in a globally declining environment (Oswald et al. 2007). Coral reefs make up approximately 123 million km^2 of tropical oceans and are among the most complex ecosystems in the world however, they are also among the most threatened (Angelo et al. 2008). With an ever increasing threat of climate and pollution related issues bearing down on coral reef habitats it is becoming more and more important to develop better ways to monitor coral health. Observations in laboratory settings are useful in understanding the general mechanics of what a coral could do in response to stressors. However, field observation and monitoring tools offer better overall understanding of corals in nature. It is only through gaining an understanding of how corals behave in their changing environment that we can determine how we might best help preserve them.

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REPORT

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Abundance and diversity of fluorescent anemone species across reef habitats off the coast of Bonaire, Dutch Caribbean

Abstract Marine anemones influence oceanic food webs, partake in symbiotic relationships with many marine phyla, and can prove detrimental to coral reef ecosystems in excess. Few descriptive studies have been conducted on anemone communities. The present study examined anemone abundance and diversity using fluorescence across reef habitats in a coral reef ecosystem for the first time on Bonaire, Dutch Caribbean. A total of 110 fluorescent anemones belonging to at least 9 species were documented. Anemones exhibited species-specific ranges in one or multiple reef habitats including the reef flat (2-6 m depth), reef crest (6-9 m depth), and reef slope (9-15 m depth). Four possibly unidentified species were documented. Fluorescent anemone abundance varied significantly between reef flat (5.5 ± 0.7 individuals) and reef crest (27.5 ± 6.4 individuals) habitats. Although fluorescent anemone diversity was highest on the reef flat and lowest on the reef slope, there was no significant difference among the reef habitats. The study contributed to current knowledge on fluorescent anemone ecology by documenting species habitat ranges. It suggested that among species and as a whole, anemones are habitat-specific. The results also provided habitat ranges for obligate anemone symbionts. The study may be valuable for a variety of scientific fields. Descriptive studies such as the present project in Bonaire facilitate the possible discovery of new and groundbreaking species and model organisms. Tracking distribution and diversity could also inform of anemone bleaching and serve as a bioindicator of reef health and climate change ramifications.

Keywords Coral reef • anemone distribution • anemone diversity

Introduction

Marine anemone species inhabit broad geographical ranges and ecosystem niches. As passive suspension feeders, anemones play an important role in oceanic food webs, preying upon zooplankton and macroorganisms such as arthropods (Rubenstein and Koehl 1977), mollusks (Sebens 1981), and small fish (Cintiroglou and Koukouras 1992). One study shows that a species of anemone may even prey on jellyfish (Fautin and Fitt 1991). The carnivorous organisms capture most prey with various types of cnidae, which envenomate (nematocysts) or grasp (spirocysts) the target organism (Fautin 2009). In addition to predating on several levels of the food web, marine anemones are preyed upon by carnivorous or omnivorous species of almost all marine phyla, including some herbivores in pursuit of *Symbiodimium* algae harbored within the anemones (Kuguru et al. 2007). By applying pressure to prey species and providing nutrients to predator species, marine anemones influence the movement of energy between oceanic organisms and resources.

Anemones are utilized by other species for their stinging cells (nematocysts) and as specialized habitats. Nudibranchs have been known to ingest anemones and preserve the unfired nematocysts in their dorsal appendages as chemical weaponry (Doepke et al. 2012). An obligate symbiont of anemones is the spotted sea anemone shrimp that seeks shelter among

the cnidae-laden tentacles and may feed on floating detritus caught in the passive filter feeder (Spotte 1996). Some organisms also take part in mutualistic relationships with host anemones. Various hermit crab species attach specific species of anemones to their shells for protection and camouflage, and the anemone gains motility and access to food scraps (Gusmão and Daly 2010). Another example of a mutualistic relationship includes the interaction between an anemonefish and its host *Heteractis magnifica*. The orange anemonefish lays its eggs on the hard substrate beneath the oral disc of the anemone *H. magnifica,* away from predation and water movement, and defends its host from specialized predators such as chaetodontids (Holbrook and Schmitt 2004). *Heteractis magnifica* growth has been shown to triple with symbiont anemonefish protection (Holbrook and Schmitt 2004). These symbiotic relationships range broadly in species involved and manner of involvement.

Much like scleractinian corals, anemones participate in symbiotic relationships with various clades of *Symbiodinium* (Kuguru et al. 2007). The endosymbiotic dinoflagellates provide nutrients to the host in the form of photosynthates and carbon, and contribute to anemone lipid nutrition (Venn et al. 2008). The symbiotic host provides protective housing, and may enhance photosynthesis with fluorescent pigments (Leutenegger et al. 2007). Both the zooxanthellae and host anemone species can produce florescent pigments that, apart from enhancement of dinoflagellate metabolism, may also serve to attract prey and protect the holosymbiont from excess light through the dissipation of energy in radiative and non-radiative pathways (Leutenegger et al. 2007). The fluorescent pigments are composed of molecules belonging to a family of proteins, including green fluorescent proteins or GFPs, that is utilized in laboratory research on biotechnology, gene expression, and protein production within many organisms (Labas et al. 2002).

Elevated numbers of anemones, such as corallimorphs, can be detrimental to coral reef communities, and can serve as a bioindicator of ecosystem health (Work et al. 2008). Anemones belonging to the order Corallimorpharia, commonly known as corallimorphs, exhibit similar morphological traits to Scleractinia (hard corals) and sometimes house symbiotic zooxanthellae (Chen et al. 1996). One study on the Palmyra atoll in the U.S. Line Islands in the central Pacific documented a phase shift from coral to aggressive corallimorph species triggered by a shipwreck in 1991 (Work et al. 2008). After the ship weakened and destroyed colonies of coral within the atoll, *Rhodactis howesii* corallimorphs began to overgrow the damaged reef (Work et al. 2008). The study highlighted the infestation capabilities of *R. howesii* when coupled with human impact that debilitated the coral's ability to thwart the anemone competitors (Work et al. 2008). The corallimorph cover originated at the shipwreck and various buoys, and radiated outward along the reef, indicating one of the many ways that man-made structures can have long-term negative ramifications in marine ecosystems (Work et al. 2008). Because aggressive anemones such as *R. howesii* are capable of inflicting substantial coral mortality in weakened habitats, it is important to monitor their densities in coral reef ecosystems.

Another study conducted in 2012 off the Northern coast of Venezuela and nearby islands surveyed the depth, habitat, and species of anemones across six research sites (González-Muñoz et al. 2016). The study provided updated information on identified anemone species' taxonomy and substrate preference. The present study aimed to record similar data on Bonairean reef anemone species, and to document abundance and diversity in various reef habitats for the first time on Bonaire. Florescence was utilized as an effective method for locating individuals, and therefore only fluorescent anemones were studied. A detailed analysis of anemone species within reef flat, crest, and slope habitats were performed using depth distribution.

Anemones inhabit a range of depths and benthic substrates across multiple reef habitats

(Humann and Deloach 2003). Habitat characteristics such as structural complexity, benthic substrate, and level of light exposure vary with depth across the coral reef ecosystem (personal observation). The reef flat usually exhibits low structural complexity, a sandy benthic layer, and high levels of light exposure (personal observation). The reef slope usually exhibits high structural complexity, a rock and coral benthic layer, and low levels of light exposure (personal observation). The reef crest, located between the flat and slope, exhibits characteristics favorable to anemone species of both neighboring habitats (personal observation). The crest provides a high level of light exposure for anemones hosting endosymbiotic zooxanthellae, and also provides hard substrates with crevices for settlement and protection.

- H_1: Fluorescent anemone abundance will differ significantly between reef flat, reef crest, and reef slope habitats, with the highest abundance occurring on the reef crest
- H_2: Fluorescent anemone diversity will differ significantly between reef flat, reef crest and reef slope habitats, with the highest diversity occurring on the reef crest

The relative abundance among species was evaluated at specific depths and grouped into depth intervals according to habitat. The abundance and diversity of anemones in reef flat, reef crest, and reef slope habitats was analyzed and compared. The results provide data comparable to past studies, and document unique characteristics of the study site anemone community. This data updates the known information on Bonairean anemones, provides an analysis of abundance and diversity across different reef habitats, and deepens the understanding of Anthozoan ecology.

Materials and methods

Study site

The research was conducted in the southern Caribbean Sea off the leeward side of Bonaire, an island of the Dutch Caribbean about 110 km from the coast of Venezuela. The study site, located south of Something Special dive site (12°09'35"N, 68°16'55"W, Fig. 1), begins as a sand flat extending about 40 m from the shore to a fringing reef crest at a depth of ~6 m. The forereef slopes to a depth of ~30 m. The selected reef provides an excellent study site as it includes a variety of reef habitats in a compact area. Recreational fishing and diving are permitted and frequent at the study site, which may affect the density and diversity of fluorescent anemone communities. Human impact at this particular site should be kept in mind if the results of the present study are compared to other sites.

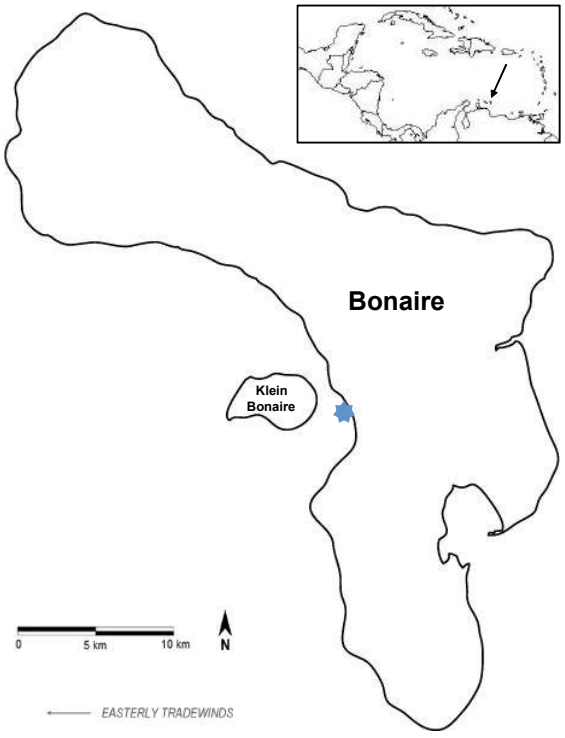


Fig. 1 Map of study site shown with *blue star* located south of Something Special dive site. Position of Bonaire in the Caribbean Sea shown at top right

Photography and surveys

Photographs of fluorescent anemones were taken at night under both blue and white lights between depths of 3 and 14 m. The photos

documented species-specific fluorescence patterns and facilitated identification of cryptic individuals. To record data on target organisms, two randomly selected side-by-side 350 m^2 areas were surveyed (Fig. 2). Dives were conducted at night using Nightsea blue light flashlights to locate fluorescent anemones. Depth and species of the target organisms within the box transects were recorded.

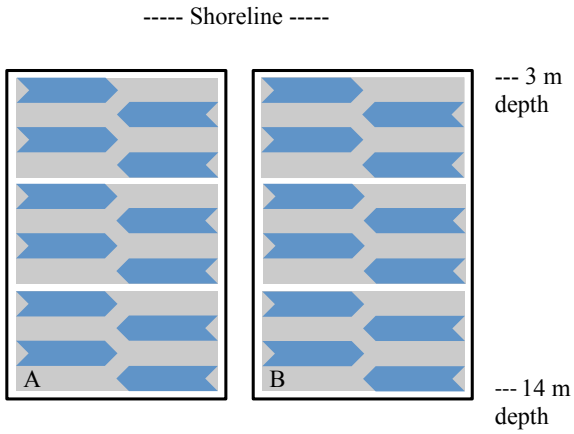


Fig. 2 Diagram of survey area. *Black lines* indicate border of the two 350 m^2 box transects (A, B). *Grey boxes* indicate area surveyed during a single dive using multiple belt transects. *Blue arrows* indicate direction and area of surveyed non-overlapping belt transects

Data analysis

A bar graph was used to show the overall abundance per species. A stacked bar graph of abundance per species between the reef flat (2.1 m - 5.8 m depth range), the reef crest (6.1 m - 9.1 m depth range), and the reef slope (9.4 m - 14.3 m depth range) habitats was used to analyze species range between habitats. A bar graph of fluorescent anemone abundance per reef habitat was used to compare habitat range of fluorescent anemones in all species. A two-tailed t-test was used to analyze the differences in mean anemone abundance between (1) reef flat and crest, (2) reef crest and slope, and (3) reef flat and slope habitats. A bar graph of mean Simpson's diversity indices per reef habitat was used to compare habitat range of fluorescent anemones in all species.

Results

Fluorescent anemone species abundance

Anemone abundance across the entire study was tallied and summed for each species (Fig. 3, Table 1). The most abundant species was *Isarachnanthus nocturnus*, which represented

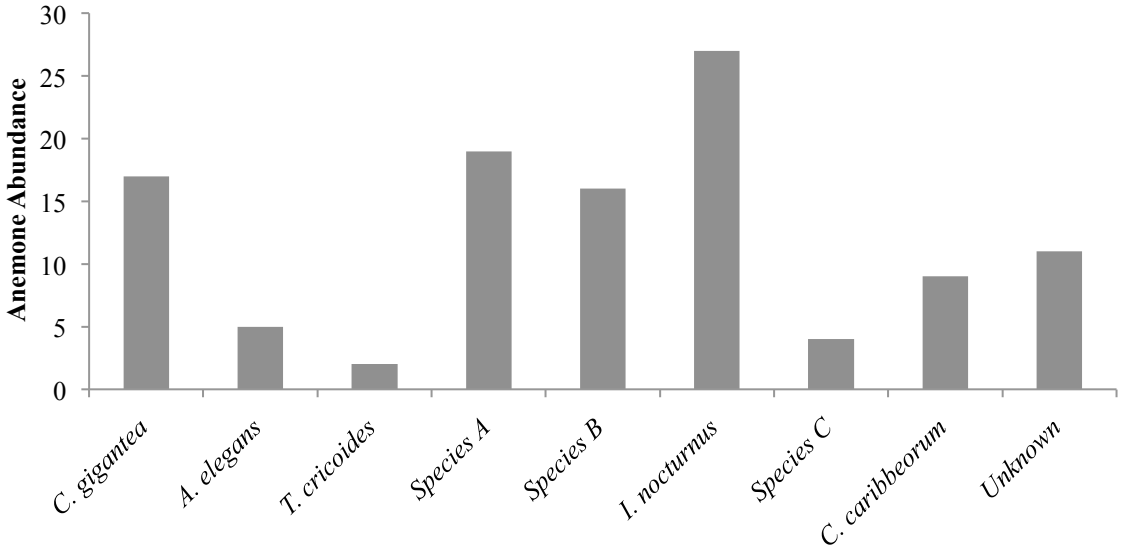


Fig. 3 Abundance of fluorescent anemone species located at night using Nightsea blue light flashlights and filters during March 2016 near Something Special dive site (N 12°09'35"N, 68°16'W). Depth of reef surveyed was 2-15 m, including the reef flat, crest, and slope habitats (area = 700 m^2). Unknown category includes multiple unidentified species, including Species D

Table 1 Habitat and depth distribution of fluorescent anemone species found in study site including digital photographs taken at night with a white light flashlight with no filter and a Nightsea blue light flashlight with yellow filter

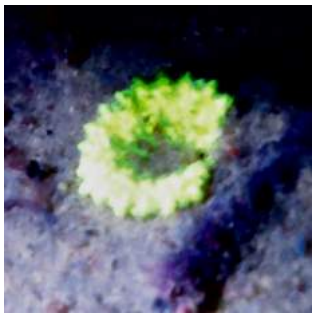
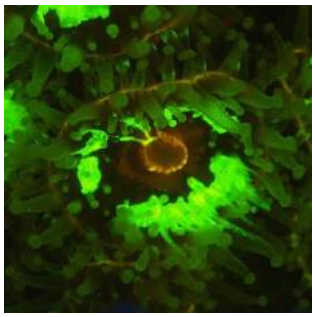
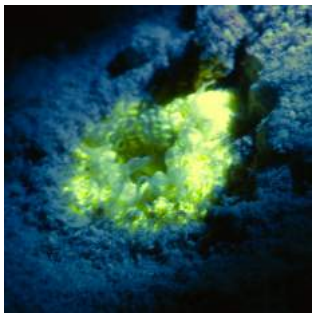
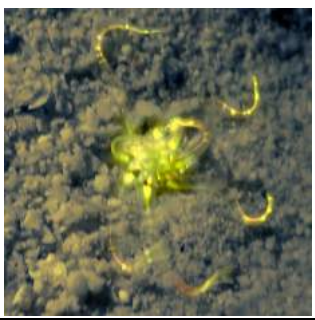
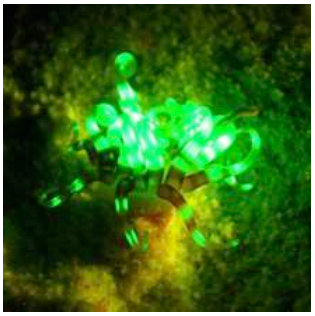
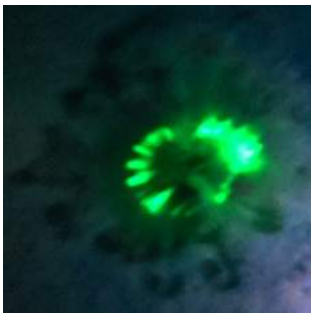
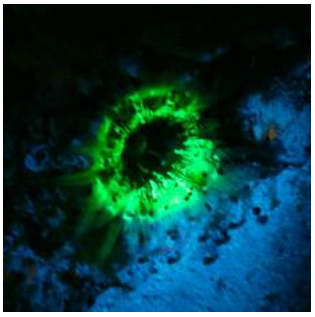
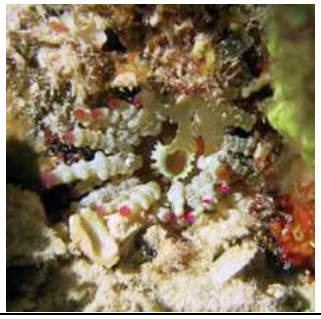
Species Name (Common Name)	Habitat	White Light	Blue Light
Actiniaria *Condylactis gigantea* (Giant Anemone)	Found at depths between 0.5 and 40 m on coral reefs, seagrass meadows, sandy bottoms, rocky shores, and in mangroves.[2] In present study found at depths between 4 and 10 m.[3]		
Actinoporus elegans (Elegant Anemone)	Found at depths between 1 and 2 m on sandy patches.[2] In present study found at depths between 7.5 and 10 m.[3]		
Telmatactis cricoides (Club-Tipped Anemone)	Found at depths between 1.5 and 20 m on coral reefs and in caves and dark recesses.[1&2] In present study found at a depth of 8.2 m.[3] Photos not taken during present study.[4&5]		
Species A	In present study found at depths between 5.5 and 14 m on sandy patches.[3]		
Species B	In present study found at depths between 5 and 13 m on sandy patches.[3]		

Table 1 continued

Ceriantharia *Isarachnanthus nocturnus* (Banded Tube-Dwelling Anemone)	A solitary and nocturnal polyp in tube found at depths between 10 and 27 m and shallow water on soft substrate.[1&2] In present study found at depths between 5 and 14 m.[3]		
Species C (Wideband Tube-Dwelling Anemone)	A solitary polyp in tube found at depths between 2 and 18 m on clean sand.[1] In present study found at depths between 2 and 5 m.[3]		
Corallimorpharia *Corynactis caribbeorum* (Orange Ball Corallimorph)	A solitary and nocturnal polyp found at depths between 15 and 20 m on coral reefs and sandy areas.[1&2] In present study found at depths between 6 and 14.5 m.[3]		
Unknown Order Species D	A solitary polyp in present study found in coral and rock crevices.[3]		

[1](Humann and Deloach 2003)
[2](González-Muñoz et al. 2016)
[3]Present study 2016
[4]Printed without permission http://doris.ffessm.fr/Especes/Telmatactis-cricoides-Anemone-americaine-1267
[5]Printed without permission http://diver.net/bbs/posts002/84301.shtml

24.5% of total fluorescent anemones (Fig. 3, Table 1). Other abundant anemones included *Condylactis gigantea* (15.5% of total) and two possibly unidentified anemone species (Species A, 17.3% of total) and (Species B, 14.5% of total) (Fig. 3, Table 1). Less abundant species out of the total fluorescent anemones included *Telmatactis cricoides* (1.8% of total), Species C (3.6% of total), *Actinoporus elegans* (4.5% of total), and *Corynactis caribbeorum* (8.2% of total) (Fig 3, Table 1). The 'Unknown' category included Species D (Table 1) as well

as other scarce and unknown species (10% of total) (Fig. 3).

Fluorescent anemone species habitat distribution

Anemone species depth distribution was tallied across the entire study site (Fig. 4). Some species exhibited narrower depth ranges, such as Species C (2-6 m), *T. cricoides* (8-9 m), and *A. elegans* (7-10 m) (Fig. 4). Some species exhibited wider depth ranges, such as *C. caribbeorum* (6-15 m), *I. nocturnus* (4-14 m), Species A (6-14 m) and Species B (6-14 m) (Fig. 4). Most anemones were documented at depths between 6 and 10 m (Fig. 4). The analysis of distribution within depth intervals demonstrates a relationship between depth and fluorescent anemone abundance per species.

Fluorescent anemone abundance across reef habitats

To analyze species abundance of fluorescent anemone communities among reef habitats, anemone abundance was grouped according to reef flat, crest and slope (Fig. 5). Mean anemone abundance was 5.5 ± 0.7 on the reef flat, 27.5 ± 6.4 on the reef crest, and 22 ± 15.6 on the reef slope (Fig. 5). Mean anemone abundance was significantly different between the reef flat and crest (Student's t-test; n = 2, df = 2, p = 0.04), and not significantly different between the reef flat and slope (Student's t-test; n = 2, df = 2, p = 0.27) or the reef crest and slope (Student's t-test; n = 2, df = 2, p = 0.69). The reef crest had a five-fold increase in mean anemone abundance when compared to

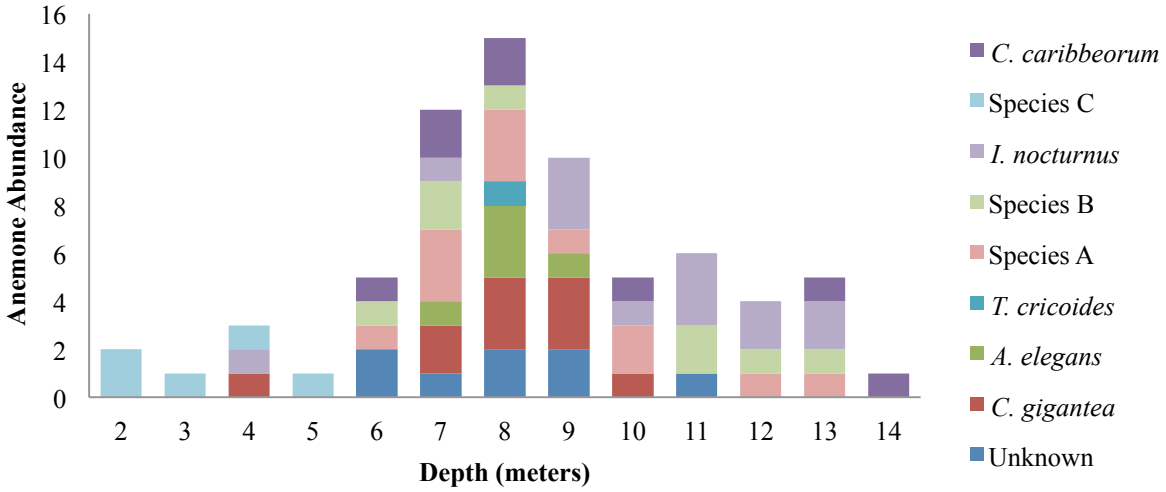


Fig. 4 Distribution of fluorescent anemone species located at night using Nightsea blue light flashlights and filters during March 2016 near Something Special dive site (12°09'35"N, 68°16'55"W). Depth of reef surveyed was 2-15 m, including the reef flat, crest, and slope habitats (area = 700 m^2). Depth intervals [2, 3), [3, 4), etc. Unknown category includes multiple unidentified species, including species D

the reef flat. The results suggested that some anemone species may be habitat-specific.

Fluorescent anemone diversity across reef habitats

To analyze species diversity of fluorescent anemone communities across reef habitats, Simpson's diversity index was calculated according to habitat depth ranges (Fig. 6). Mean anemone diversity index was 0.87 ± 0.09 on the reef flat, 0.83 ± 0.02 on the reef crest, and 0.69 ± 0.10 on the reef slope (Fig. 6). Mean diversity index was not significantly different between the reef flat and crest (Student's t-test; n = 2, df = 2, p = 0.61), reef flat and slope (Student's t-test; n = 2, df = 2, p = 0.22), and reef crest and slope (Student's t-test; n = 2, df = 2, p = 0.20). The results show that fluorescent anemone diversity is constant across reef habitats. Diversity indices were high within each studied reef habitat.

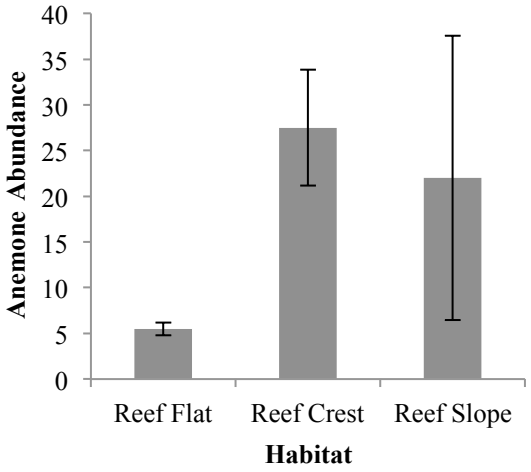


Fig. 5 Mean fluorescent anemone abundance by habitat located at night using Nightsea flashlights and filters during March 2016 near Something Special dive site (12°09'35"N, 68°16'55"W). Three habitats were categorized with the following depth parameters: reef flat (2-6 m), reef crest (6-9 m), and reef slope (9-15 m). Error bars show standard deviation

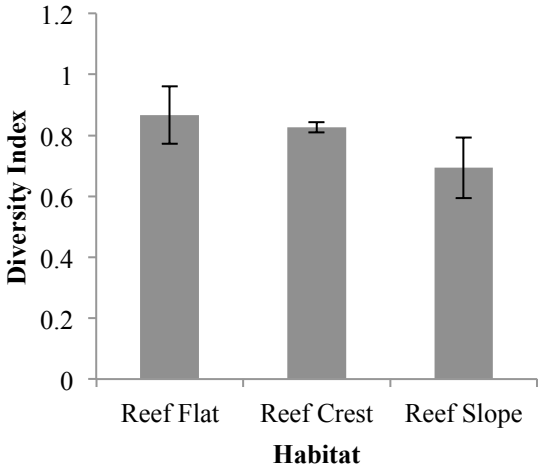


Fig. 6 Mean Simpson's diversity index of fluorescent anemones located at night using Nightsea flashlights and filters during March 2016 near Something Special dive site (12°09'35"N, 68°16'55"W). Three habitats were categorized with the following depth parameters: reef flat (2-6 m), reef crest (6-9 m), and reef slope (9-15 m). Error bars show standard deviation

Discussion

This descriptive study on fluorescent anemones suggests that anemone communities exhibit complex and diverse qualities. At least nine species were recorded, and the abundance of fluorescent anemones varied according to the species. Documented species also exhibited ranges across one, two, or all of the reef habitats. The significant difference between mean reef flat and reef slope anemone abundance partially supported the hypothesis that abundance varies with reef habitat and that the highest abundance occurs on the reef crest. The lack of a significant difference in anemone abundance between the (1) reef flat and slope or the (2) reef crest and slope refuted the hypothesis that abundance varies with habitat and that the highest abundance occurs on the reef crest. The lack of a significant difference in species diversity among reef habitats refutes the second hypothesis. Three abundant and possibly unidentified fluorescent anemone species (Species A, B, and C) were documented, as well as various infrequent and possibly unidentified species (Species D). Based on similar morphologies, Species A may be related to a type of sand anemone, and Species B may be related to *Nematostella vectensis* (Starlet Anemone) (Darling et al. 2005).

Fluorescent anemone species abundance

The different abundance of each fluorescent anemone species proposes that some anemones thrive in the Bonairean coral reef ecosystem more than others. The species *Condylactis gigantea, Isarachnanthus nocturnus*, Species A, and Species B may be the most adapted of all recorded species to the study site based on their relative abundance. The variation in species abundance may also indicate that some anemones prefer to live in close proximity to other organisms of the same species, whereas other anemones prefer to live isolated from all other anemones. For example, *I. nocturnus* tended to be located within close range of other anemones of the same species, sometimes forming groups of five or six individuals (personal observation). This could point to a reproductive strategy in which larva or clone polyps settle within centimeters of the mother polyp, or possible advantages of feeding in close quarters. On the other hand, *Telmatactis cricoides* and *Corynactis caribbeorum* tended

to be separated from any anemone individuals (personal observation). This suggests that proximity to other anemones is species specific. Furthermore, *I. nucturnus*, *T. cricoides*, and *C. caribbeorum* belong to three different anemone orders (Ceriantharia, Actiniaria, and Corallimorpharia), suggesting that proximity to other anemones is also order specific. In a study on the *Actinia tenebrosa* anemone, genotypic trends suggested that sexually produced anemone juveniles (scarce) dispersed over thousands of kilometers in small numbers, whereas asexually brooded juveniles (abundant) were rarely found more than a few centimeters from an adult (Ayre 1984). The *A. tenebrosa* study further suggests that proximity to other anemones depends on reproductive strategies.

Fluorescent anemone species habitat distribution

The observed tendencies for fluorescent anemone species to either extend across the three reef habitats or only establish in one or two reef habitats suggest that anemones exhibit species-specific habitat ranges. The species with higher abundance on the reef crest (*C. gigantea*, *Actinoporus elegans*, and *T. cricoides*) may be selecting habitat based on a combination of substrate preference and symbiotic zooxanthellae light requirements. In other Cnidaria species such as scleractinian corals, symbiotic zooxanthellae clades vary between hosts (Toller et al. 2001). Corals exhibit habitat range variance because some dinoflagellate clades are more stress-resistant and therefore tolerate a variety of irradiances (Toller et al. 2001). Anemone endosymbiont clades may vary in a similar way to coral endosymbiont clades, resulting in anemone habitat ranges. The reef crest species may prefer to establish on rock and coral substrates, and therefore are not located on the sandy reef flat. The reef crest species may also house endosymbiotic dinoflagellates that cannot photosynthesize in low light (Toller et al. 2001), and therefore are not located on the deeper, light-deficient reef slope. The tube anemone located only on the sandy reef flat (Species C) may share similar morphological traits to edwardsiid anemones that have evolved long and vermiform bodies without basilar musculature, allowing a habitat shift from sessile attachment to burrowing (Daly et al. 2002). Specialized morphology would explain the high abundance of Species C on the sandy reef flat. The species located on both the reef crest and reef slope (Species A, Species B, *I. nocturnus,* and *C. caribbeorum*) may house endosymbiotic dinoflagellates that exhibit higher photosynthesis efficiency in low-light than symbionts in reef crest species (Toller et al. 2001). It is also possible that some species do not partake in a symbiotic relationship with zooxanthellae, and therefore can establish at any depth. Thus, the observed habitat preference of documented anemones suggests potential for substrate, energy dependence, and symbiotic relationship variation among species.

Fluorescent anemone abundance across reef habitats

The difference in anemone abundance between the reef habitats may indicate that fluorescent anemones tend to settle in habitats with rock or coral structures such as the reef crest and reef slope, and do not tend to settle in habitats with a shallow sandy bottom such as the reef flat. The findings aligned with a study on an Australian sand shelf that observed anemone abundance across five strata and concluded that the lowest anemone abundance occurred in shallow sandy areas (Peterson and Black 1986). In the present study, this tendency could be attributed to substrate preference for fluorescent anemone settlement, but not all the species on the reef crest and slope were attached to hard rock or coral. Some species were often in patches of sand between coral heads. The high abundance of anemones in the crest/slope habitat range could therefore be attributed to prey access. The organisms preyed upon by various fluorescent anemones such as arthropods (Rubenstein and Koehl 1977), mollusks (Sebens 1981), and small fish (Cintiroglou and Koukouras 1992) may prefer

hard substrates and habitat complexity, limiting the anemone species to habitats with complex reef structure habitats, unlike the reef flat. Peterson and Black (1986) also attributed varying anemone abundance across habitats to access to resources such as refuge from predators, prey, and hard substrates for attachment.

Fluorescent anemone diversity across reef habitats

The similarity in Simpson's diversity index between the habitats suggests that fluorescent anemone diversity does not change across the three habitats. However, the similarity could mask differences in species richness and evenness between the habitats. For example, the reef flat may exhibit low species richness and high species evenness, while the reef crest may exhibit high species richness and low species evenness. Even though the habitats would be showing opposite trends, their Simpson's diversity index could be relatively similar because the index incorporates both factors into the calculation. Therefore it is possible for the diversity index of each habitat to be similar, but for different reasons. The constancy of diversity across the three reef habitats could mean that each zone houses different, but equally diverse, fluorescent anemone communities.

Because fluorescence was used as a tool for locating anemones, the species that did not exhibit florescence were not studied. Therefore, to complete a more comprehensive report of anemone communities in reef habitats, future studies should incorporate data on non-fluorescent anemones using a locating method other than fluorescence. For successful extrapolation of data to other local reefs or the entire Bonairean reef ecosystem, future studies should include a higher number of replicates. However, these findings may guide future study questions and research that provides knowledge of model organisms, coral reef bleaching events, and symbiotic organism ecology.

Descriptive studies such as the present project in Bonaire facilitate the possible discovery of new and groundbreaking species. The anemone *Nematostella vectensis* (Starlet Anemone) has recently surfaced as a valuable model organism in studies on metazoan evolution, embryogenesis, larval development, and other fundamental biological questions (Darling et al. 2005). The process of sequencing *N. vectensis* is underway, and will result in the first genome-wide comparisons between Bilaterian and non-Bilaterian organisms (Darling et al. 2005).

This study also contributes information on Bonairean anemone abundance and distribution that can be used in the future to track changes in the reef ecosystem. Because most anemone species maintain symbiotic relationships with dinoflagellates much like scleractinian corals (Kuguru et al. 2007), anemone bleaching could serve as a bioindicator of reef health and climate change ramifications. The cellular mechanisms of cnidarian bleaching remain relatively unsolved, but a study on cell death in sea anemones of the genus *Aiptasia sp*. and their endosymbiotic dinoflagellates suggested that programmed cell death (PCD) and necrosis in both the anemone and algae coincides with bleaching (Dunn et al. 2004). Anemone bleaching can also negatively affect organisms involved in symbiotic relationships with host cnidarians (Saenz-Agudelo et al. 2011). Bleached host anemones have been found to inhibit panda anemonefsh reproduction and recruitment (Saenz-Agudelo et al. 2011). The present study could provide information on habitat health and availability of a variety of marine organisms that utilize anemone species as specialized habitats. The results also predict habitat ranges for obligate anemone symbionts. In addition, this study highlights the effectiveness of utilizing fluorescence in monitoring reef anemones, a technique that can serve as an important baseline for future monitoring methods.

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REPORT

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Cephalopholis cruentata (graysby) behavior and interspecific response to invasive *Pterois volitans* (lionfish) in Bonaire, Dutch Caribbean

Abstract The invasion of *Pterois volitans* (lionfish) is a serious concern for Caribbean coral reef health. The morphology and behavior of lionfish is novel to the reef in Bonaire, which allows lionfish to take advantage of resources at the expense of native reef fish. *Cephalopholis cruentata* (graysby) is a native grouper on a similar trophic level as lionfish. Other groupers show congeneric aggression, but documentation of graysby behavior is scarce. This study observed graysby behavior and investigated whether graysbys recognize lionfish as competitors. A model-bottle experiment was used to present lionfish to graysbys. Graysby responses, aggressive, neutral, and submissive, were observed. Behavior was quantified using a reactive index. No significant difference in the frequency distribution of behavior types was observed between treatments. A moderate correlation was observed between graysby size and reactive index, suggesting that graysby reactions may be size-dependent. Future studies should consider size when analyzing graysby behavior towards other species, native or invasive.

Keywords Tropical marine ecology • Graysby behavior • Competition

Introduction

Pterois volitans (lionfish) is an invasive species to the Caribbean coral reefs, with the first sighting in Bonaire occurring in 2009 (STINAPA Bonaire 2009). Originally from the Indo-Pacific, lionfish were likely introduced to the Atlantic through the exotic fish trade (Morris and Whitfield 2009). Lionfish invasion in the Caribbean is a serious ecological concern; previous studies have documented the negative effects of lionfish on native fish and invertebrates within coral reef communities (Albins and Hixon 2011; Albins 2013; Côté et al. 2013; Curtis-Quick et al. 2013). In the Caribbean, lionfish prey upon juveniles of large fish species and small reef fish (Albins and Hixon 2011). Lionfish are successful at outcompeting native fish species partly because of the naïveté of the surrounding coral reef fish community, as it is not recognized either as prey or as a predator (Albins and Hixon 2011).

The unique morphology of the lionfish contributes to its success; the movement of its long, cryptically-colored fins mimics the appearance of seaweed (Albins and Hixon 2011). It hunts by herding prey with its fins and then attacks the prey quickly, and has also been observed to blow jets of water at prey to facilitate hunting; this latter behavior is unique to the lionfish (Albins 2013). It is unlikely that small Caribbean reef fishes had a similar predator in their evolutionary histories, and thus display prey naïveté towards lionfish (Albins and Hixon 2011). This allows lionfish to exploit a wide variety of small native fish species (Côté et al. 2013). Compared to native mesopredators, the presence of lionfish significantly reduced recruitment and diversity of small fish species in Bahamian reefs (Albins 2013).

Cephalopholis cruentata (graysby) is a species that occupies a similar trophic level as lionfish (Curtis-Quick et al. 2013), and is native to the coral reef community of Bonaire. The graysby, like the lionfish, is a mesopredator; unlike lionfish, graysbys are recognized by

native prey fish species (Steneck et al. 2015). Typical conspecific aggressive behavior in *Cephalopholis* include changes in coloration (Shpigel and Fishelson 1989). Other species of *Cephalopholis* have been observed to have 'color fights' to establish dominance; darker coloration indicates dominance, while a pale coloration signals submission (Shpigel and Fishelson 1989). The larger fish is usually victorious during intergeneric competition, but in the case of similarly sized opponents, physical fights also occur if the dispute cannot be solved by color signaling (Shpigel and Fishelson 1989). Other aggressive behaviors include raising the dorsal fin, gill flaring, and chasing away an opponent (Erisman et al. 2010). Feuding individuals may also open their mouths while facing each other, and occasionally bite each other (Erisman et al. 2010).

While several papers utilize members of *Cephalopholis* as a comparable native predator to invasive lionfish (Albins and Hixon 2011; Albins 2013; Curtis-Quick et al. 2013), there is little literature focused specifically on the behavior of graysbys. The first goal of this study is to observe and document graysby behavior in Bonaire, paying attention to the species' response to competitors and any aggressive or submissive behavior they may exhibit.

Graysbys must compete for resources on the reef with lionfish, particularly prey (Albins 2013). Lionfish have been shown to outcompete similarly-sized native fish of a similar trophic level, including *Cephalopholis fulva*, the coney grouper (Albins 2013), which also inhabits Bonairean reefs. In addition, it is possible that graysbys do not recognize lionfish as a threat, due to the species' unique physical and behavioral features (Albins 2013). However, it is possible for native species to adapt to the presence of invasive species (Strauss et al. 2006). Many of these adaptations were not observed until decades after the arrival of the introduced species, but changes in response to competition have been recorded as short as 19 years post-invasion (Strauss et al. 2006).

Although it has only been seven years since lionfish were sighted in Bonaire, rapid microevolution has been observed in natives in response to invasive species (Strauss et al. 2006). The second aim of this study was to determine if there are any signs that graysbys have developed an awareness of lionfish as a competitor species by observing the number and types of reactions to a lionfish. Investigating potential behavioral adaptations of native reef species towards invasive lionfish would also provide insight into how graysbys in Bonaire are reacting to an exotic species. In addition, if behavioral adaptation is observed in graysbys, it may indicate the types of selective pressures lionfish are exerting on the native population.

H_1: Graysbys will exhibit behavior similar to other species in *Cephalopholis*, particularly in regards to aggression and submission.

H_2: If graysbys do recognize lionfish as competitors, they will display size-dependent reactive behavior. Neutral behavior will be shown if graysbys do not recognize lionfish as competitors.

Materials and methods

Study site

Bonaire's reefs rank among the healthiest in the Caribbean, due to the coral density and abundance of other organisms, particularly reef fish (Steneck et al. 2015). The entire experiment was conducted at Yellow Submarine (Fig. 1).

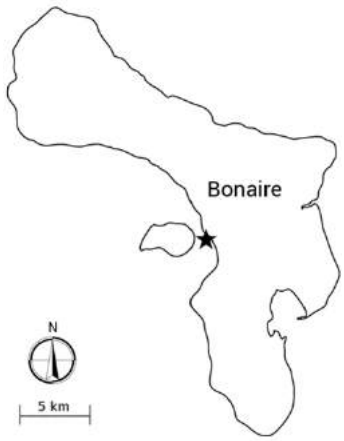

Fig. 1 Map of Bonaire, located in the Dutch Caribbean. Black star indicates Yellow Submarine study site for current study

The reef at Yellow Submarine is a fringing reef in the harbor of Kralendijk, Bonaire (12°16'01" N, 68°28'22" W). The reef crest begins about 61 m from shore, at a depth of 6 m, and extends down to 36 m.

Experimental design

Data collection took place over the course of five weeks. Dives occurred twice per week, starting between 16:30 and 17:00. Individual graysbys were observed at a depth between 6 – 10 m in order to establish a behavioral baseline. Behaviors were recorded from a 3-m distance for 15 min, with particular attention focused on aggressive and submissive behaviors. Fish length, surrounding substrate, initial color, and total number of color changes were also recorded. For the study on graysby behavior towards lionfish, lionfish were collected from Yellow Submarine by CIEE Staff or lionfish collectors and maintained in aquaria at the research station.

A model-bottle design, based on Kindinger's (2014) work, was used to test graysby reactions to lionfish. A control jar was presented to graysby subjects found at a depth of 6 – 10 m, containing only seawater. The experimental jar was a replicate of the control, but also contained a 10-cm lionfish. The lid of both jars was replaced with mesh to allow for any chemical signaling that lionfish or graysby may use. The jar was attached to a PVC pipe apparatus in order to lower the jar onto the substrate and minimize diver interference. Jars were presented to adult graysbys of various sizes at a horizontal distance of 1 m (Fig. 2). Behavior was observed and recorded from at least a 2-m distance for 15 min. In the event the subject left the area, another diver watched the jar apparatus while the subject graysby was followed and any behaviors recorded. A GoPro Hero3 video camera was also attached to the top of the jar to record any close interactions, and video was reviewed to confirm initial data collection.

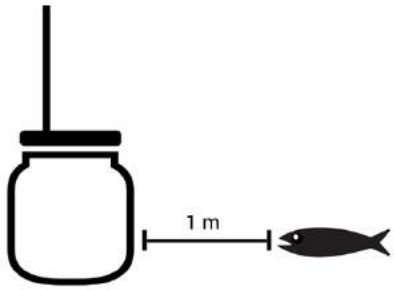

Fig. 2 Setup for model-bottle experiment. Jar, including PVC pipe apparatus, was placed 1 m away from where graysby was initially observed

Behavioral quantification

Based on existing data of *Cephalopholis* behavior (Shpigel and Fishelson 1989), each aggressive and submissive behavior was recorded. The total number of aggressive, neutral, and submissive behaviors for each treatment was calculated, and analyzed using Pearson's Chi-square test. Behavior towards the control and experimental jars were also quantified using a score based on the intensity of the action (Table 1).

Relative aggression or submission of individual fish were then calculated using the following equation:

$$\frac{\Sigma(aggressive + neutral + submissive\ scores)}{\#\ total\ behaviors\ observed}$$

These quantities were assessed for each condition. The correlation between graysby size and reactive index was then examined to determine the strength of the relationship between these two variables.

Table 1 Graysby behaviors quantified and recorded to calculate reactive index

Behavior type	Weighted score	Behavior observed
Aggressive	3	Physical attack (biting)
	2	Open mouth display while facing target
	1	Change to dark coloration
	1	Raise dorsal fin
	1	Flare gills
Neutral	0	No change in coloration
	0	No movement towards or away from jar
	0	Interactions not including the jar
Submissive	-1	Change to light coloration
	-2	Retreat

Results

Baseline observation

Baseline aggressive and submissive behavior in graysbys was observed to be exactly like behaviors in other *Cephalopholis*. Reactions witnessed included gill flares, dorsal fin raising, color fights, retreating behavior, attacking behavior, and open-mouth fighting.

To determine whether graysbys responded more towards the lionfish, the type and number of reactions between the two treatments was determined. The total number of behaviors observed for each type was compared using Pearson's Chi Square test. Over a span of 15 min, the mean number (± SD) of aggressive reactions towards the control jar was 1.25 (± 0.46) reactions, the mean number of neutral reactions was 1.00 (± 0.89) reactions, and the mean number of submissive reactions was 1.13 (± 0.52) reactions. Overall, the types of behaviors displayed were about the same in number.

Frequency distribution of behaviors

The mean number of aggressive reactions towards the experimental treatment was 2.00 (± 1.85) reactions, the mean number of neutral reactions was 0.75 (± 0.71) reactions, and the mean number of submissive reactions was 1.38 (± 0.74) reactions. This indicates that on average, graysbys tended to display more aggressive behavior towards the lionfish, and remained less neutral than the control. However, no significant difference was found in the frequency distribution of reactions displayed by the graysbys towards the lionfish as compared to the control (X^2 = 1.78, n = 8, p = 0.41), indicating that there were no observed behavioral differences exhibited by graysby subjects between treatments.

Reactive index in relation to size

To analyze size-dependent variations in behavior, graysby reactions were considered in relation to size. The mean size of graysbys surveyed under control conditions (n = 8) was 17.13 (± 4.82) cm, while the mean size of graysbys surveyed under experimental conditions (n = 8) was 13.88 (± 4.16) cm. The mean reactive index for control conditions was -0.27 (± 0.46), while the mean reactive index for graysbys exposed to the experimental jars was -0.10 (± 0.66). Both treatments yielded negative mean reactive indices, indicating that overall, the observed graysbys were submissive. There was no correlation between the size of the graysby and reactive index under control conditions (R^2 = -0.08), suggesting that size is independent from the reactive index of graysbys given this treatment. However, when exposed to the lionfish, there was a moderately positive correlation between size and reactive index in graysbys (R^2 = 0.42), indicating that size of the graysby had some effect on how submissive or aggressive its reactions were to the lionfish.

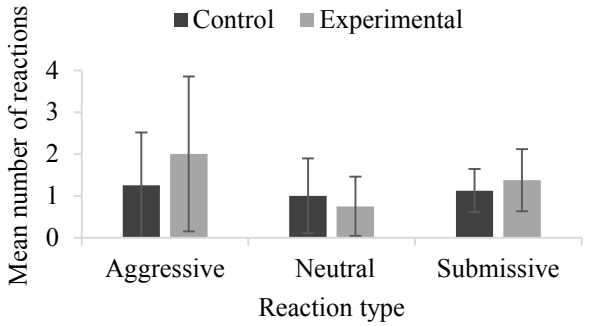

Fig. 3 Mean number of reactions displayed by graysbys (n = 8 for each treatment) towards the model-bottle treatments, categorized by reaction type. Treatments consisted of an empty control jar (dark) or an experimental jar containing a lionfish (light). Error bars represent standard deviation

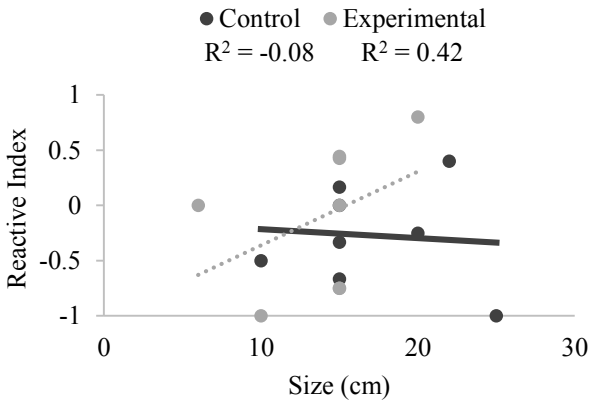

Fig. 4 Individual size and reactive index of graysbys exposed to the control jar and jar containing a lionfish. Linear best-fit lines were presented for control jars (dark, n = 8) and for experimental jars containing lionfish (light, n = 8)

Discussion

The hypothesis that graysbys would recognize lionfish as competitors and react to their presence was not supported by statistical tests run on the data. Graysbys did not display a significant increase in reactive behavior towards the invasive lionfish than the control jar. This lack of significance may be due to the posture of the lionfish (Kindinger 2014), which varied for each trial, as well as other factors such as sample size, observation length, the small size of the lionfish, and inadvertent diver interference. However, the lack of significance more likely indicates that graysbys do not yet recognize lionfish as a competitor.

No correlation between size and reactive index indicates that the control conditions did not have any impact on the graysby subject. However, a moderately positive correlation among experimental subjects between size and reaction index suggests that larger graysbys tend to be more aggressive, while smaller graysbys under 15 cm tend to be more submissive towards the experimental lionfish. Since smaller *Cephalopholis* are typically more submissive in intergeneric interactions (Shpigel and Fishelson, 1989), it may indicate that graysby reactions to an intruder are size-dependent. *Epinephelus striatus* (Nassau grouper), another ecologically similar mesopredator, displayed size-dependent behavior, using different methods of avoidance depending on how large the lionfish was relative to the grouper (Raymond et al. 2015). Although the lionfish used in this study was 10 cm, its elongated pectoral fins may have helped it to appear larger to the graysby (Tullock, 2006), thus explaining why the reactive index was correlated with graysby size over or under 15 cm.

While the frequency distribution of reactions displayed did not differ between the two groups, the difference in the correlation between size of the graysby and reactive index suggests that graysbys are recognizing lionfish and displaying aggressive or submissive behavior, as opposed to neutral behavior. This discrepancy may be due to sample size, as well as graysby size as a confounding factor in the behavioral frequency distribution analysis. Further limitations include the usage of the reactive index, which was an artificial equation made to quantify graysby behavior on a basic level. While the scores for each behavior were not completely arbitrary, it is difficult to accurately measure the impact of a single behavior, due to other factors such as length of time the behavior was displayed.

As previous evidence suggests (Strauss et al. 2006), it is unlikely that graysby naïveté towards

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REPORT

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Cephalopholis cruentata (graysby) behavior and interspecific response to invasive *Pterois volitans* (lionfish) in Bonaire, Dutch Caribbean

Abstract The invasion of *Pterois volitans* (lionfish) is a serious concern for Caribbean coral reef health. Previous studies have already explored the negative effects of lionfish on fish species native to Caribbean reefs. In Bonaire, the morphology and behavior of lionfish is novel to the reef community, which allows lionfish to take advantage of resources at the expense of native fish. *Cephalopholis cruentata* (graysby) is a native grouper on a similar trophic level as lionfish. Other *Cephalopholis* groupers show congeneric aggression, but documentation specifically focusing on graysby behavior is scarce. This study observed graysby behavior, focusing on aggressive and submissive displays, and investigated whether graysbys recognize lionfish as competitors. A model-bottle experiment was used to present lionfish to graysbys. Graysby responses, aggressive, neutral, and submissive, were observed and recorded. The magnitude of observed behaviors was quantified using a reactive index. No significant difference in the frequency distribution of graysby responses was observed between treatments. However, there was a moderate positive correlation observed between graysby size and reactive index. These results suggest that graysby reactions towards lionfish may be size-dependent. Future studies should consider the size of the graysby when analyzing graysby behavior towards other species, native or invasive.

Keywords Tropical marine ecology • graysby behavior • competition

Introduction

Pterois volitans (lionfish) is an invasive species to the Caribbean coral reefs, with the first sighting in Bonaire occurring in 2009 (STINAPA Bonaire 2009). Originally from the Indo-Pacific, lionfish were most likely introduced to the Atlantic through the exotic fish trade (Morris and Whitfield 2009). Lionfish invasion in the Caribbean is a serious ecological concern; previous studies have documented the negative effects of lionfish on native fish and invertebrates within coral reef communities (Albins and Hixon 2011; Albins 2013; Côté et al. 2013; Curtis-Quick et al. 2013). In the Caribbean, lionfish prey upon juveniles of large fish species and small reef fish (Albins and Hixon 2011). Lionfish are successful at outcompeting native fish species partly because the surrounding coral reef fish community is naïve, as lionfish are not recognized either as prey or as a predator (Albins and Hixon 2011).

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H_1: Graysbys will exhibit behavior similar to other species in *Cephalopholis*, particularly in regards to aggression and submission.

H_2: If graysbys do recognize lionfish as competitors, they will display size-dependent reactive behavior. Neutral behavior will be shown if graysbys do not recognize lionfish as competitors.

Materials and methods

Study site

Bonaire's reefs rank among the healthiest in the Caribbean, due to the coral density and abundance of other organisms, particularly reef fish (Steneck et al. 2015). The entire experiment was conducted at Yellow Submarine, a local dive site south of Something Special (Fig. 1).

The reef at Yellow Submarine is a fringing reef in the harbor of Kralendijk, Bonaire (12°09'36.2"N, 68°16'55.2"W). The reef crest begins about 61 m from shore, at a depth of 6 m, and extends down to 36 m.

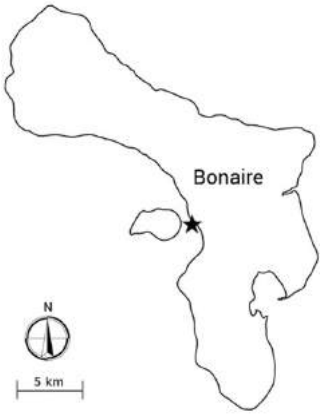

Fig. 1 Map of Bonaire, located in the Dutch Caribbean. Black star indicates Yellow Submarine study site for current study

Experimental design

Data collection took place over the course of five weeks and occurred twice per week, starting between 16:30 and 17:00. Individual graysbys were observed at a depth between 6 – 10 m in order to establish a behavioral baseline. Behaviors were recorded from a 3-m distance for 15 min, with particular attention focused on aggressive and submissive behaviors. Fish length, surrounding substrate, initial color, and total number of color changes were also recorded. For the study on graysby behavior towards lionfish, lionfish were collected from Yellow Submarine by CIEE staff or lionfish collectors and maintained in aquaria at the CIEE research station.

A model-bottle design, based on Kindinger's (2014) work, was used to test graysby reactions to lionfish. A control jar was presented to graysby subjects found at a depth of 6 – 10 m, containing only seawater. The experimental jar was a replicate of the control, but also contained a 10-cm lionfish. The lid of both jars was replaced with mesh to allow for any chemical signaling that lionfish or graysby may use. The jar was attached to a PVC pipe apparatus in order to lower the jar onto the substrate and minimize diver interference. Jars were presented to adult graysbys of various sizes at a horizontal distance of 1 m (Fig. 2). Graysby behavior was observed and recorded from at least a 2-m distance for 15 min. In the event the subject graysby left the area, another diver watched the jar apparatus while the subject graysby was followed and any behaviors recorded. A GoPro Hero3 video camera was also attached to the top of the jar to record any close interactions, and video footage was reviewed to confirm initial data collection.

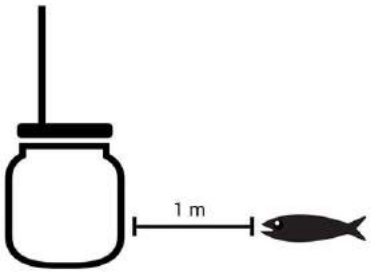

Fig. 2 Setup for model-bottle experiment. Jar, including PVC pipe apparatus, was placed 1 m away from where graysby was initially observed

Behavioral quantification

Based on existing data of *Cephalopholis* behavior (Shpigel and Fishelson 1989), each aggressive and submissive behavior was recorded. The total number of aggressive, neutral, and submissive behaviors for each treatment was calculated, and analyzed using Pearson's Chi-square test. Behavior towards the control and experimental jars were also quantified using a score based on the intensity of the action (Table 1).

Relative aggression or submission of individual fish was then quantified into a reactive index using the following equation:

$$\frac{\Sigma(aggressive + neutral + submissive\ scores)}{\#\ total\ behaviors\ observed}$$

These quantities were assessed for each treatment given. The correlation between graysby size and reactive index was then examined to determine the strength of the relationship between these two variables.

Table 1 Graysby behaviors quantified and recorded to calculate reactive index

Behavior type	Weighted score	Behavior observed
Aggressive	3	Physical attack (biting)
	2	Open mouth display while facing target
	1	Change to dark coloration
	1	Raise dorsal fin
	1	Flare gills
Neutral	0	No change in coloration
	0	No movement towards or away from jar
	0	Interactions not including the jar
Submissive	-1	Change to light coloration
	-2	Retreat

Results

Baseline behavior observation

Baseline aggressive and submissive behavior in graysbys was observed to be like behaviors in other *Cephalopholis.* Reactions witnessed included gill flares, dorsal fin raising, color fights, retreating behavior, attacking behavior, and open-mouth fighting (n = 4).

Frequency distribution of behaviors

To determine whether graysbys responded more towards the lionfish, the type and number of reactions between the two treatments was analyzed. Over a span of 15 min, the mean number (± SD) of aggressive reactions towards the control jar was 1.25 (± 0.46) reactions, the mean number of neutral reactions was 1.00 (± 0.89) reactions, and the mean number of submissive reactions was 1.13 (± 0.52) reactions. Overall, the types of behaviors displayed were about the same in number.

The total number of behaviors observed for each type was compared using Pearson's Chi-square test. The mean number of aggressive reactions towards the jar containing the lionfish was 2.00 (± 1.85) reactions, the mean number of neutral reactions was 0.75 (± 0.71) reactions, and the mean number of submissive reactions was 1.38 (± 0.74) reactions. This indicates that on average, graysbys tended to display more aggressive behavior towards the lionfish, and remained less neutral than the control (Fig. 3).

However, no significant difference was found in the frequency distribution of observed reactions displayed by the graysbys towards the lionfish as compared to the control (X^2 = 1.78, n = 8, p = 0.41), indicating that there were no observed behavioral differences exhibited by graysby subjects between treatments.

Reactive index in relation to size

To analyze size-dependent variations in behavior, graysby reactions were considered in relation to size. The mean size of graysbys surveyed under control conditions (n = 8) was 17.13 (± 4.82) cm, while the mean size of graysbys surveyed under experimental conditions (n = 8) was 13.88 (± 4.16) cm. The mean reactive index for control conditions was -0.27 (± 0.46), while the mean reactive index for graysbys exposed to the experimental jars was -0.10 (± 0.66).

Both treatments yielded negative mean reactive indices, indicating that overall, the observed graysbys were submissive. There was no correlation between the size of the graysby and reactive index under control conditions (R^2 = -0.08), suggesting that size is independent from the reactive index of graysbys given this treatment. However, when exposed to the lionfish, there was a moderately positive correlation between size and reactive index in graysbys (R^2 = 0.42), indicating that size of the graysby had some effect on how submissive or aggressive its reactions were to the lionfish.

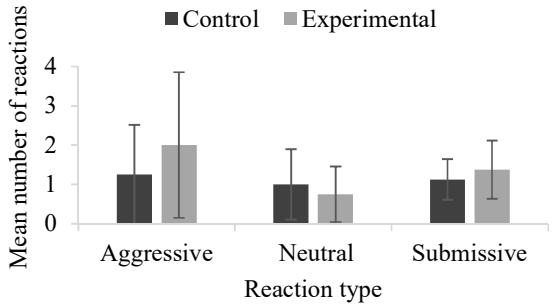

Fig. 3 Mean number of reactions displayed by graysbys (n = 8 for each treatment) towards the model-bottle treatments, categorized by reaction type. Treatments consisted of an empty control jar (dark) or an experimental jar containing a lionfish (light). Error bars represent standard deviation

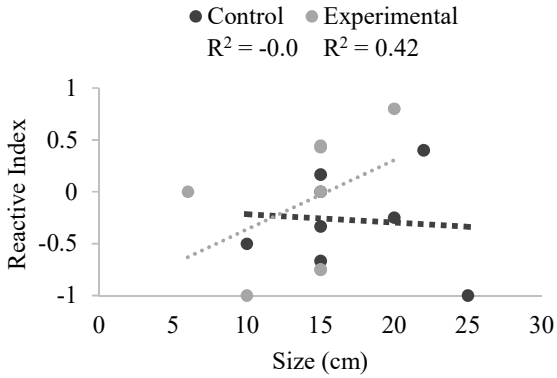

Fig. 4 Individual size and reactive index of graysbys exposed to the control jar and jar containing a lionfish. Linear best-fit lines were presented for control jars (dark, n = 8) and for experimental jars containing lionfish (light, n = 8)

Discussion

The hypothesis that graysbys would recognize lionfish as competitors and react to their presence was not supported by statistical tests run on the data. Graysbys did not display a significant increase in reactive behavior towards the invasive lionfish than the control jar. This lack of significance may be due to the posture of the lionfish (Kindinger 2014), which varied for each trial, as well as other factors such as sample size, observation duration, the small size of the lionfish, and inadvertent diver interference. However, the lack of significance more likely indicates that graysbys do not yet recognize lionfish as a competitor.

No correlation between size and reactive index indicates that the control conditions did not have a noticeable impact on the graysby subject. However, a moderately positive correlation among experimental subjects between size and reaction index suggests that larger graysbys tend to be more aggressive, while smaller graysbys under 15 cm tend to be more submissive towards the experimental lionfish. Since smaller *Cephalopholis* are typically more submissive in intergeneric interactions (Shpigel and Fishelson, 1989), it may indicate that graysby reactions to an intruder are size-dependent. *Epinephelus striatus* (Nassau grouper), another ecologically similar mesopredator, displayed size-dependent behavior, using different methods of avoidance depending on how large the lionfish was relative to the grouper (Raymond et al. 2015). Although the lionfish used in this study was 10 cm, its elongated pectoral fins may have helped it to appear larger to the graysby (Tullock, 2006), thus explaining why the aggressive or submissive behavior was correlated with graysby size over or under 15 cm.

While the frequency distribution of reactions displayed did not differ between the control and experimental groups, the difference in the correlation between size of the graysby and reactive index suggests that graysbys are recognizing lionfish and displaying size-dependent aggressive or submissive behavior, as opposed to neutral behavior. This discrepancy may be due to sample size, as well as graysby subject size as a confounding factor in the behavioral frequency distribution analysis. Future studies may choose to perform this analysis with consideration to graysby subject size. Further limitations include the usage of the reactive index, which was an artificial equation made to quantify graysby behavior on a basic level. While the scores for each behavior were not completely arbitrary, it is difficult to accurately measure and quantify

the magnitude a single behavior, due to factors such as length of time the behavior was displayed.

As previous evidence suggests (Strauss et al. 2006), it is unlikely that graysby naïveté towards the lionfish can disappear within seven years, as the process usually takes decades. There are few studies on interspecific interactions between lionfish and other species, but current evidence suggests that other reef fish also do not display full awareness of lionfish as a member of the reef community. Nassau groupers have shown size-dependent reactions to lionfish when competing for shelter (Raymond et al. 2015). However, *Stegastes planifrons* (three-spot damselfish), which are non-competitors with lionfish, did not display significant reactions to the presence of lionfish as opposed to the presence of coney groupers (Kindinger 2014).

During the observation period, graysbys were seen acting aggressively towards competitors, such as chasing bar jacks away, while ignoring non-competitive species, such as parrotfish. Graysbys displayed the same aggressive and submissive behaviors towards control and experimental jars as other *Cephalopholis* did in interspecific interactions, such as changes in coloration and gill flaring (Shpigel and Fishelson 1989; Erisman et al. 2010). This study indicates that graysbys may recognize invasive lionfish as a competitor, and react towards lionfish in a size-dependent manner. Any subsequent studies on interspecific graysby behavior, especially those focusing on lionfish, should take this into account. Further research can provide insight into graysby behavior and elucidate the factors in graysby reactions to lionfish, as well as help understand how naïveté towards invasive species changes over time.

Acknowledgements I would like to thank my research advisors, Nathaniel Hanna Holloway and Austin Lin, for guiding this research and answering any and all questions I had. I would also like to thank everyone in the class for being amazing, but I owe special thanks to my dive buddy, Lydia Tobin, for her role in my project, as well as Samuel Barrett and Susan Burke for their help. Finally, I would like to acknowledge my lionfish, Mufasa, for its sacrifice.

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REPORT

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Does an increasing gradient in population create a bottom-up effect on the intertidal community in Kralendijk, Bonaire?

Abstract In the microbial loop, heterotrophic bacteria utilize dissolved organic matter (DOM) as an energy source. DOM becomes remineralized into inorganic material and nutrients available for primary production. As the amount of nutrients increase, the abundance of each trophic level increases, which is known as the bottom-up effect. This study investigates the effect of the increasing human population density on an intertidal community along the waterfront of Kralendijk, Bonaire. DOM, fecal indicator bacteria (enterococci, *Escherichia coli,* and coliform bacteria), nutrients (nitrate, phosphate, and ammonia), primary producers (percent cover of macroalgae), and herbivorous consumers (density of sea urchins) were sampled. There was no pattern between the variables and the increase of the adjacent population density. Factors such as rainfall, changes over time, tides, and herbivore grazing may have influenced the results. When graphed over time, rainfall impacted the concentrations of nutrients and fecal indicators. Nitrate, ammonia, and coliform bacteria increased, while phosphate, enterococci, and *E. coli* decreased. Concentrations of ammonia were found to exceed the threshold for a healthy coral reef ecosystem (6x). No correlation was found between DOM and heterotrophic bacteria, although concentrations of *E. coli* and nutrients were high at one site. This intertidal ecosystem does not appear to be influenced by bottom-up controls, as there was neither a correlation found between the percent cover macroalgae and nutrients or the density of sea urchins. The site with the highest percent cover of macroalage had the lowest density of urchins and vice versa.

Keywords Heterotrophic bacteria • sea urchins • macroalgae

Introduction

Nitrogen (NO_3) and phosphorus (PO_4) are limiting nutrients essential for algal growth and survival (Carpenter et al. 1998). Nitrogen in the form of nitrate in the ocean originates from the upwelling of deep nutrient-rich water (Zehr and Ward 2002). However, ammonia (NH_3) is the preferred form of nitrogen since this form is directly utilized for algal growth (Pal and Kumar 2014). Phosphorus in the form phosphate enters the ocean primarily through rivers from sediments resulting from the weathering of rocks (Paytan and McLaughlin 2007). If a large amount of either nutrient enters a water system, such as through runoff, it can cause eutrophication, or the explosive growth of algae and phytoplankton (Carpenter et al. 1998). This may degrade water quality and lead to an unhealthy ecosystem such as degraded coral reefs (Carpenter et al. 1998).

As nutrients become available, producers such as phytoplankton and macroalgae are able to utilize these nutrients for growth. Macroalgae can then become prey for herbivores such as sea urchins. If nutrients are the limiting factor for primary production, then the ecosystem is known to have a bottom-up control (Hobbie and Villeger 2015). Likewise, an experiment by Nielsen (2001) showed that the abundance of algae increased through the addition of nutrients. This is an indication of an ecosystem under a bottom-up control, where the biomass of each trophic level increases as

the level of nutrients increases. While nutrients are essential for algal growth, abiotic factors such as temperature, pH, salinity, sunlight, and the amount of dissolved oxygen available also contribute to algae abundance in an ecosystem (Juneja et al. 2013).

Algal growth is also increased indirectly through heterotrophic bacteria, which contributes to the overall food web through the microbial loop (Graham et al. 2014). Within the microbial loop, heterotrophic bacteria use dissolved organic matter as their energy source (Pomeroy et al. 2007). Dissolved organic matter (DOM) then becomes remineralized into inorganic material and nutrients that are reabsorbed by producers such as phytoplankton and macroalgae to facilitate their growth (Pomeroy et al. 2007). A second way heterotrophic bacteria contributes to the grazing food chain is by becoming prey for protozoans, consumers of heterotrophic bacteria (Graham et al. 2014). At the end of its lifecycle, heterotrophic bacteria decays to become available DOM, or prey for protozoans, which can then fuel the grazing food chain (Graham et al. 2014).

Previous studies by Ma et al. (2014) and Zhang et al. (2012) have noted that little research has been conducted on the complex mutualistic relationship between bacteria and algae. This research will therefore provide a better understanding of the role of heterotrophic bacteria in the food web by investigating if nutrients are solely responsible for the bottom-up effect or if the amount of heterotrophic bacteria results in this as well. All sizes and species of macroalgae and sea urchins will be documented in this research to represent producers and consumers in the trophic levels of the food web.

H_1: Areas closest to heavily impacted areas (sites 7-9) will have higher concentrations of heterotrophic bacteria and nutrients due to human population influences, such as contaminated groundwater and stormwater runoff

H_2: Areas with higher concentrations of heterotrophic bacteria will have lower levels of DOM and higher concentrations of nutrients. The higher concentrations of nutrients will result from the decomposition of DOM by heterotrophic bacteria

H_3: High concentrations of nutrients will result in the bottom-up effect by increasing the abundance of the trophic levels: producers (macroalgae) and consumers (sea urchins)

Materials and methods

Study site

Data was collected along the waterfront of Kralendijk, Bonaire (12°9'22.06"N, 68°16'45.66"W to 12°9'11.20"N, 68°16'41.59"W). Bonaire is an island located in the southern Caribbean about 80 km north of Venezuela. Kralendijk is an urbanized town on the west coast of Bonaire with high variability in the ranges of low to highly populated areas. The intertidal zone along the waterfront of Kralendijk is comprised mostly of hard, rough substrate or dead coral. Nine sites along the waterfront, approximately within 50 m of each other, were chosen for sampling (Fig. 1). These areas run along a gradient of low to high impacted areas. Low impacted areas contain few houses or single-family homes, while higher impacted areas contain more buildings and more residents within 100 m of the shoreline.

Fig. 1 *Yellow pins* represent the nine sampling sites along the waterfront of Kralendijk, Bonaire. The sites are approximately 50 m apart and increasing site number corresponds to population density increases

Data collection

To avoid biased sampling, random numbers were chosen for each round of data collection to determine the distance from the starting point of each of the nine sites. The distance ranged from 0-10 m to ensure sampling covered a 10-m wide area or 5 m left and right from the starting point for each of the nine sites. At each site, a transect was laid perpendicular to where the tide meets the shore, and a 1-m^2 gridded quadrat was placed on the center of the transect at the 2-m mark. The sites were sampled a total of seven times, biweekly, over the course of three and a half consecutive weeks.

Macroalgae and sea urchin abundance

Within each quadrat, the number of sea urchins, all species and sizes, was counted and recorded. Urchins touching the border of the quadrat, underneath ledges or on concrete slabs within the quadrat were also included. The percent of macroalgae of all species considered, was visually estimated based on its coverage within the gridded quadrat. The attachment of the macroalgae had to be within the quadrat to be included in the estimate.

Nutrients and DOM

A water sample was collected at each site to test for DOM and the nutrients: nitrate (NO_3), phosphate (PO_4), and ammonia (NH_3). To collect water, a plastic 100 mL or 500 mL bottle was submerged underwater at the center of the quadrat. The sample was stored in a cooler then transferred to a freezer to ensure the external temperature would not affect the composition of the samples before analysis. A list of procedures was followed, and a fluorometer was then used to determine the amount of DOM (ppb), phosphate (mg L^{-1}), nitrate (mg L^{-1}), and ammonia (μg L^{-1}) for each sample.

Bacteria

A second and third water sample, each 100 mL, was collected to test for the presence of enterococci, coliform bacteria, and *Escherichia coli.* The collect water, the same methodology to collect nutrient analysis was used. The samples were then stored in a cooler before analysis to ensure the composition of the sample was not affected by the external temperature. To test for coliform bacteria and *E. coli*, IDEXX Colilert test kits were used. After 24-28 hours of incubation at 35°C, the yellow coloring of the wells was compared to the control to determine if coliform bacteria were present in the sample. A black light was then used to detect fluorescing wells, which determined if *E. coli* was present in the sample. The IDEXX Enterolert test kit was used to detect the presence of enterococci bacteria. After incubating the samples for 24-28 hours at 41°C, a black light was then used to detect the number of fluorescing wells. The number of colony forming units for enterococci and *E. coli* per 100 mL sample, and coliform bacteria per 10 mL sample, was analyzed with the IDEXX 2000 MPN Table.

Abiotic factors

The temperature, salinity, and the amount of dissolved oxygen in the water were recorded at each site using a PRO2030 YSI meter. The probe was placed at the center of each quadrat halfway between the bottom and the surface of the water.

Data analysis

The results were graphed using Excel to analyze correlations between variables. Bar graphs were used to determine the effect of population increase on the concentrations of DOM, nutrients, fecal indicator bacteria, macroalgae, and density of sea urchins. The correlation coefficients were found using the site number as a proxy for population density. The correlation coefficients of the level of DOM versus the concentrations of enterococci, *E. coli*, and coliform bacteria, as well as the percnt coverage of macroalgae versus the density of sea urchins were determined using Excel. Any correlation coefficient less than

± 0.1 was considered to support the null hypothesis.

Results

Population density vs. fecal indicator bacteria

At the study, as site numbers increase, the adjacent population density increases. However, none of the fecal indicator bacteria increased as predicted (Fig. 2, Fig. 7). The highest mean coliform bacteria was found at site 5 (772.52 CFU 10 ml^{-1}); the highest mean enterococci was at site 5 (1.68 CFU 100 ml^{-1}); and the highest concentration of *Escherichia coli.* was at site 7 (9.24 CFU 100 ml^{-1}). Similarly, the lowest values of fecal indicator species showed no correlation to the adjacent population density. For coliforms, the lowest value was at site 8 (487.23 CFU 10 ml^{-1}); whereas no enterococci were found at site 2 or 3, and the lowest concentration of *E. coli* was at site 4 (0.667 CFU 100 ml^{-1}) (Fig. 2). However, when graphed over time, there is an increase in mean concentration of coliform bacteria after 9 March 2016 (Fig. 5) and levels remained high throughout the study. The opposite pattern was found for mean *E. coli* where the concentration decreased after 9 March 2016; there was no pattern for the concentration of enterococci over time (Fig. 3).

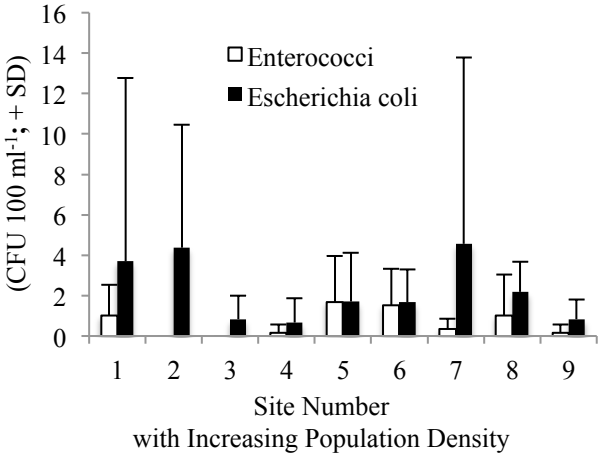

Fig. 2 Mean concentration of enterococci and *Escherichia coli* bacteria in colony forming units (CFU 100 ml^{-1}) in water samples (n = 9) collected biweekly at each site from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

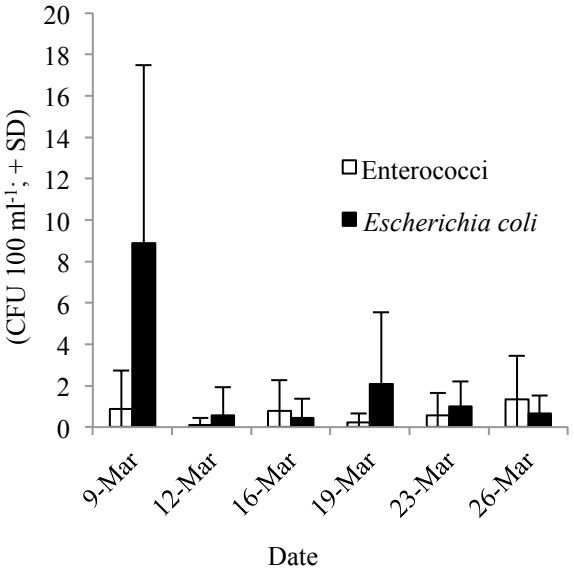

Fig. 3 Mean concentration of enterococci and *Escherichia coli* bacteria in colony forming units (CFU 100 ml^{-1}) per day (n = 9) in water samples collected biweekly from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

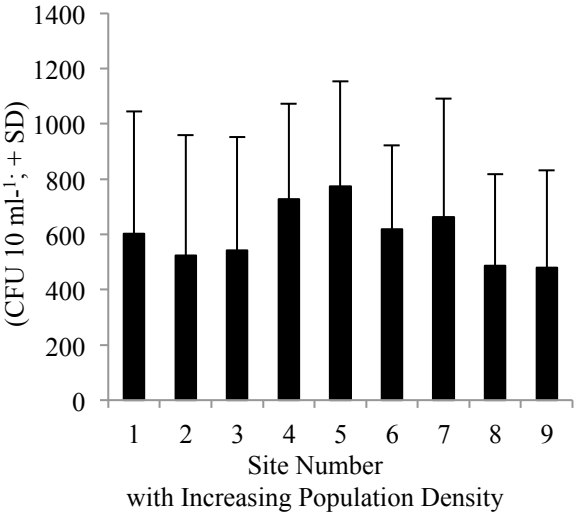

Fig. 4 Mean concentration of coliform bacteria in colony forming units (CFU 10 ml^{-1}) in water samples (n = 9) collected biweekly at each site from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

Population density vs. nutrients and DOM

As site numbers increased and areas increased with population density, neither nutrients nor DOM increased as expected. The highest mean concentration of nitrate was at site 7 (0.058 mg L^{-1}) and the highest mean concentration of phosphate was highest at site 3 (0.082 mg L^{-1})

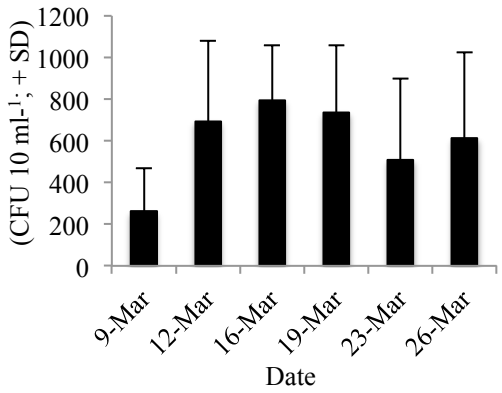

Fig. 5 Mean concentration of coliform bacteria in colony forming units (CFU 10 ml^{-1}) per day (n = 9) in water samples collected biweekly from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

(Fig. 6). The lowest concentration of the amount of nitrate and phosphate were found at sites 9 (0.031mg L^{-1}) for nitrate and site 5 (0.011 mg L^{-1}) for phosphate (Fig. 6). The mean concentration of ammonia was found to be close in range throughout the sites with the highest concentration found at site 7 (97.153 µg L^{-1}) (Fig. 7). All ammonia concentrations exceeded the threshold (14 µg L^{-1}) by six fold for a healthy coral reef system (Fig. 7). When graphed over time, there is a noticeable decrease of phosphate and an increase of nitrate and ammonia after 9 March 2016 (Fig. 8, 9). These observed variations in nutrient levels are likely resulting from temporal fluctuations.

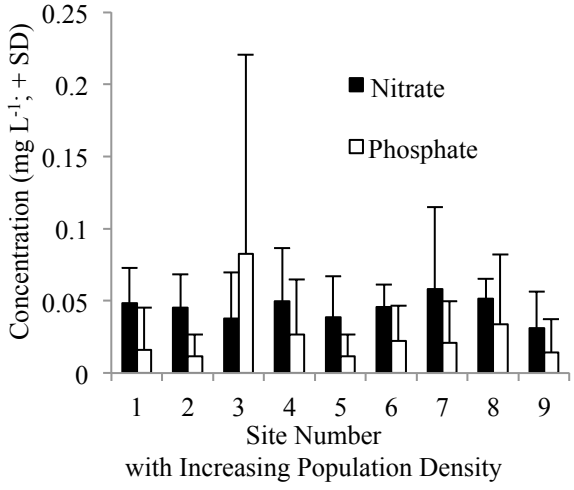

Fig. 6 Mean concentration of nitrate and phosphate (mg L^{-1}) collected biweekly (n = 9) at each site from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

It was predicted that there would be a negative correlation between the levels of DOM and heterotrophic bacteria due to the breakdown of DOM by heterotrophic bacteria. However, there was no correlation found between DOM and heterotrophic bacteria: coliform bacteria (r = -0.039), enterococci (r = -0.017), and *E. coli* (r = 0.052). The lack of correlation between nutrients and bacterial content shows that these factors may be independent of one another.

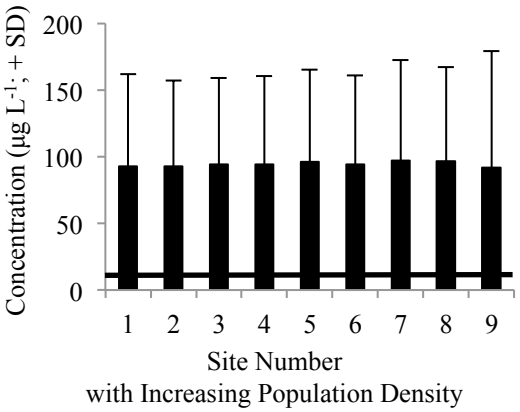

Fig. 7 Mean concentration of ammonia (µg L^{-1}) collected biweekly (n = 9) at each site from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1). The line at 14 µg L^{-1} represents the threshold for a healthy coral reef ecosystem (Bell, 1992)

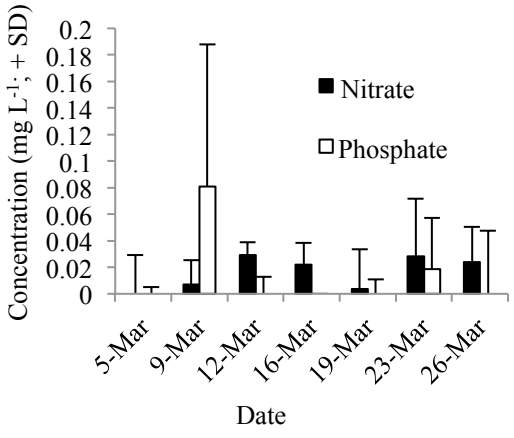

Fig. 8 Mean concentration of nitrate and phosphate (mg L^{-1}) per day (n = 9) in water samples collected biweekly from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1)

Percent cover macroalgae vs. sea urchin abundance

It was predicted that areas with a higher

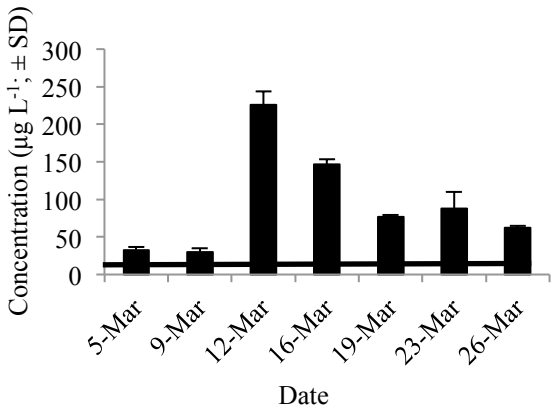

Fig. 9 Mean concentration of ammonia (µg L^{-1}) per day (n = 9) in water samples collected biweekly from 9-26 March 2016. Sites were located along the waterfront (see Fig. 1). The line at 14 µg L^{-1} represents the threshold for a healthy coral reef ecosystem (Bell, 1992)

concentration of nutrients would result in a higher percent coverage of macroalgae and density of sea urchins. However, there was no correlation found between the percent coverage of macroalgae and the concentration of nutrients: nitrate (r = 0.088), phosphate (r = 0.006), and ammonia (r = 0.107). There was no correlation found between the percent coverage of macroalgae (r = 0.036) and increasing adjacent population or the mean density of sea urchins (r = 0.071) and increasing adjacent population (Fig. 10). The mean percent coverage of macroalgae and mean number of sea urchins fluctuated throughout the sites, with the highest percent coverage of macroalgae found at site 4 (55.0%) and the highest number of sea urchins found at site 7 (20 individuals m^2) (Fig.10). The lowest percent coverage of macroalgae was found at site 7 (5.0%) and the lowest mean number of sea urchins was found at site 4 (0.143 individuals m^2) (Fig. 10). The mean percent cover of macroalgae increased slightly over time, while there was no discernable pattern with the mean number of sea urchins over time (Fig. 11). The most common urchin observed was *Tripneustes ventricosus*, although *Diadema antillarum* and *Echinometra viridis* were observed occasionally.

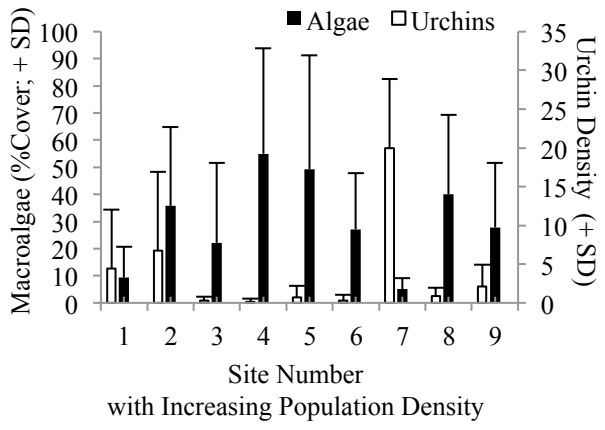

Fig. 10 Mean number of sea urchins (individuals m^{-2}) and percent cover macroalgae per 1 m^2 quadrat (n = 9) sampled biweekly from 9-26 March 2016 at each site along the waterfront (See Fig. 1)

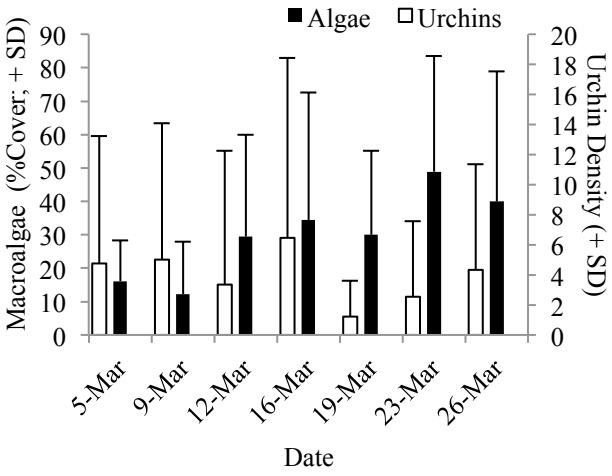

Fig. 11 Mean number of sea urchins (individuals m^{-2}) and percent cover macroalgae per day over time (n = 9) sampled biweekly from 9-26 March 2016. Sites were located along the waterfront (see Fig.1)

Discussion

Heterotrophic bacteria, nutrient input and population density

The amount of heterotrophic bacteria, nutrients, DOM, macroalgae, and sea urchins was found to be highly variable along the gradient of population density at the study site. It was predicted that areas closest to heavily impacted areas (sites 7-9) would have higher concentrations of heterotrophic bacteria and nutrients due to human population influences resulting in contaminated groundwater and stormwater runoff. It was also predicted that

areas with higher amounts of heterotrophic bacteria would have lower levels of DOM and result in high levels of nutrients.

Previous studies have found that heterotrophic bacteria in the microbial loop can break down DOM into inorganic nutrients that can then be utilized by algae, thus forming a mutualistic relationship with algae and impacting the food chain (Zhang et al. 2012, Graham et al. 2014). Although no correlation was found between the amount of heterotrophic bacteria and the amount of DOM, site 7 was found to contain the highest concentrations of *Escherichia coli,* nitrate, and ammonia. The highest value for *E. coli* (9.24 CFU 100 ml^{-1}) was found at site 7; this is congruent with my hypothesis. The high amount of nutrient input may be resulting from the breakdown of DOM by heterotrophic bacteria, particularly *E. coli*. The observed concentration could also be the result of excessive nutrient input of nitrate (0.058 mg L^{-1}) and ammonia (97.153 μg L^{-1}) at site 7 due to the groundwater or stormwater runoff potentially containing more pollutants than other sites.

Only three types of heterotrophic bacteria, all fecal indicators, were tested for. Further research would include studying the effects of the diversity of heterotrophic bacteria in order to fully understand its role in the microbial loop and its complex relationship to algae. Distance between sites was only 50 m apart, covering a range of 400 m. This distance may have resulted in there being no distinguishable differences in the detection of nutrients, DOM, and fecal indicators compared to if sites were closer or further apart.

There was a rainfall that occurred in the afternoon of 9 March 2016 and ended on 10 March 2016. Rain is likely to be a factor resulting in the high variability. Data was collected on the morning of 9 March 2016 and collected three days later on 12 March 2016. This rainfall may be responsible for the reduction of enterococci, *E. coli,* and phosphate (Fig. 3, 8) and the increase of coliform bacteria, nitrate, and ammonia (Fig. 5, 8, 9). These graphs indicate that rain is an important factor responsible for the influx of nutrients and fecal bacteria into the marine environment. Rainwater entering the marine environment may have washed away buildup of enterococci, *E. coli,* and phosphate, or may have introduced pollutants such as coliform bacteria and excess nitrate and ammonia into the marine environment via groundwater and stormwater runoff. Groundwater is likely to be entering the marine environment from the shoreline equally at all sites rather than at a gradient from low to high heavily impacted areas. Fig. 7 suggests an equal distribution of groundwater at each site since a relatively equal concentration of ammonia was found at each site.

Primary production and nutrient input

It was predicted that areas with a higher concentration of nutrients would have a higher percent coverage of macroalgae due to the predicted bottom-up effect. However, neither of the nutrients were found to have the highest concentrations at site 4, where the highest percent coverage of macroalgae was found (Fig. 10). There was a canal draining into the waterfront near site 4 and may be facilitating excess input of nutrients in this area. However, it is unknown if eutrophication is occurring since neither of the highest concentrations of nitrate or phosphate were found at site 4 (Fig. 6).

Currents and tides are factors to take into consideration that may have affected the distribution of nutrients in the water to result in the high variability. Data was not collected with the peak of high or low tide. Nielson (2001) performed a similar study and found that nutrient concentration decreased in areas that were exposed to waves resulting from high tide. This indicates that currents and tides can greatly impact the availability of nutrients and may deliver nutrients at a faster rate to be readily utilized by algae. The tides and strength of the currents in this experiment may have resulted in the high variability of nutrients and bacteria found throughout the sites and the area as a whole over time.

The concentrations of ammonia were found to exceed the threshold (14 μg L^{-1}) over six fold for all forms of nitrogen for a healthy coral reef system (Bell 1992). High levels of

ammonia can be toxic to the marine environment and can be detrimental to fish, resulting in an imbalance of ionic regulation, altered behavior, and even death (Eddy 2005). If high amounts of nutrients enter a water system such as through runoff, it can cause eutrophication (Carpenter et al. 1998).

Percent coverage of macroalgae and sea urchin density

It was predicted that areas with a higher percent coverage of macroalgae would have a higher density of sea urchins due to the predicted bottom-up effect. However, results suggested otherwise. Out of all the sites, site 4 contained the highest percentage of macroalgae and the lowest density of sea urchins, while site 7 contained the highest density of sea urchins and the lowest percent coverage of macroalgae (Fig. 10). If sea urchins prefer areas with habitat complexity, then the low urchin densities found at sites 3, 4, and 6 may be due to the flat, open sandy areas allowing urchins to be vulnerable to predation.

Time of data collection and habitat structure at the study sites are factors that may have greatly impacted the distribution of sea urchins that were sampled. Some sea urchins such as *Diadema antillarum* are found to remain hidden under shelters during the day but re-emerge at night to graze (Andrew 1993). The type of substratum and habitat complexity may also impact the distribution of the urchins sampled. Lee and Hessen (2006) found that a higher density of urchins in areas with increasing habitat complexity ultimately resulted in a decreased percent coverage of macroalgae due to urchin grazing. This trend of high urchin density and low macroalgae cover was observed at site 7.

Urchin grazing may be a factor that affects the coverage of macroalgae and may explain why the percent coverage of macroalgae fluctuated throughout the sites and over time. It may also explain why a positive correlation resulting from a bottom-up effect is not observed between the coverage of macroalgae and sea urchin density. The results indicate that this ecosystem is not under bottom-up controls, as there was neither a correlation found between the percent coverage of macroalgae and the density of sea urchins nor between the percent coverage of macroalgae and nutrients. The effects of heterotrophic bacteria in the microbial loop were not completely observed in this research since no correlation was found between DOM and heterotrophic bacteria, although the high concentrations of *E. coli* at site 7 were found to parallel the high concentrations of nitrate and ammonia.

Herbivores, such as the damselfish, sea snails, and schools of parrotfish may be disrupting this food web from being directly under bottom-up controls. Further research would include the study of top-down controls such as the impact herbivores may have on this food web

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