Physis

Journal of Marine Science

CIEE Research Station
Volume XX, Fall 2016

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Table of Contents.....	Ive Pieterick, Brian Anschel, Levi Dodge, Savannah D'Orazio, Danielle Goldberg, Nick Hobgood, Georgette Douwma, Brian Anschel, Shannon Richardson
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Physis

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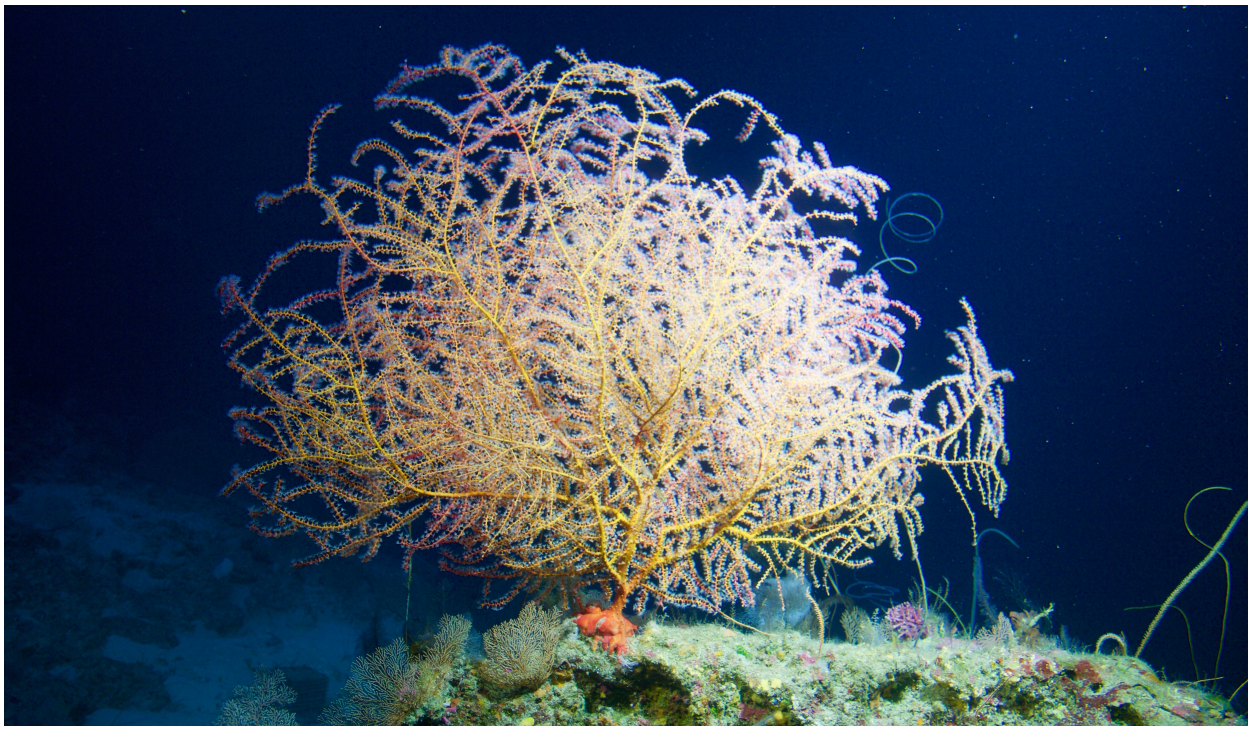


CIEE Research Station
Tropical Marine Ecology and Conservation Program
Volume XX, Fall 2016

PHYSIS

Close your eyes. Now picture yourself on a pristine, white sandy shoreline. Admire the sapphire blue water, but do not see with just your eyes. Listen to the wallowing waves kiss the shore, inhale the salty air from the ocean's breeze, and enjoy the wind gliding across your face as the sand tickles your toes. The existence of life is everywhere. Now open your eyes. Let the cognition of reality sink in. The roaring of bustling streets, the pungent odor of industry, and the unsightly world of concrete rears its ugly head. We, the human race, have diminished Mother Nature to nothing more than a servant. For she has been bent, broken and stripped repeatedly to satisfy our selfish desires.

Modern society has pioneered numerous technological advancements, but at what unseen costs? Too often, people do not stop and ask "and what then?" – Rod Fujita. They fail to fully comprehend the consequences of their actions. But the past remains as an ominous reminder that we live to fight for today. Now as we watch our world die in front of our eyes, do we finally see that this is the answer to the never-ending question, and what then?

We have forged a way to inhibit the natural breathing of our world's ecosystems and the organisms that reside within them. These ecosystems have been suffocated beyond the point of return. For a lucky few, however, there are potential methods to reduce and reverse existing anthropogenic stressors. For the ocean, deceivingly too immense, too vibrant with life to be affected, has experienced the most harmful human impacts. The complexity of our oceans are not yet even fully understood, but we continue to witness the services they have provided for generations slowly disintegrate with every rising tide.

To restore our planet into a thriving, beautiful home once more, we must let her breath. We vow to let her rebuild her mountains, replenish her forests, restore her oceans once more so that we can live with her, in harmony. We must allow *physis*, the process in which Mother Nature is freed to heal herself. Nature has been, and always will be, the best restorative agent.

Studying beautiful treasures hidden beneath the waves strengthens our knowledge and understanding of the intricate and naturally occurring processes essential to restoring the oceans. With this knowledge comes the ability to enlighten the minds of others, to see what is unseen, the good and the bad, and to take part in assisting Mother Nature in freeing herself from servitude. People will protect what they love, and can love what they understand.

Here, we present Volume XX of *Physis: A Journal of Marine Science.*

FOREWORD

Publication of Volume XX of the student journal *Physis: Journal of Marine Science* was a major goal of the Independent Research in Marine Ecology/Biology course. The course is part of the semester program in Tropical Marine Ecology and Conservation at the CIEE Research Station Bonaire. Fadilah Ali, PhD Candidate, Franziska Elmer, PhD, and Kelly Hannan, MS co-taught the course. Additionally, student projects were supported by an intern, Nicole Jackson, BS or Emily Dawson, BS. The academic advisors guided the projects through course content delivery and weekly meetings with each student. Astrid de Jager Verstappen directed the Dive Safety Program for the semester.

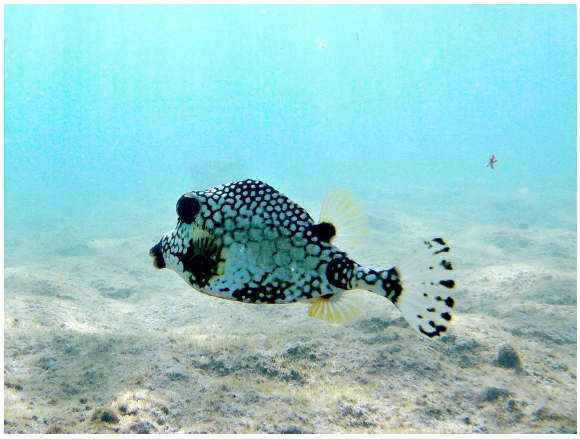
Research was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature. Projects were conducted near the research station, which is located on the leeward side of Bonaire to the north of the town of Kralendijk. The students presented the findings of their research projects in a public forum on the 30th of November, 2016 at the CIEE Research Station lecture room.

The Tropical Marine Ecology and Conservation program in Bonaire is designed for upper level undergraduates majoring in Biology/Ecology. There is a field-based orientation to the program with a strong focus on research-skills acquisition. In addition to the Independent Research course, students enroll in five courses: Coral Reef Ecology, Marine Ecology Field Research Methods, Advanced Scuba, Tropical Marine Conservation Biology, and Cultural & Environmental History of Bonaire. A noteworthy accomplishment is that students earned a Scientific Dive certification with the American Academy of Underwater Sciences during the program.

Part of the mission of CIEE Foundation, which is a Bonairean not-for-profit organization, is: ***"to provide outstanding educational opportunities to students in Tropical Marine Ecology and Conservation. We strive to provide interdisciplinary marine research opportunities for CIEE students as well as visiting scientists and their students from around the world."***

Thank you to the students and staff that participated in the program this semester. A final word to the students: **Congratulations on publishing this volume of *Physis* and best of luck as you embark on your future careers!**

Dr. Rita BJ Peachey



Faculty

Dr. Rita Peachey is the founding Director at CIEE. She has a PhD in Marine Sciences from University of South Alabama. Her research specialization is larval ecology, phototoxicity, fish ecology, and invasive species ecology. Her current research interests in Bonaire are a shark tagging project in Lagun, a coral gardening project along the leeward side of Bonaire and the effects of cruise ship tourism on Lac bay.

Franziska Elmer is the Coral Reef Ecology Faculty for CIEE and co-teaches Independent Research and Marine Ecology Field Research methods. She has a Master's in Ecology and Evolution from ETH Zurich (Switzerland) and a PhD in Marine Biology from Victoria University in Wellington (New Zealand). For her PhD, she researched how biological and physical factors affect coral recruitment and calcium carbonate accretion by CCA.

Fadilah Ali is the Tropical Marine Conservation Biology Faculty at CIEE. She is also the co-instructor on the Independent Research Course and serves as the Outreach Coordinator. Fadilah's specialty is in Biodiversity and Conservation, and she has a Masters in Environmental Science and is currently finishing up her PhD in Ocean and Earth Sciences at the University of Southampton. She has been studying the lionfish invasion for the last six years and has conducted research on many Caribbean islands including Bonaire and Curacao.

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

Kelly Hannan is the Marine Ecology Field Research co-instructor and Independent Research co-instructor. She has a Bachelor's degree in Comprehensive Science from Villanova MSc. Natural Resources and Environmental Sciences from University of Illinois. She is currently a PhD candidate in Marine Biology from James Cook University. Previous research involves physiology research relating to climate change.

Staff

Sara Buckley is the Office and Laboratory Manager. She received a B.S. in Oceanography from the University of North Carolina at Wilmington. She is a PADI/SDI SCUBA instructor. She studied UV effects on zooplankton. After a year Internship, she was hired on as a full-time staff member to be the Laboratory and Office Manager and to finish the zooplankton study.

Luigi Eybrecht is the residence hall manager and logistics coordinator. His study interests include environmental conservation. He is also a proud local Bonairean.

Marc Tsagaris is the facilities manager at CIEE and instructor for the Advanced Scuba course. He is interested in diver impacts on coral reefs and rebreather research.

Mary DiSanza was born and raised in Colorado where she was committed to protecting the environment. Computers, banking and law gave way to scuba dive and travel. 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.

Interns

Emily Dawson is a teaching assistant for Coral Reef Ecology, Advanced Scuba, and Independent Research. She has two bachelor's degrees from Florida Institute of Technology (FIT) - Conservation Biology and Ecology along with Marine Biology. She worked as a research assistant at FIT for 3 years studying a variety of marine topics.

Nikki Jackson is a TA for Tropical Marine Conservation Biology and Marine Ecology Field Research Methods. She also assists with the Advanced Scuba course and is a co-advisor for Independent Research. She has a Bachelors in Biological Sciences from Florida State University. She intends to obtain a Master's degree in Biological Oceanography.

Martijn Koot is the laboratory intern at CIEE and knows everything about nutrients and a little bit about DNA. He is still studying at the Technical Collage of Rotterdam as a Chemical/physical Analyst and studied biotechnology for a year. He is interested in the amount of nutrients that are floating in the water and what kind of effect that have on the animals living in the sea.

Students

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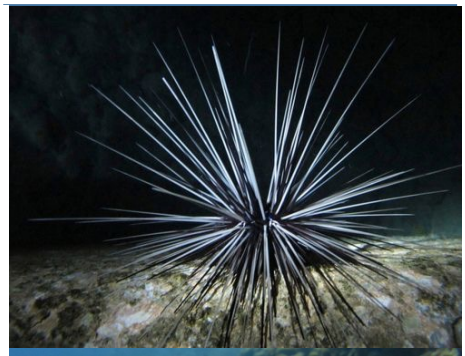
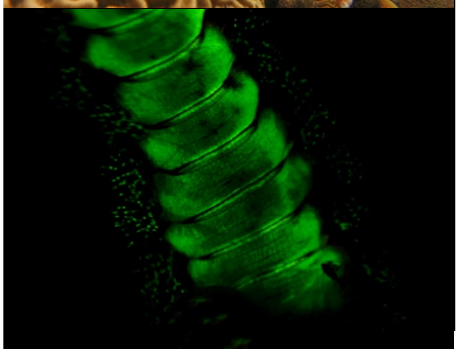
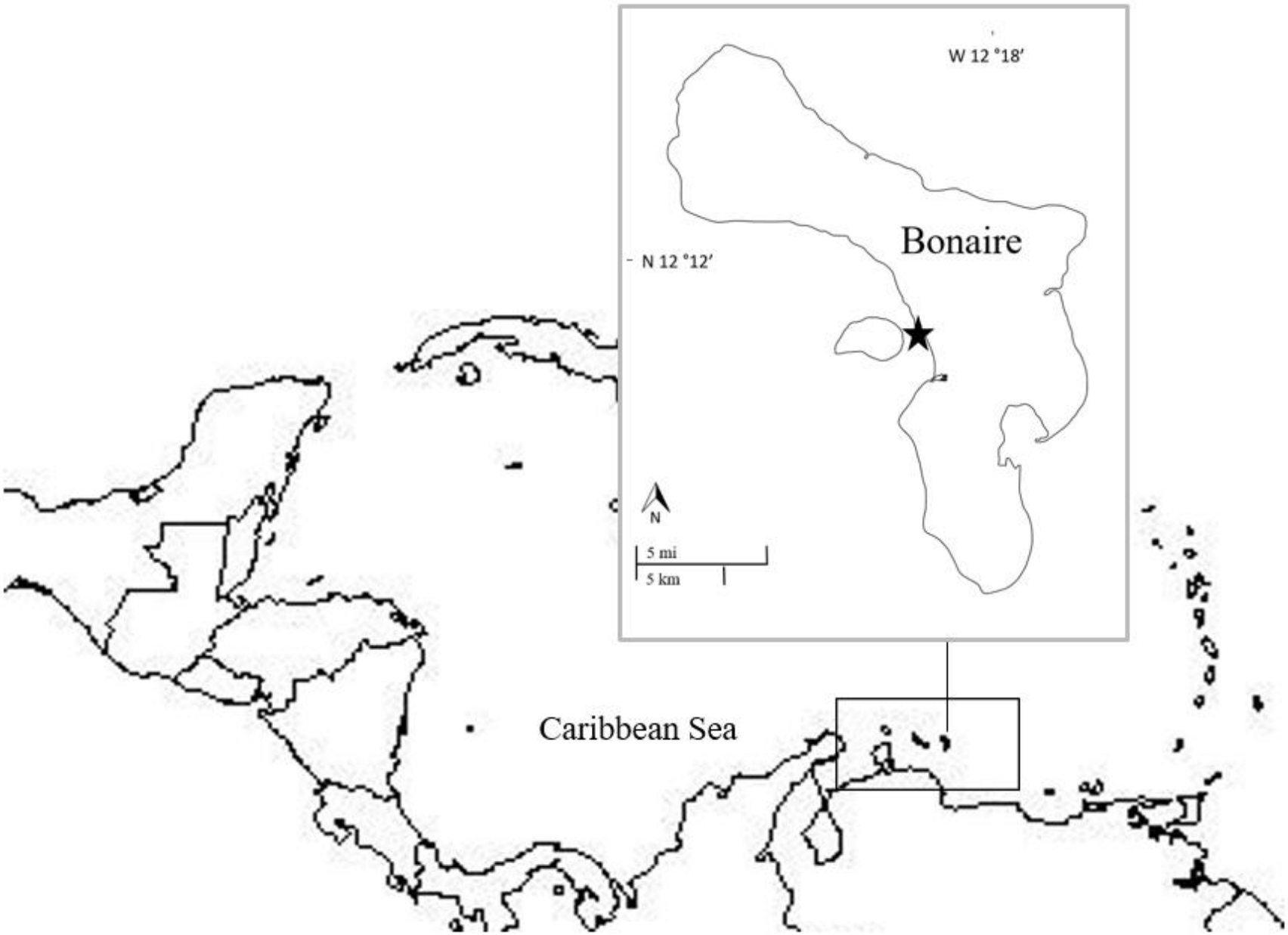
Table of Contents



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REPORT

Brian Anschel • University of Colorado Boulder • Brian.Anschel@colorado.edu

Diel variation in the biomass of *Diadema antillarum* predators linked to post urchin larval densities

Abstract Black spiny sea urchins, *Diadema antillarum*, are herbivorous, keystone species that remove macroalgae from coral reefs, facilitating coral growth, and thereby acting as an essential component towards coral reef health. An underwater pathogen killing a majority of the population has caused a slow recovery of the species with densities still remaining far below pre-die-off observations. There have been many studies which look at probable causes for *D. antillarum*'s stagnant recovery although few have taken into account species' behavior as a factor. This study looked at the unique relationship between the possible correlation of predation on *D. antillarum* and urchin density. Comparison of data collected at day and dusk examined the relationship between behavior and fish predation of *D. antillarum*. Average density of *D. antillarum* observed during the day (1.2 ± 1.1 per 100 m^2) was lower than at dusk (2.0 ± 2.7 per 100 m^2) and average weighted predator biomass during the day (17.73 ± 22.58 per 100 m^2) was also lower than at dusk (69.11 ± 148.64 per 100 m^2). These results suggest that there could potentially be increased predation as an overlapping response to the nocturnal foraging behaviors of *D. antillarum.* The population density of *D. antillarum* found at Yellow Sub dive site in Bonaire was minimal. Therefore, no direct negative correlation was found with a predation intensity index using weighted predator biomass, providing insight on *D. antillarum* recovery.

Keywords Predation • recovery • compare

Introduction

The spiny black sea urchin, *Diadema antillarum* plays a unique role within the Caribbean coral reef ecosystem as a herbivorous keystone species (Edmunds and Carpenter 2001). *Diadema antillarum* provide a service to coral reefs by eating and removing macroalgae, yielding additional space for coral recruits to settle and grow (Edmunds and Carpenter 2001). This is exemplified in shallow reef zones high in *D. antillarum* density. Densities as high as five urchins per 1 m² show a reduction in macroalgae coverage, with juvenile coral densities 2-11x higher than in areas lacking *D. antillarum* coverage (Edmunds and Carpenter 2001). Furthermore, they contribute significantly to the bioerosion of reef calcium carbonate by feeding on the surfaces of some corals (Stearn et al. 1977) and are known competitors with other herbivorous reef fish that eat macroalgae (Williams 1981). Other behaviors examined from *D. antillarum* show they prefer areas of high structural complexity and take shelter inside enclosed nooks of live or dead coral for protection (Bodmer et al. 2015). They may aggregate occasionally and will forage at night for macroalgae, since many hide for protection during the day (Carpenter 1984).

In 1983, scientists first reported a mass mortality event affecting *D. antillarum*. An underwater pathogen targeting *D. antillarum* spread an estimated 3.5 million km^2 throughout the Caribbean and Atlantic, killing more than 97% of the total population (Lessios et al. 1984; 2001). Recovery is still an ongoing process for *D. antillarum* in the Caribbean. For

both local and regional scales, *D. antillarum* populations at present remain only a shadow of their once dominant populations along Caribbean coral reefs (Carpenter and Edmunds 2006). They can still be seen at a variety of locations with some local habitats supporting relatively high densities and recovery rates, but not at levels higher than before the mass mortality event (Carpenter and Edmunds 2006). Because of this dramatic loss of *D. antillarum*, many reef habitats with once abundant corals now host higher amounts of macroalgae, a result of the loss of herbivorous trophic level control from a major keystone species (Carpenter and Edmunds 2006). Rates of successful recovery are thus variable and presumably controlled by a number of primary factors. This includes the success of connected larval supplies from *D. antillarum* from different locations (Cowen et al. 2006), the available rugosity a reef provides for urchins to find shelter (Bodmer 2015), and the amount of urchin predators a location supports which may prevent the maturation of *D. antillarum* (Harborne et al. 2009).

Some local habitats still support relatively high densities and recovery rates of *D. antillarum* (Carpenter and Edmunds 2006). For example, recruitment rates from Curaçao in 2005 were shown to be similar to the recruitment rates from 1982-1983, before the mass mortality event. Yet densities of *D. antillarum* at the same site still remain low compared to levels recorded before the die off (Vermeij et al. 2010). Comparing different locations exhibiting high and low densities of *D. antillarum* showed that the locations with lower densities had a 22-fold higher proportion of juveniles within the population (Bodmer et al. 2015). This evidence suggests recovery is likely to be related to post-settlement mortality.

A study done inside and outside of a reserve in the Bahamas found predator biomass affects *D.* antillarum density by describing the expected negative correlation between predator biomass and prey density (Harborne et al. 2009). Therefore, to investigate causes of post-settlement mortality, this study focused on predation pressure on *D. antillarum* by fishes of various trophic levels at two distinct times: day and dusk. Predation was assumed to be associated with the behavior of *D. antillarum* as it is a nocturnally active herbivore and could overlap with higher predation intensity at dusk (Carpenter 1984). Dynamics of predator-prey relationships within the coral reef community often adjust based on time of day (Bosiger and McCormick 2014). Diel migratory movement is an innate behavior for many reef species that perform different functions at different times in the day (Bosiger and McCormick 2014). Diel periods account for behavioral changes and abundance among urchins and their predators during times of day that would affect the data collected if gathered from only a single point in time during the diel cycle. Therefore, this study observed the predatory and prey behaviors at day and dusk.

The purpose of this study was to provide an assessment of the biomass of *D. antillarum* predators during two distinct times of day to provide insight into recovery limiting levels of post settlement mortality as seek a result of predation. This study aimed to provide additional information within the local context of a single coral reef site that may contribute toward a broader perspective of *D. antillarum* success in relation to levels of predation. The aim was to compare these two factors in tandem with observable changes in diel behavior to form a predicted negative correlation between *D. antillarum* density and weighted predator biomass. Thus, the hypotheses were as follows:

H_1: Areas with greater amounts of *D. antillarum* predator biomass will have lower densities of *D. antillarum*

H_2: Lower amounts of *D. antillarum* predator biomass and higher densities of *D. antillarum* will be seen at dusk

Materials and methods

Study site

All research was conducted at the Yellow Sub dive site on the western coastline of Bonaire, an island that is part of the Dutch Caribbean (12°09'36.5" N 68°16'54.9" W; Fig. 1). The habitat is characterized by a typical fringing reef, with small sand and rubble patches along the slope. All data was collected at a depth of ~10 m. The location is prone to frequent visits by recreational divers because of its close proximity to dive shops. The coastline is occupied by urban residential homes, hotels, and apartments; commercial fishing is not allowed due to Yellow Sub and the rest of Bonaire's coastline being protected as a marine park.

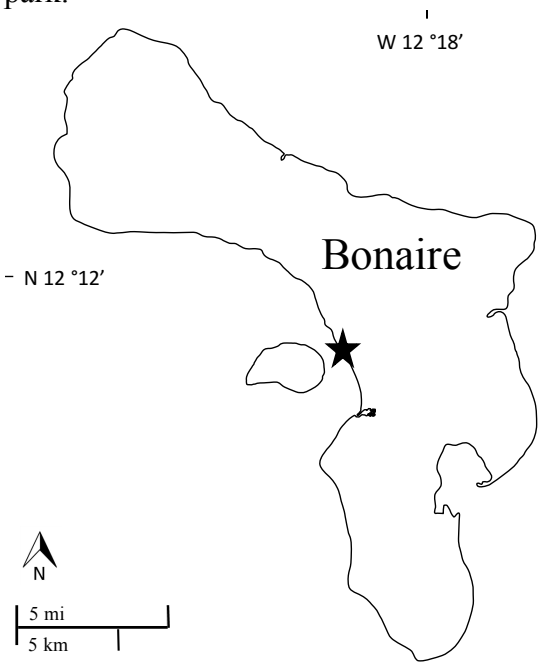

Fig. 1 The Dutch Caribbean island of Bonaire marked with a star to indicate the dive site of Yellow Sub (12°09'36.5" N 68°16'54.9" W) located on the sheltered, westward side of the island

Data collection

Data was collected between late September and October 2016. All data was collected using eight 30 x 4 m belt transect surveys during the day within the hours of 1100-1300 and eight 30 x 4 m belt transect surveys during dusk within the hours of 1700-1900. Transect locations were picked at random going directly off the Yellow Sub site entry out to the reef slope at depths of ~10 m.

Data was gathered from the transects in two passes, the first pass was done to collect data on fish type, species size, and abundance. The second pass was done to collect data on *D. antillarum* size and abundance.

For the first pass, predator fishes were surveyed over belt transects following a two-minute acclimation period. T-bars were used with two divers on each side of the transect recording data. Target fish were marked down by name if they entered the transect and their size was estimated to the nearest centimeter. Fish biomass was calculated from the Bayesian fish biomass formula ($a \times length^b$) with 'a' and 'b' values obtained from FishBase. Target fish species were taken from a specific list of known *D. antillarum* predators examined by Randall et al. (1964) which allowed for the weighing of predator biomass by frequency of collected fish with *D. antillarum* remains (Table 1). As an example to the reasoning behind this methodology, only 2% of *Haemulon sciurus* caught from Randall et al. (1964) were found to have *D. antillarum* remains as compared to 19.23% of *Bodianus rufus*. Therefore, the biomass of *B. rufus* would be weighted more heavily than the biomass of *H. sciurus* when the results were ready to be analyzed, providing a more accurate analysis of predator biomass since not all predators of *D. antillarum* have the same preference for *D. antillarum* consumption.

Scientific name	Common name	% with *D. antillarum* remains (n)	Biomass weighting
Haemulon sciurus	Blue striped grunt	2.00 (50)	0.033
Diodon hystrix	Porcupinefish	2.70 (27)	0.061
Spheroides splengleri	Bandtail puffer	7.14 (14)	0.117
Calamus calamus	Saucereye porgy	7.69 (13)	0.126
Haemulon carbonarium	Caesar grunt	8.33 (24)	0.137
Haemulon plumieri	White grunt	10.53 (19)	0.173
Trachinotus falcatus	Permit	12.50 (8)	0.205
Lactophrys bicaudalis	Spotted trunkfish	14.29 (7)	0.235

Bodianus rufus	Spanish hogfish	19.23 (26)	0.316
Halichoeres radiatus	Puddingwife	22.73 (22)	0.373
Canthidermis sufflamen	Ocean triggerfish	25.00 (4)	0.410
Anisotremus surinamensis	Black margate	38.89 (54)	0.638
Calamus bajonado	Jolthead porgy	40.00 (10)	0.657
Haemulon macrostomum	Spanish grunt	48.15 (27)	0.790
Balistes vetula	Queen triggerfish	60.92 (87)	1.000

Table 1 List of target species, *D. antillarum* predators, looked for during all transects, the number of fishes containing *D. antillarum* remains as quantified by Randal et al. (1964), and the biomass weighting of fishes as calculated by Harborne et al. (2009)

The second pass of the belt transect was used to determine the abundance and size of *D. antillarum* within the 30 x 4 m area. The number of *D. antillarum* were counted per transect. *Diadema antillarum* density was calculated from the number of individuals seen per 120 m² and adjusted to density of individuals per 100 m². Each *D. antillarum* was then categorized as a juvenile (< 10 cm) or an adult (≥ 10 cm). Size was recorded by placing a ruler next to each individual urchin, with many urchin lengths only partially estimated because of their positions inside of the reef structure. Data was gathered to determine the stage of their maturity (Randall et al. 1964; 1984). Searching for *D. antillarum* involved looking through tiny crevices and holes in the reef structure, where the majority of *D. antillarum* were found. Lights were used at dusk to count and measure *D. antillarum* since many of the structural openings that hide urchins are otherwise too dark to see because of the low light conditions.

Data analysis

Mean *D. antillarum* density at dusk and day was compared using a statistical t-test.

A Pearson correlation was used to compare fish weighted biomass and *D. antillarum* density. Mean predator biomass at dusk and day was compared using a statistical t-test.

Results

Diadema antillarum density

Diadema antillarum was recorded in 11 of the 18 total transects completed at Yellow Sub and a total of 35 observations of *D. antillarum* were made. The average density of *D. antillarum* from all 18 transects was 1.6 ± 2.0 per 100 m^2. *Diadema antillarum* density from the 9 transects during the day was 1.2 ± 1.1 per 100 m^2 and density from the nine transects during dusk was 2.0 ± 2.7 per 100 m^2. Average population density was higher at dusk rather than at day, but this difference was not significant (t = -0.864, df = 11, p = 0.407; Fig. 2).

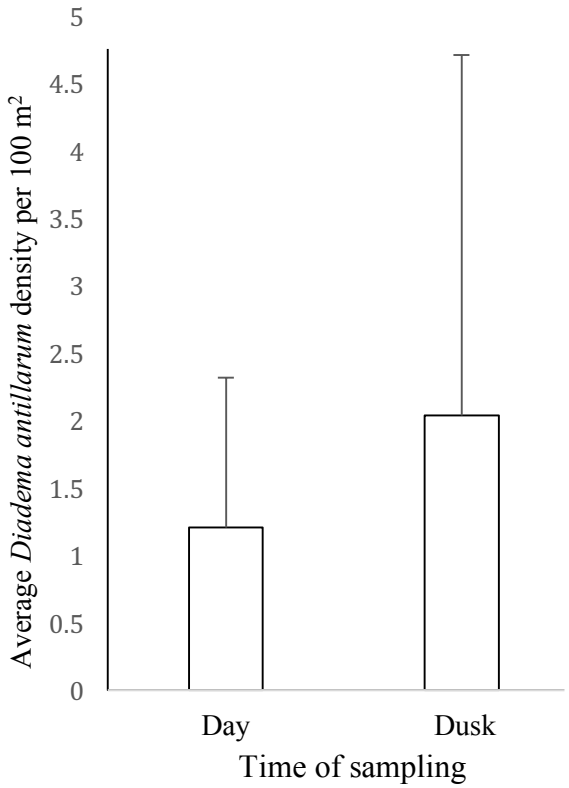

Fig. 2 Average *Diadema antillarum* density (±SD) at Yellow Sub compared at daytime and dusk per 100 m^2 (n = 9)

Diadema antillarum population dynamics

Data collected on *D. antillarum* size revealed that the majority (82.9%) of urchins observed were juveniles (< 10 cm) (Fig. 3a). During the day, no adult (≥ 10 cm) *D. antillarum* were

recorded. During dusk, 72.7% of *D. antillarum* observed were juveniles (Fig. 3b).

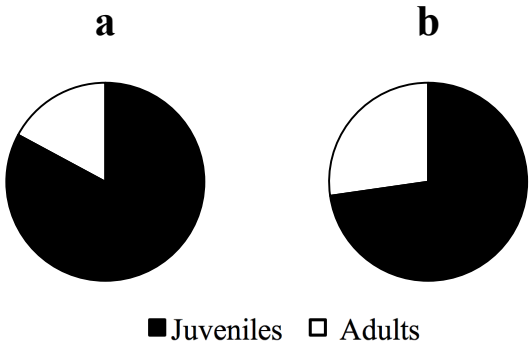

Fig. 3 The percentage (b) of juvenile (< 10 cm) and adult (≥ 10 cm) *Diadema antillarum* observed at Yellow Sub during dusk (n = 9). The percentage (a) of juvenile (< 10 cm) and adult (≥ 10 cm) *Diadema antillarum* observed at Yellow Sub during all observations (n = 18)

Predator biomass and *Diadema antillarum* density

A scatterplot showing weighted *D. antillarum* predator biomass and *D. antillarum* density tested with a Pearson correlation showed no significant trend in the data ($R^2 = 0.0004$, p = 0.938; Fig. 4). One transect containing the weighted predator biomass of a black margate was removed as it was an outlier in the data and skewed the representative data.

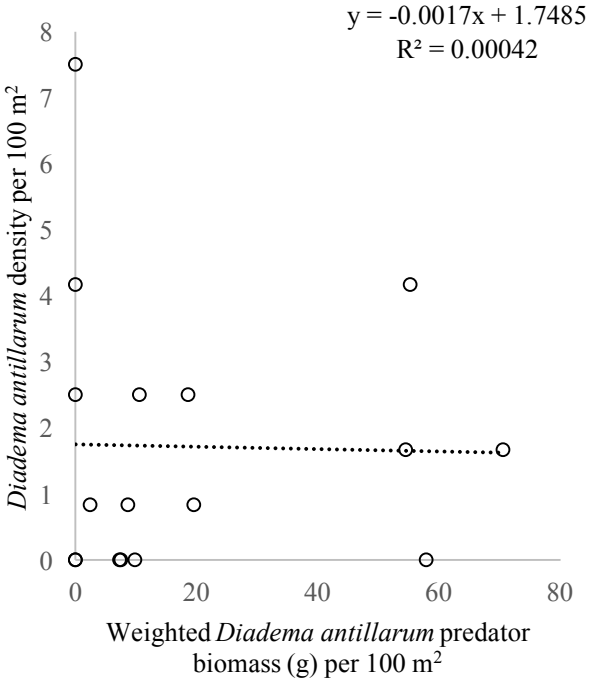

Fig. 4 Weighted *D. antillarum* predator biomass compared with *D. antillarum* density as recorded per transect, with one instance of outlier data removed, adjusted per 100 m^2 (n = 18)

Weighted vs unweighted predator biomass at dusk versus day

Both weighted and unweighted predator biomass was higher at dusk than at day. However, there was neither a significant difference in unweighted biomass compared between day and dusk (t = -0.287, df = 12, p = 0.779; Fig. 5), nor was there a significant difference in weighted biomass between day (17.73 ± 22.58 per 100 m^2) and dusk (69.11 ± 148.64 per 100 m^2)(t = -1.025, df = 8, p = 0.334; Fig. 5).

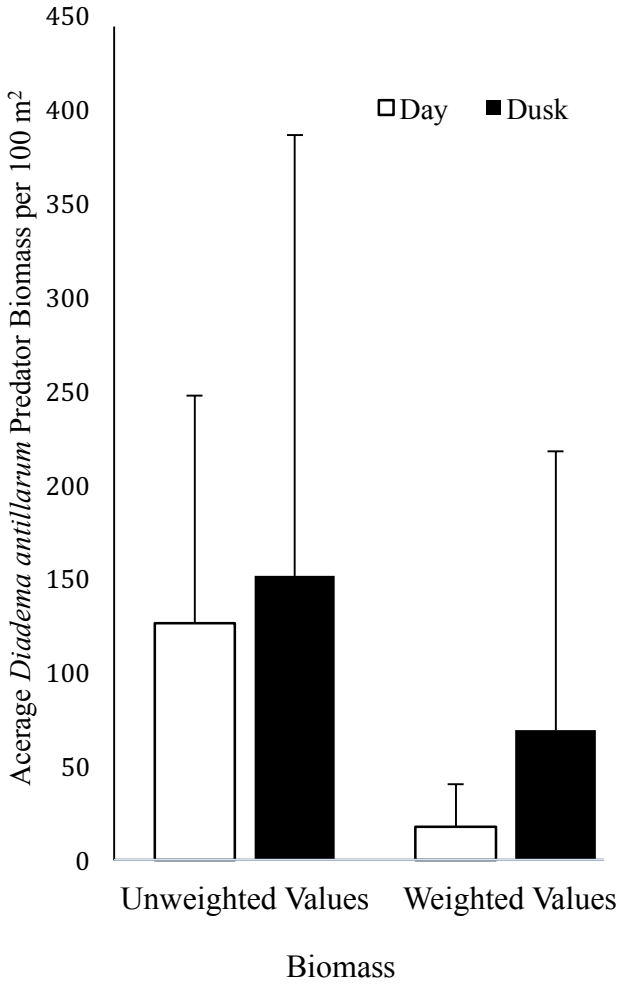

Fig. 5 The average weighted and unweighted values of *Diadema antillarum* predator biomass (±SD) at Yellow Sub compared at daytime and dusk (n = 9)

Diadema antillarum predator population

Of the 15 predators that were included in the survey, only six were identified within the data collection area: the bandtail puffer (*Sphoeroides spengleri*), puddingwife (*Halichoeres radiatus*), spotted trunkfish (*Lactophrys bicaudalis*), blue striped grunt (*Haemulon sciurus*), Spanish hogfish (*Bodianus rufus*), and black margate (*Anisotremus surinamesis*) (Fig. 6). The Spanish hogfish and blue striped grunt were the most abundant predators. When weighting factors based on *D. antillarum* consumption were considered, the Spanish hogfish and black margate had the highest weighted biomass.

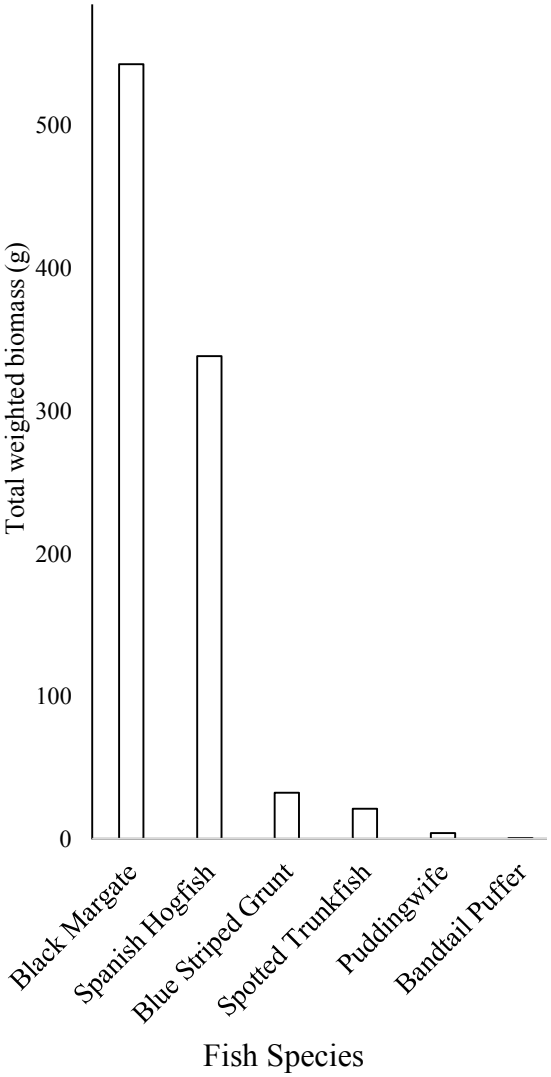

Fig. 6 Total weighted biomass (g) of all six observed *Diadema antillarum* predators at Yellow Sub calculated per fish species (n = 18)

Discussion

Diadema antillarum density was low on the reef slope at Yellow Sub. This value comes close to total average *D. antillarum* density recorded by Steneck et al. (2013) (0.012 ± SE 0.004 per 1 m^2) from an average of 11 different sites in Bonaire that did not include Yellow Sub. From this it can be determined that Yellow Sub does not differ in *D. antillarum* density when compared to the rest of Bonaire's western coastline. This observation of low *D. antillarum* density is common for many coastal coral reefs throughout the Caribbean as populations tend to be clustered in a few small locations or spread widely throughout the reef slope (Vermeij et al. 2010). Observations of *D. antillarum* are common in shallow waters near the coastal edge of the back-reef around Yellow Sub. In coastal zones North of Yellow Sub near Karpata however, snorkeled observations found densely grouped patches of *D. antillarum* in shallow sections of water. These patches were higher in observable density than anything seen around Yellow Sub. *D. antillarum* grouping behavior is positively correlated with higher levels of predator abundance and negatively correlated with urchin density (Carpenter 1984). It may be that different locations in Bonaire are prone to varying urchin behaviors that might be a result of *D. antillarum* predator abundance.

There was no significant difference between the lower observed *D. antillarum* densities at day versus the higher observed densities at dusk. Further data collection might prove to be significant and should be subject to further study. This slight increase in *D. antillarum* density at dusk could be related to their behavior. *Diadema antillarum* have been observed to forage for macroalgae more frequently at night since they mostly hide during the day (Carpenter 1984). They are sensitive to light exposure which is why they are commonly found hiding beneath cracks and under crevices (Carpenter 1984). Taking their nocturnal behavior into account, there may not

be an actual change in *D. antillarum* density at all and they are simply more difficult to observe during the day. Alternatively, *D. antillarum* may be moving to the reef slope at dusk to forage on macroalgae.

The majority of *D. antillarum* observed were smaller juveniles. No adult *D. antillarum* were observed on the transects during the day although a few were seen off the transects near the top of the reef crest, in between mooring blocks, or in less than a few meters of water under debris. This could indicate that the majority of *D. antillarum* on the reef slope do not survive until maturity and are subject to high levels of post settlement mortality (Bodmer et al. 2015). Besides predation, limited hiding spaces available to accompany a fully grown *D. antillarum* due to low rugosity may be inhibiting size potential for the urchins (Bodmer et al. 2015). Data from Steneck et al. (2013) showed that the trend of high ratios of juveniles to adults is not a new phenomenon for Bonaire and suggests that this has been common for the last 15 years. Thus, current conditions on Bonaire suggest factors inhibiting *D. antillarum* populations from returning to pre-die-off numbers due to post settlement mortality have remained mostly consistent.

There have been several studies showing a negative trend in *D. antillarum* density when compared to weighted *D. antillarum* predator biomass (Harborne et al. 2009; Steneck et al. 2013). Assuming different transects between dusk and day would vary in species composition, it was hypothesized that there would be a negative trend between urchin density and a weighted predator index as seen from the other studies. The Pearson correlation model produced however was inconclusive and not statistically significant. Interestingly, the linear model produced from Steneck et al. (2013) comparing FPA (Fish Protected Area) sites with non-FPA sites using the same comparison of *D. antillarum* density and an Urchin Predator Index (similar to weighted biomass) produced insignificant results as well. In the case of this study, there was no significant difference in weighted biomass or density between day and dusk which may have resulted in the insignificant negative trend between weighted biomass and density due to a lack in variability between day and dusk. It could also be, as the work in Steneck et al. (2013) supports, that the negative trend is an ineffective determinant in the relationship between *D. antillarum* and their predators. It could be that *D. antillarum* populations are at present too small to express any type of negative trend concerning their predators within the geographic extent of Bonaire.

Diadema antillarum predation was attributed most to the black margate and second to the Spanish hogfish. The black margate was only seen once on a single transect but because of its large body size and high weighting factor for consuming *D. antillarum*, it contributed the most weighted predator biomass. Unlike the black margate, spotted trunkfish were seen a total of four times along transects. Their minimal impact on weighted biomass comes from their relatively small body size compared to the other fish species and consumption of *D. antillarum*. As day transitions to dusk and then to night, diel behavioral changes occur amongst reef fish that follow a pattern of migration in examples such as resting to eating. It is known that grunts exhibit nocturnal foraging behaviors for invertebrates (Burke 1994). Although there were more blue striped grunts observed during the day, data from Burke (1994) still suggests there could be a behavior that overlaps advantageously with *D. antillarum*'s nocturnal foraging habits (Carpenter 1984). This may also explain the increase in average weighted predator biomass during the hours of dusk as compared to hours of day for the diel behaviors of other *D. antillarum* predator species.

Recovery of urchins at Yellow Sub and throughout Bonaire remains a relevant issue that must be addressed if we are to recuperate coral reefs. Looking further into small population booms for *D. antillarum* with densities of up to an average 178 ± 38.74 individuals per 100 m^2 in locations like the Banco Capiro reef may be the key in understanding the recovery of urchins in terms

of habitat and structural complexity (Bodmer et al. 2015). If structural complexity is the primary component to urchin recovery as Bodmer (2015) suggests, then it could be that the threshold for coral reef complexity on Bonaire is not at a level capable of sustaining a fully recoverable population of *D. antillarum*. This would also suggest that the presence of *D. antillarum* predators are more independently associated with urchin density due to their smaller and less mature population sizes. Regardless of causation however, the pathogen that targeted *D. antillarum* in 1983 had a massive effect on the overall health of coral reefs (Lessios 1984). *D. antillarum*, a keystone herbivorous species essential for maintaining low levels of macroalgae (Edmunds and Carpenter 2001) was nearly wiped out within a matter of a year. Although *D. antillarum* no longer exists within its former levels of success throughout the Caribbean, it continues to persist and occupy many places it once did in the past (Carpenter and Edmunds 2006). Populations of *D. antillarum* are struggling to recover and yet continue to persist around many coral reefs. So, for the sake of improving coral reef health, an invaluable resource for millions of individuals, human kind must persist in trying to understand how to continually improve upon supporting coral reefs, and the planet.

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REPORT

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The effects of varying algae cover on fish species diversity below the reef crest

Abstract Corals reefs are experiencing a period of extreme change as algae are slowly becoming the dominant benthic organisms on the reef. Without important grazers and limited nutrients to keep them in check, the growth of algae is largely uninhibited. As algae biomass increases, it outcompetes coral species and reduces the structural complexity that has allowed coral reefs to become the most diverse ecosystems in the ocean. While there is abundant research examining how herbivorous fish populations are adjusting to increasing algae cover, there is little information on how reef fish diversity is affected as a whole. This study focused on the effect of increasing algae cover on fish species diversity as well as fish community structure. Additionally, it examined whether herbivorous fish species are flourishing in environments with increased algae cover. First, it was determined that fish biomass and diversity were higher in areas with low algae cover. Secondly herbivore, piscivore, planktivore and invertivore abundance increased as algae cover decreased. This data indicated that fish have a preference for areas of low algae cover. Further algae growth and subsequent reef deterioration could reduce viable reef fish habitat and reduce species diversity and total population. A deterioration of the reef on a global scale would directly impact the livelihoods of millions and indirectly effect the majority of the world's population.

Keywords Fish diversity • algae • coral reef

Introduction

Coral reefs provide far more than biodiversity. Globally, more than 500 million people live in close proximity to coral reefs, many of whom rely on them for food, employment, and recreation (Costanza et al. 1997). The deterioration of structural complexity in conjunction with lower densities of several groups of important marine organisms is a serious concern (Graham and Nash 2013). It is important to remember that substantial changes in either of these factors would affect the food supply and job security of millions of people worldwide (Jackson et al. 2001).

Since the mass die-off of the Caribbean sea urchin, *Diadema antillarum*, in 1983 and 1984 Caribbean coral reefs have been struggling to maintain homeostasis as algae biomass continues to increase (Lessios et al. 2001; Macia et al. 2007). Phase shifts from coral dominated to algal dominated reefs are taking place on numerous islands in the region and scientists are concerned that once a shift has taken place it will become exceedingly difficult for reefs to return to their previous state (McManus and Polsenberg 2004). In addition to the urchin die-off, other human stressors have exacerbated algae growth and accelerated phase shifts (Mora 2008). Nutrient pollution from farming and waste water is of particular concern. The Caribbean waters are naturally nutrient poor which limits algae growth, however nutrients from fertilizer and animal waste shift this balance and algae growth is no longer limited by nutrient deficiency (Aronson 2001). The issue with this phase shift is that stony coral reefs provide an abundance of habitat for thousands of fish species, largely due to their complex three dimensional shape (Graham and Nash 2013). Overgrowth of algae prevents adequate coral calcification, and structural complexity of the reef slowly deteriorates (Graham and Nash 2013). Branching corals, *Acropora spp.* in particular,

provide some of the most structurally complex habitat on the reef and are the preferred habitat of a variety of fish species (Brooker 2013). These coral types are also typically more adversely affected by external stressors and are therefore more likely to disappear from the reef (Brooker 2013). There is strong evidence that algae overgrowth is preventing corals from growing at their normal rates as well as negatively affecting food web resilience (Pereira et al. 2014; Carmichael and Boyer 2016). Without an effective consumer to keep their population in check, algae outcompete coral by growing faster and therefore decreasing much of the available sunlight (Conklin and Stimson 2004). After the die-off of *D. antillarum*, herbivorous fish began to consume far more algae (Sotka and Hay 2009). The populations of many herbivorous fishes have grown due to a decrease in competition from the absence of *D. antillarum* (Carpenter 1988). However, this increase in herbivory is not enough to prevent continued overgrowth of algae populations (Carpenter 1988).

This particular study focused on the relationship between algae and fish species diversity as well as how herbivorous fish populations responded to increased algae cover. *Orbicella annularis,* was chosen as the benthic organism on which data was to be collected. This coral has a non-continuous structure, creating numerous protected interior pockets that shelter fishes (Weil and Knowton 1994). Numerous coral heads were examined and the following hypotheses were tested:

- H_1: Areas with higher algae cover will have lower species diversity.
- H_2: Areas with higher algae cover will have a higher abundance of herbivorous fish.
- H_3: Areas with higher algae cover will have lower fish biomass in all fish functional groups observed (herbivore, invertivore, piscivore, planktivore) with the exception of herbivores, whose biomass will increase with algae cover.

As previously stated, algae's effects are well documented and have been found by some to affect reef structure and fish distribution more than any other biotic factor (Pereira et al. 2014). In addition, links have been observed between herbivorous fish and reef community structure. Burkepile and Hay (2008) found that herbivorous fish aid in coral recruitment. Understanding the global impacts of increased algae is of vital importance. The biodiversity of coral reefs has few parallels in the natural world. A deteriorating benthos could drastically reduce not only the biodiversity of coral reefs but also create circumstances in which it is unlikely they will be able to full recover from deterioration (McManus and Polsenberg 2004; Bellwood et al. 2006).

Gathering information on the effect of expanding algae biomass on coral reefs and its subsequent effect on reef fish will aid in convincing commercial interests as well as the general public that action needs to be taken to maintain structural complexity and fish diversity on the reef. While there is a fair amount of research examining how changing algae communities have effected herbivores, the data on other fish groups is limited. The intention of this study was to draw attention to the effects of increased algae cover and strengthen the argument that this change in habitat structure will detrimentally effect both the reef's fish populations and commercial interests dependent on it.

Materials and methods

Study site

Data was collected on Bonaire, a tropical desert island in the Dutch Caribbean approximately 90 km off the north coast of Venezuela. The island has a fringing reef with the reef crest beginning at approximately 8 m depth. Collection took place across the Yellow Submarine (12°09'36.47''N, 68°16'55.44''W) and Something Special (12°09'41.50''N, 68°17'00.8''W) dive sites located adjacent to

one another on the western side of Bonaire, in between the main island and a smaller island named Klein Bonaire (Fig. 1). The sites were selected for their ease of access as well as the abundance of *O. annularis*, algae and fish species.

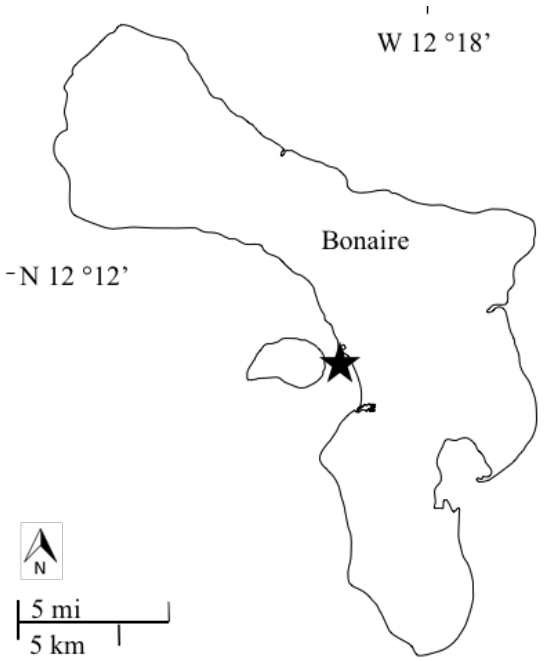

Fig. 1 Island of Bonaire. Located 90km north of Venezuela in the Dutch Caribbean.

Data collection

Quadrats were placed approximately 25 m from shore at depths ranging from 8 m to 12 m. Two divers swam in a line, parallel to shore, looking for *Orbicella annularis.* coral colonies with varying levels of algae cover. Replicates were found for low algae cover (0-33%; n = 5), moderate algae cover (33-66%; n = 9) and high algae cover (66-100%; n = 6). When a coral head was chosen, its algae cover was estimated using a 1 m^2 quadrat divided into 100 equally sized squares in its interior. The quadrat was placed flat on top of the coral head and the percent cover of algae was estimated using the number of interior squares that were more than 50% full of algae. Next, the divers gently removed the quadrat and fastened another 1 m^2 quadrat, without interior divisions, in the exact place of the first by weighting each corner of the quadrat. A PVC pipe was attached to one corner of the quadrat so that it extended 0.5 m toward the surface of the water and 0.5 m below the quadrat towards the reef. This delineated the upper and lower limits of the data collection zone. After this, the divers moved on to find a different coral head for the next replicate, allowing 10 min to pass as fish acclimated to the recently placed quadrat. At the end of this 10 min period the divers returned to the quadrat and began recording the species of fish they observed. Fish were only recorded upon entering the borders of the 3 dimensional data collection zone created by the quadrat and PVC pipes. Additionally, in order for fish to be recorded, they needed to stay in the data collection zone for more than 3 sec in an attempt to remove transient fish from the data. Once the survey began, each diver was responsible for recording the species, quantity, and size of fish on their pre-assigned data sheet. Each fish was classified into its respective functional group based on diet. Omnivorous fish were grouped by their primary prey or added to two functional groups if they consumed similar quantities of two different food categories. Data collection took place for 5 min at the end of which the divers gently removed the quadrat and moved on to the next coral head.

Data analysis

All analyses examined the differences and patterns between varying amounts of algae cover. Shannon diversity index values (H) were calculated for each quadrat and compared to algae cover values. A linear regression was used to determine whether this relationship was statistically significant. A linear regression examined the relationship between the total fish biomass and algae cover of each quadrat. An ANOVA single factor analysis was used to determine whether high (66-100%), medium (33-66%) and low (0-33%) algae cover groups had any effect on the biomass, size distribution and abundance of different fish functional groups.

Results

Species diversity

A total of 352 fish were recorded across the 20 quadrats surveyed during data collection. Among these fish, 36 different species were recorded. These species were members of 17 different families, the most numerous being the Pomacentridae family of which there were 247 individuals recorded. A negative linear relationship was found between algae cover and Shannon diversity index values (H) (R2 = 0.113, p = 0.150; Fig. 2).

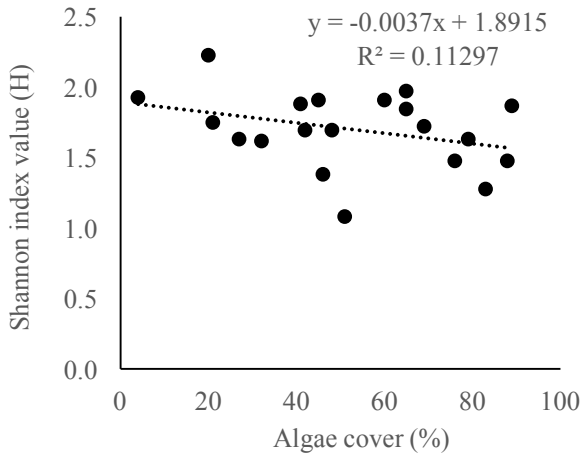

Fig. 2 Comparison of Shannon diversity values (H) with corresponding algae cover (%).

A linear regression also revealed a statistically significant relationship between fish biomass and algae cover percentage (R^2 = 0.265, p = 0.020; Fig. 3). As algae cover increased, fish biomass per quadrat decreased (Fig. 3).

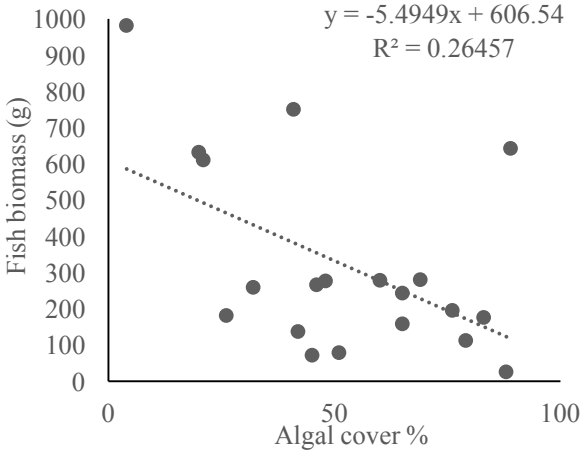

Fig. 3 Comparison of fish biomass (g) in each quadrat and algae cover (%) in each quadrat.

Size classes

Fish from 6-10 cm were the most numerous with 169 individuals recorded. The next most common size class was 0-5 cm with 119 individuals. The larger size classes had comparatively fewer fish with 57 individuals in the 11-20 cm size class and 7 in the >21 cm size class. Between algae cover groups, as algae cover increased, the abundance of fish increased as well for size classes 0-5 cm (F = 3.494, df = 2,17, p = 0.054), 11-20 cm (F = 2.991, df = 2,17, p = 0.077) and 21->40 cm (F = 1.609, df = 2.17, p = 0.229) (Fig. 4). The 6-10 cm size class was the only exception to this pattern as the difference between its 33-66% and 66-100% abundance values was minimal (F = 0.270, df = 2,17, p = 0.766; Fig. 4).

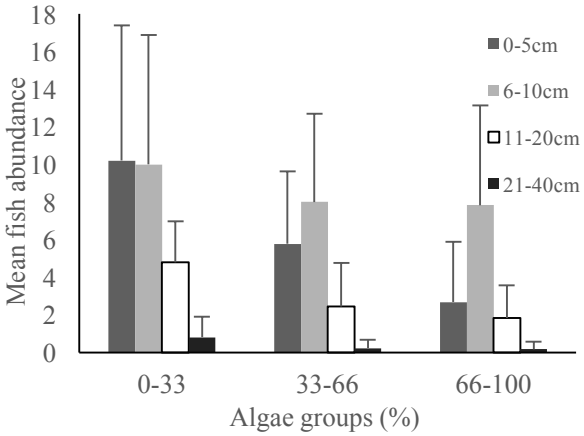

Fig. 4 Comparison of fish abundance to algae cover groups (%) by fish size class. Error bars represent standard deviation.

Functional groups

Among the functional groups (invertivore, piscivore, herbivore and planktivore) the most abundant were the herbivores with 139 individuals recorded. Planktivores were recorded at a slightly lower abundance with a total of 122 total individuals followed by invertivores and piscivores with 72 and 41 recorded respectively. The total of these values was slightly higher than the previously mentioned fish total as some species were counted in two functional groups due to the

variety of their diet. The biomass of each functional group was assessed at low, medium and high algae cover groups. Piscivore and planktivore biomass increased as algae cover decreased (piscivores: $F = 2.513$, $df = 2,17$, $p = 0.111$; planktivores: $F = 1.073$, $df = 2, 17$, $p = 0.364$; Fig. 5). Herbivore and invertivore biomass decreased as algae cover increased from 0-33% to 33-66% but increased again at 66-100% (herbivore: $F = 0.795$, $df = 2, 17$, $p = 0.468$; invertivore: $F = 1.518$, $df = 2, 17$, $p = 0.247$; Fig. 5).

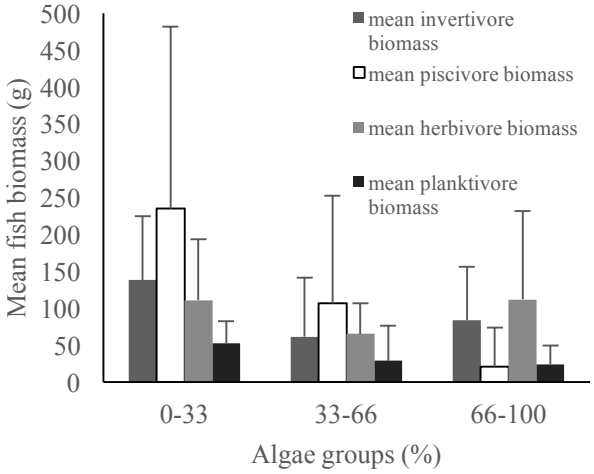

Fig. 5 Comparison of fish biomass to algae cover groups (%) across functional groups. Error bars represent standard deviation.

Fish abundance increased in relation to decreasing algae cover for invertivores ($F = 0.349$, $df = 2, 17$, $p = 0.710$), piscivores ($F = 2.890$, $df = 2, 17$, $p = 0.083$) and herbivores ($F = 1.248$, $df = 2, 17$, $p = 0.312$)(Fig. 6). Planktivores also showed this increase in abundance as algae cover decreased and was the only functional group that had a statistically significant relationship ($F = 4.513$, $df = 2.17$, $p = 0.027$; Fig. 6).

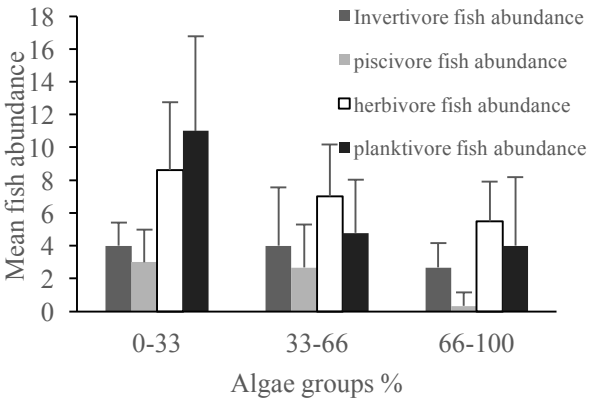

Fig. 6 Comparison of fish abundance to algae cover groups (%).Error bars represent standard deviation

Discussion

This study provides support for the claim that fish prefer habitats with low algae cover rather than high algae cover as fish biomass was significantly higher in areas of low algae cover. This was not simply due to a few common species skewing the trend as it was consistent across all functional groups. While it may be the case that fish biomass increased as algae cover decreased, the evidence that species diversity increased with decreased algae cover is less strong. While diversity estimates were found to increase with decreased algae cover, the trend was not found to be significant. However, this trend in conjunction with decreased diversity and fish abundance across all observed functional groups, could mean that fish are more successful in areas of low algae cover. For example, previous studies have found that young reef fish are less susceptible to predation in algae dense areas but are unable to forage or grow as efficiently as in reef habitats (Dahlgren and Eggleston 2000). It could be that fish associate these areas of heavy algae cover with lower abundance of food and typically avoid them. It could also be that because the fish on the reef are no longer juvenile, having spent their juvenile stages in other habitats (Nagelkerken 2000), they are less susceptible to predation. In this case, the fish no longer need algae for protection and will spend the majority of their time in areas with better foraging (Schlosser 1988).

Contrary to what was originally hypothesized, the abundance of herbivorous fish decreased as algae cover increased. While there is evidence that algae is one of the major determinants of reef structure (Pereira 2014), herbivore distribution has also been found to be influenced by proximity of shelter, predator abundance and density of territorial competitors (Lewis and Wainwright 1985). It could be that due to the great abundance of algae on Bonaire (Bak 2005) food availability is not a major concern for herbivores. This could make them more prone to staying in areas with an abundance of other fish, reducing their susceptibility to predation (Major 1978). While abundance of herbivorous fish did decrease with increasing algae cover the total herbivore biomass actually increased from medium (33-66%) to high (66-100%) algae cover plots. This would indicate that while the majority of herbivores did prefer lower algae cover there were a few larger and heavier herbivorous fish that brought the average biomass up in the high algae cover plots. Larger herbivores grazing in the areas of higher algae cover further strengthens the theory that smaller herbivores stay away from these areas because of increased susceptibility to predation (Schlosser 1988).

The hypothesis that all fish functional groups, with the exception of herbivores, would decrease in biomass as algae cover increased was not supported by the data collected. The potential reasons for the patterns in herbivore biomass were discussed above. Invertivore biomass decreased from low (0-33%) to medium (33-66%) algae cover but then increased from medium to high algae cover (66-100%) mimicking the pattern of the herbivores. Invertivore abundance, like the herbivores, decreased from low to high algae cover which would again indicate it was a few larger individuals foraging in the high algae cover quadrats that caused the increase in biomass from medium to high algae cover. This makes sense as invertivores often dig through the sand in order to find prey (McCormick 1995) and digging in an area with less invertivore competition would likely yield more food. However, because foraging in an area with less fish makes one more susceptible to predation (Major 1978) it is likely that only the larger invertivores would be able to do this safely. It stands to reason that piscivores and planktivores, both of whose abundance and biomass decreased as algae cover decreased, did not follow the same trend as herbivores and invertivores due to different feeding habits. Piscivores have a better chance of capturing prey in areas of high fish biomass as there is more prey available and planktivores often use particulate feeding in the water column (Lazzaro 1987) so staying around other fish is advantageous for them as their risk of being eaten is lower (Major 1978).

Much of the data collected in this experiment seems to indicate that reef fish have a preference for habitats with low algae cover. Fish of all functional groups were found in higher numbers when algae cover was lower. While some fish groups such as herbivores and invertivores could benefit from increased algae in the short term, a phase shift could undoubtedly be very detrimental to the population's health. As algae dominance spreads throughout the Caribbean, the ability of reef fish to adapt to changing environments will become increasingly apparent. If fish have a preference for reefs with low algae cover, it is likely that fish will migrate to the healthiest available section of their reef system thereby increasing competition for the already limited resources. A reduction in habitat may lead to a reduction in population size and genetic diversity. In order for regions to maintain healthy fish populations on their reefs, they must make environmental protection their number one priority.

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Physis (Fall 2016) 20: 16-23

REPORT

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Relationship between planktivore abundance and zooplankton abundance at two different depths in a Caribbean coral reef system

Abstract Zooplankton play an important role in the transfer of energy from primary production to higher trophic levels. The extent that resource availability (bottom-up) as opposed to predation (top-down) control plankton dynamics has been a topic of much debate. The effects of top-down and bottom-up controls on zooplankton populations have been shown to vary across both temporal and spatial scales. The extent to which top-down and bottom-up factors structure zooplankton communities has not been thoroughly explored in Caribbean coral reef systems. To investigate the effect of predation on zooplankton abundance in a Caribbean coral reef system, the relationship between planktivore abundance and zooplankton abundance was documented at two different depths (7.5 ± 1 and 15 ± 1 m). Zooplankton abundance was quantified using individual copepod counts and the dry weight of the sample. Planktivore abundance and planktivore diversity had no significant effect on zooplankton abundance at both depths. Although not statistically significant, copepod abundance decreased as planktivore abundance increased indicating that predation may have an effect on zooplankton abundance in Caribbean coral reef systems. Additionally, there was no significant difference in zooplankton abundance between the two depths. Though not measured, the data suggest that bottom-up factors regulate zooplankton abundance in Bonaire's coral reef system more than top-down factors. Further study of the relationship between planktivores and zooplankton abundance as well as resource availability is necessary to determine the extent of bottom-up versus top-down controls in shaping zooplankton community structure in Caribbean coral reef ecosystems.

Keywords Top-down • bottom-up • predation

Introduction

The relationship between plankton community structure and primary production was originally thought to be simple: resource availability (e.g. light and nutrients) controls plankton community structure (Verity 1998). Community structure, in this sense, refers to planktonic species abundance and distribution as well as interactions between different planktonic species. The assumption that resources such as light and nutrient availability control the patterns and processes in marine communities oversimplifies the complex nature of marine food webs (Verity 1998). The traditional notion that ecosystems are controlled by bottom-up factors (i.e. resource availability) has been controversial in the past few decades (Reid et al. 2000). While it is still accepted that bottom-up factors play a part in controlling aquatic community structure, the role of predation, or top-down controls is a subject of debate (Reid et al. 2000). Verity and Smetacek (1996) discussed the evolution of predation avoidance strategies among zooplankton as support for the idea that top-down controls may be equally important in structuring marine communities. Reid et al. (2000) suggested that planktivore-plankton interactions may at times modulate planktonic community structure, contradicting the

traditional notion that resource availability is responsible for plankton dynamics.

Zooplankton is a taxonomically and morphologically diverse group (Kiørboe 2011), ranging in size from microns to centimeters and meters (Bathmann et al. 2001). This group plays many important roles in the food web and includes both herbivorous and omnivorous species (Daewel et al. 2013) which function as consumers, producers, and prey in marine ecosystems (Bathmann et al. 2001). They also facilitate the transfer of energy from primary producers, like phytoplankton to species in higher trophic levels, like fish (Daewel et al. 2014).

The relationship between planktivorous fish and zooplankton has been explored in many marine and freshwater environments (Kingsford and MacDiarmid 1988; Lazzaro et al. 1992; Jeppesen et al. 1997; Daewel et al. 2014). Brooks and Dodson (1965) examined an example of predation affecting zooplankton; in lakes with intense predation the larger dominant crustaceans were replaced by smaller species. Daewel et al. (2014) determined that planktonic community structure is regulated more by predation when there is low species diversity and extreme environmental conditions (i.e. low salinity, low temperature) compared to when there is high species diversity and moderate environmental conditions. Although nutrients are largely responsible for zooplankton abundance, predation has been shown to affect their densities and composition (Kingsford and MacDiarmid 1988) as well as reduce species richness (Shurin 2001). Additionally, planktivore biomass regulates zooplankton abundance more than planktivore type (Lazzaro et al. 1992).

Pressures implemented by planktivores have influenced the evolution of diel vertical migration in zooplankton where zooplankton ascend in the water column during the night to feed on phytoplankton and avoid predators (Gliwicz 1986). Therefore, more zooplankton are expected to be observed at deeper depths during the day to avoid predators active in the water column during the day (Gliwicz 1986). On the other hand, Motro et al. (2005) found that planktivores are more abundant near the bottom compared to higher in the water column in order to avoid piscivores that forage in intermediate depths. Accordingly, Motro et al. (2005) found that plankton had a greater chance of survival higher in the water column compared to below 1.5 m from the bottom. The distribution of zooplankton populations are site specific depending on the pressures implemented by predation (Daewel et al. 2014).

This study aimed to explain the relationship between zooplankton abundance and planktivore abundance at two depths along a Caribbean coral reef in Bonaire, Dutch Caribbean. Little research has been conducted on the relationship between zooplankton and planktivores in the Caribbean. Thus, this study aimed to identify the effect of a top-down control, namely predation, on zooplankton dry weight, and copepod abundance by testing these hypotheses:

- H_1: During the day there will be more zooplankton at 15 ± 1 m than at 7.5 ± 1 m
- H_2: As planktivore abundance and diversity increases the abundance of zooplankton decreases
- H_3: Zooplankton dry weight is a sufficient proxy for copepod abundance

Materials and methods

Study site

This study was conducted at Yellow Submarine dive site (12°09’36.2”N, 68°16’55.2”W) on the western side of Bonaire, Dutch Caribbean just north of the island’s capital, Kralendijk (Fig. 1). Fish surveys and plankton collections occurred at 15 ± 1 and 7.5 ± 1 m along the fringing reef slope approximately 50 m off shore. The coral and fish diversity is relatively high and the most abundant planktivore at this location is the brown chromis (*Chromis multilineata*).

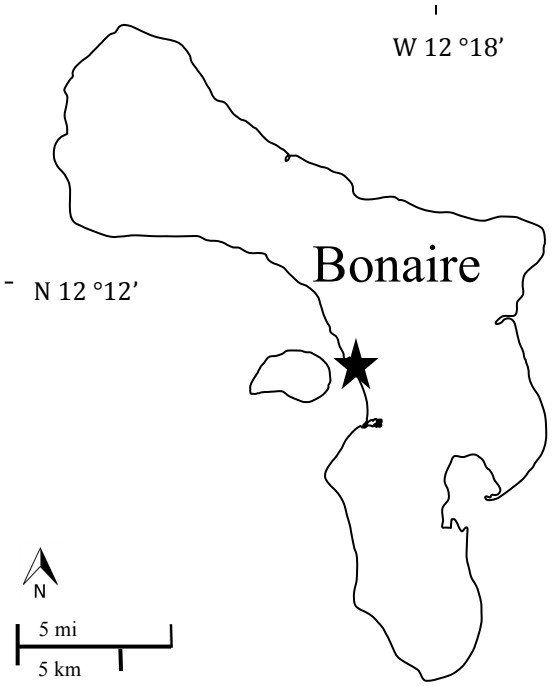

Fig. 1 Map of Bonaire, Dutch Caribbean, located in the Caribbean Sea. The study site at Yellow Submarine dive site (12°09'36.2"N, 68°16'55.2"W) is marked by a black star

Plankton collection

Zooplankton samples (N = 17) were taken at two different depths (15 ± 1 and 7.5 ± 1 m) on SCUBA throughout October 2016. The opening of the plankton nets had a diameter of 30 cm and a mesh size of 30 μm. The collection container attached to the end of the tow had a mesh size of 20 μm. Each net was towed along a 20 m transect filtering 1.42 m^3 (area of net opening × the length of transect) of seawater. Two tows were completed parallel to the shoreline during each dive, the first at a depth of 15 ± 1 m and the second at 7.5 ± 1 m. The plankton nets were stored in a waterproof bag to avoid contamination while moving between depths. After each tow the net was quickly twisted up to avoid contamination and placed back in its respective bag. To avoid plankton clinging to the mesh of the collection container, the zooplankton samples were submerged in a bucket of seawater upon reaching the shoreline for transport to the lab (time < 15 min). Additionally, 4 ml of magnesium chloride ($MgCl_2$) was added to the bucket to sedate the zooplankton.

Plankton analysis

After the samples were transferred to the lab, they were emptied into a jar and the mesh from the collection container was rinsed with filtered seawater to ensure that all of the organisms were transferred to the jar. To ensure proper sedation of the zooplankton, an additional 2 ml of $MgCl_2$ was added to the jar. Then, to fix and stain the sample, 100 ml of formalin mixed with rose bengal was also added to the jar. After 48 h the sample was poured through a 150 μm sieve in order to separate the phytoplankton from the zooplankton. Zooplankton were then rinsed from the sieve into a small glass vial with 70% ethanol. The zooplankton in the glass vial were then used for quantification of zooplankton abundance. Two methods were used to quantify zooplankton abundance: copepod abundance and dry weight. Copepods were individually counted under a digital stereo zoom microscope (Motic DM143). Copepods were chosen to represent zooplankton abundance due to their ease of differentiation from other zooplankton and their relatively large size which aided counting. Following the copepod count, each sample was transferred into an identical glass vial and placed in an oven at 60°C for 24 h (McCauley 1984) to obtain dry weight of the samples. The glass vials were weighed prior to the addition of the sample and following the 24 h drying time to the nearest 0.0001 g (Mettler Toledo XS205 dual range).

Planktivore survey

A planktivore assessment was conducted at every plankton tow site to measure the abundance of planktivores prior to the collection of the plankton samples. The planktivore assessment was created by combining planktivores from the AGGRA fish diversity survey and the REEF survey into a single survey. The fish included in the survey were the banded butterflyfish (*Chaetodon striatus*), blue chromis (*Chromis cyanea*), brown chromis (*Chromis multilineata*),

beaugregory (*Stegastes leucostictus*), bicolor damselfish (*Stegastes paritus*), cocoa damselfish (*Stegastes variabilis*), smallmouth grunt (*Haemulon chrysargyreum*), tomtate (*Haemulon aurolineatum*), bluehead wrasse (*Thalassoma bifasciatum*), creole wrasse (*Clepticus parrae*), and the creole fish (*Paranthias furcifer*). The survey was conducted along a 20 × 2 m transect parallel to the shoreline at both the shallow and deep depths. After the transect was placed, the surveyor waited three minutes to begin the survey. The number of each planktivore species observed within the 20 × 2 m transect was recorded.

Data analysis

A Student's t-test was used to determine if there was a statistically significant difference for mean dry weight and mean copepod abundance between the two depths. A linear regression was used to determine how plankton abundance (measured as copepod abundance and zooplankton dry weight) was affected by planktivore abundance. Planktivore diversity was calculated using Simpson's index of diversity and a linear regression was used to determine if planktivore diversity affected copepod abundance. A linear regression was also used to determine if dry weight is a sufficient proxy for copepod abundance. All data were reported as means ± SE, where appropriate, and factors were considered significantly different when $p < 0.05$.

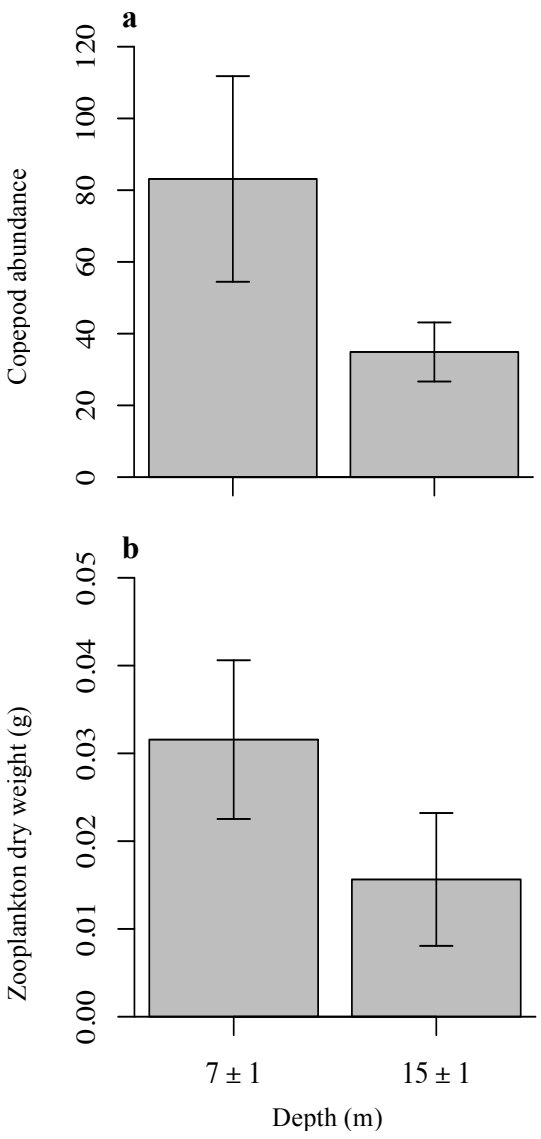

Fig. 2 Comparison of (a) copepod abundance and (b) zooplankton biomass at 7 ± 1 m (n = 8 and 6, respectively) and 15 ± 1 m (n = 9 and 8, respectively). Data are represented as means ± SE

Results

Zooplankton abundance at different depths

Copepod abundance was greater at the shallow depth (83.13 ± 28.67, n = 8) compared to the deep depth (34.89 ± 8.23, n = 9); however, there was no significant difference between abundance at the two depths (t = 1.62, df = 8.15, p = 0.144, Fig. 2a).

Likewise, zooplankton dry weight was greater at 7.5 ± 1 m (0.032 ± 0.0090) compared to 15 ± 1 m (0.016 ± 0.0076) but again there was no significant difference between the two depths (t = 1.35, df = 10.71, p = 0.205, Fig. 2b).

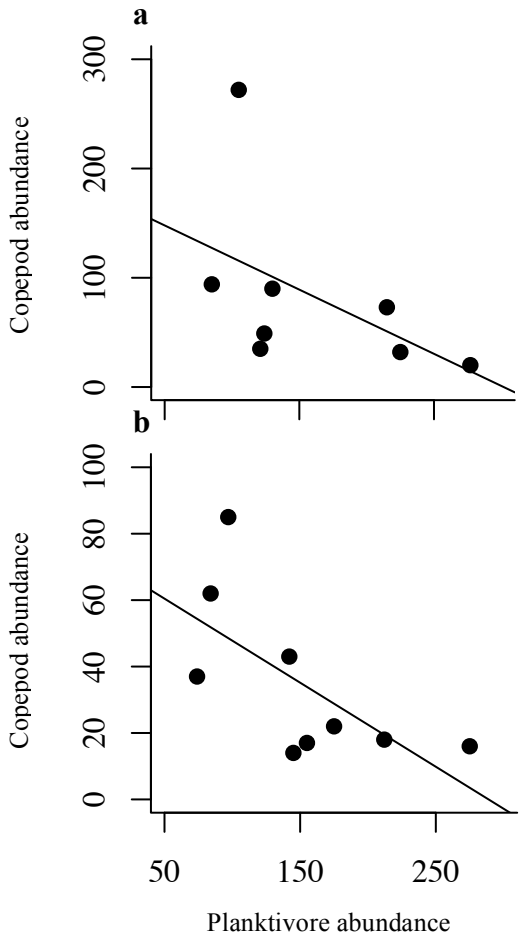

Fig. 3 Relationship between planktivore abundance and copepod abundance at (a) 7.5 ± 1 m (R^2 = 0.25, p = 0.207, n = 8) and (b) 15 ± 1 m (R^2 = 0.43, p = 0.053, n = 9)

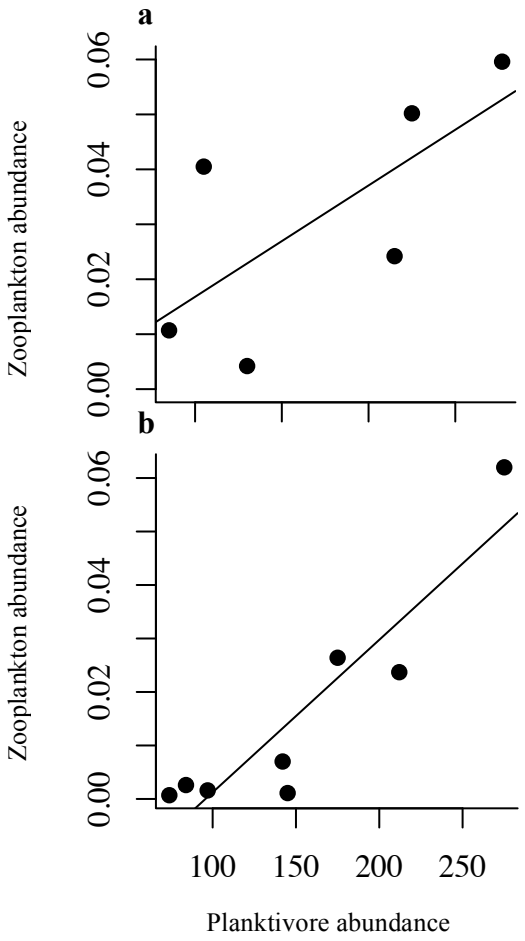

Fig. 4 Relationship between planktivore abundance and zooplankton abundance (measured as dry weight) at (a) 7.5 ± 1 m (R^2 = 0.50, p = 0.119, n = 6) and at (b) 15 ± 2 m (R^2 = 0.84, p < 0.005, n = 8)

Planktivore and copepod abundance

Planktivore abundance did not significantly affect copepod abundance at either the shallow (n = 8; R^2 = 0.25, p = 0.207, Fig. 3a) or deep depth (n = 9; R^2 = 0.43, p = 0.053, Fig. 3b). Though there was no significant affect of planktivore abundance on copepod abundance there was a negative trend observed at both the shallow (Fig. 3a) and deep depth (Fig. 3b). Similarly, planktivore diversity did not significantly affect copepod abundance at the shallow (n = 8; R^2 = 0.10, p = 0.434) or deep depth (n = 9; R^2 = 0.02, p = 0.697). Although planktivore diversity also had no significant affect on copepod abundance, there was a positive trend at the deep depth and a negative trend at the shallow depth.

Planktivore abundance and dry weight of zooplankton

When the abundance of zooplankton was estimated using dry weight, planktivore abundance did not have a significant effect on the abundance of zooplankton at the shallow depth (n = 6; R^2 = 0.50, p = 0.119, Fig. 4a), but there was a positive trend between zooplankton abundance and planktivore abundance. However, at the deep depth, planktivore abundance did have a significant positive effect on the abundance of zooplankton (n = 8; R^2 = 0.84, p < 0.005, Fig. 4b). As planktivore abundance increased, zooplankton abundance increased.

Copepod abundance and zooplankton dry weight

There was no relationship between copepod abundance and zooplankton dry weight (n = 14; $R^2 = 3.8x10^{-5}$, p = 0.983, Fig. 5).

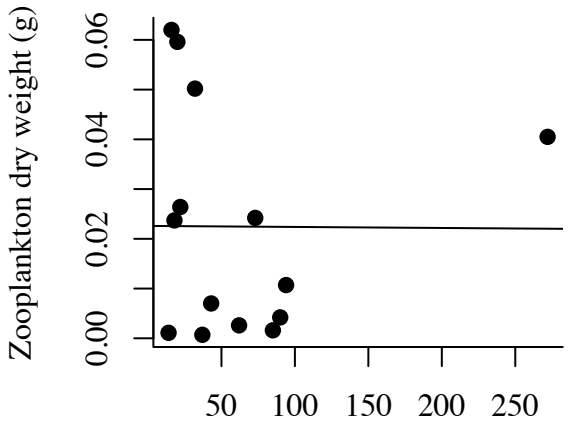

Fig. 5 Relationship between copepod abundance and zooplankton dry weight ($R^2 < .01$, p = 0.983, n = 14)

Discussion

Although none of the hypotheses were supported by the results, interesting trends were observed with biological and methodological implications. The hypothesis that zooplankton abundance would be greater at the deep depth compared to the shallow depth was not statistically supported. Although there was no statistical difference in zooplankton abundance between the two depths, the mean copepod abundance and zooplankton dry weight at the shallow depth was over two times greater compared to the deep depth. Higher abundance of zooplankton at a shallower depth could be explained by a greater survival rate higher in the water column. For example, Motro et al. (2005) found a similar trend due to planktivores foraging lower in the water column to avoid piscivores foraging higher in the water column. If the interval between the two depths of surveying and collection was increased, the data may have revealed an exaggerated or reversed trend.

The hypothesis that as planktivore diversity and abundance increases copepod abundance decreases was not statistically supported. However, a negative trend was observed at both the shallow and deep depth suggesting that planktivore abundance may have an effect on copepod abundance in certain environmental conditions. An increase in the number of samples may have emphasized this trend; possibly leading to a significant effect of planktivore abundance on copepod abundance. Although not specifically tested, the high level of variation in copepod abundance observed throughout the collection period could have been due to temporal variations in current and increased precipitation occurring on certain collection days. Leandro et al. (2007) found that during times of increased precipitation, salinity, and copepod abundance decreased. The variation in copepod abundance also may support the traditional notion that bottom-up factors affect abundance more than predation. For example, Reid et al. (2000) discussed how as ecosystem complexity increases, environmental factors (e.g. temperature, salinity) become more dominant in controlling plankton communities, making the effects of predation harder to distinguish. According to personal observation, there is a relatively high level of ecosystem complexity (i.e. high biodiversity and structural complexity) at Yellow Submarine dive site. This idea may explain the variation in the data and the trend observed between copepod abundance and planktivore abundance. Similarly, planktivore diversity did not have an effect on copepod abundance. However, at the deep depth, as planktivore diversity increased copepod abundance increased and at the shallow depth, as planktivore diversity increased copepod abundance decreased. The difference in trend direction may be explained by the high amounts of variation in copepod abundance and planktivore diversity throughout data collection. The high variation indicates weak trends and supports the notion that planktivore diversity does not affect copepod abundance. This suggests that certain planktivores may have a greater effect on copepod abundance

and that planktivore abundance plays a greater role than planktivore diversity in explaining the interaction between planktivores and copepods. This idea is in accordance with the findings of Lazzaro et al. (1992) where planktivore biomass was found to regulate zooplankton abundance more than planktivore type. Planktivore abundance is more closely related to planktivore biomass than planktivore diversity is related to planktivore biomass. This may explain why planktivore abundance yielded more consistent trends in this study.

Zooplankton dry weight measurements did not support the hypothesis that zooplankton abundance would increase with planktivore abundance. The opposite trend was observed; as planktivore abundance increased, zooplankton dry weight increased at both depths. Measurement error could possibly be responsible for this trend as the weights of the samples were very low (< 0.1 g). Some of the samples weighed less than the vial originally weighed indicating measurement error. Additionally, The results were influenced by the large amount of debris (e.g. hair, algae) in the samples. Due to other particles being included in the sample, dry weight did not accurately represent zooplankton abundance in this experiment.

An important finding from this study is that there was no trend observed between copepod abundance and zooplankton dry weight. This indicates that these two methods of estimating zooplankton abundance are not comparable, and one cannot be used as a proxy for the other. The method used to obtain the dry weight of the zooplankton sample may have been improved if the sample was rinsed through a sieve that allowed the zooplankton to pass through but not the larger particles of hair and algae. The dry weight included other substances that were not zooplankton in the measurement whereas counting individual copepods only measured zooplankton. These results further suggest that counting of zooplankton species is a more accurate representation of zooplankton abundance than dry weight.

In addition to errors in measuring the dry weight of the zooplankton samples, there could be errors in identifying planktivores, as well as collecting consistent samples. For example, there could be errors in the planktivore survey because there were two different surveyors collecting data on different days. There were slight variations in the time of day the zooplankton samples were collected which may have contributed to the observed patterns. If more data were collected, the observed trends on the relationship between planktivore abundance and zooplankton abundance may have been strengthened or different. The sources of error were consistent for both the shallow and the deep collections suggesting the errors operated at a temporal rather than spatial scale.

These results indicated that there was no significant effect of predation on zooplankton abundance in this Caribbean coral reef system. Furthermore, the results supported the notion that bottom-up controls were the dominant factor regulating plankton abundance in areas of high species diversity and moderate environmental conditions (Daewel et al. 2014). Other factors, such as time of day, salinity, current, and nutrient abundance could be responsible for the observed trends. This study supported the idea that the effects of top-down and bottom-up controls on zooplankton varied spatially and temporally (Daewel et al. 2014). The implications of this study were that bottom-up controls were responsible for zooplankton abundance and distribution in Caribbean coral reef systems. Further experiments on the relationship between planktivores and zooplankton abundance as well as resource availability are critical in order to evaluate the extent of resource availability and predation in modulating zooplankton dynamics in Caribbean coral reef systems. Future studies should also look at seasonal fluctuations in zooplankton community structure to determine different factors (e.g. temperature, salinity, planktivore biomass) regulating zooplankton dynamics.

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Physis (Fall 2016) 20: 24-32

REPORT

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Queen conch *(Lobatus gigas)* shells as shelter in reef communities: a comparison of their use in sand flats and the reef slope

Abstract Availability of refuge sites is limited in highly competitive ecosystems. A positive correlation exists between species richness, species abundance, and habitat complexity. Marine fish and invertebrates depend on rugose environments for survival in coral reef and sand flat communities. Few studies have looked at the potential value of added organic habitat in the marine environment. This experiment compared the use of empty queen conch (*Lobatus gigas*) shells in two different locations: the reef slope and the sand flats. The total number of individuals, species, and interactions near and within the shells were observed over four weeks on the island of Bonaire in the Dutch Caribbean. Exterior observations of species around shells were made sixty times for each location and interior observations were made four times per location by collecting and crushing shells to identify organisms inside. This study found that the highest number of organisms was observed on the reef slope, rather than the sand flats, both inside and outside the shells. It suggests however, that the value of the additional microhabitat in the sand flats should not be underestimated since the sand flats had a high number of recorded juveniles and numerous direct interactions. This research suggests that returning empty queen conch shells to different locations within the marine environment is important because it supports a variety of living organisms, adds rugosity and promotes species biodiversity.

Keywords Empty shells • refuge • habitat complexity

Introduction

Coral reefs are known as biodiversity hotspots with complex habitat structure. A positive correlation between habitat complexity and species richness and abundance exists because marine organisms heavily depend on those complex habitats for survival (Munday et al. 1998). Reproductive success of fish and invertebrates increases with habitat diversity. In marine environments, the future community composition depends on species composition, abundance of recruits, and the recruits' growth and survival (Shulman 1985). The survival of individuals is determined by several factors. These include abiotic sources of mortality, rate of encounters with and vulnerability to predators, access to food, and shelter availability (Shulman 1985).

Lobatus gigas, queen conch, are gastropods that are scarce in some areas of the Caribbean and abundant in others. They live in sea grass beds and sand flats around reef patches and are now more typically found in depths between 12-30 m due to overharvest (Humann and Deloach 2003). After queen conch is harvested, a hard calcareous shell with a hole from the mollusk extraction remains. Empty shells that are returned to the sea represent an additional microhabitat and potentially valuable predator refuge for a variety of organisms (Wilson et al. 2005). Increased habitat opportunities with hard substrate and small refuge holes are essential for maintaining reef fish and invertebrate biodiversity on a local scale (Gratwicke and Speight 2005). Studies have found that reefs with high topographic complexity and predators sometimes hold the

same high numbers of prey fish as reefs with no resident predators, reinforcing the idea that the availability of refuge provided by that topographic complexity is a large determinant in the livelihood of numerous reef populations (Beukers and Jones 1998). Gratwicke and Speight (2005) suggest that artificial reefs can mitigate habitat damage and help increase local fish abundance and species richness if it is a stable substrate with a rugose surface that has many small refuge holes. Empty conch shells fit these criteria and can make them an excellent substitute for artificial reefs as natural structural topography.

Adult queen conch can grow up to approximately 30 cm in length, but due to selection imposed by fishing pressure over time, a smaller sized phenotype has been selected for (Stoner et al. 2012). Variations in size, condition, and position of the individual queen conch shells may represent niches that can be exploited by different species requiring specific habitat (Wilson et al. 2005). Its shell morphology of queen conch allow a broader range of species to use it as refuge due to its spiraling shape (Claydon et al. 2011). When predators are successfully excluded, then the spaces prey hide in become valuable. If shells are morphologically advantageous sites of refuge for specific species, then those organisms benefit (Martín-Mora et al. 1995).

The increase in structural complexity that empty queen conch shells provide can attract certain species in need of protection. Briones-Fourzàn and Lozano-Álvarez (2001) provide evidence that artificial reefs using empty queen conch shells show an increase in the abundance and biomass of spiny lobsters in habitat-limited environments. Additionally, Claydon et al. (2011) showed that juveniles of *Panulirus argus* (Caribbean spiny lobster), *Epinephelus striatus* (Nassau grouper), and *Epinephelus guttatus* (red hind) all used large-scale artificial reefs made with numerous empty queen conch shells. The matrix of the coral reef limits certain species so providing diverse shelter can encourage biodiversity and give insight about which species might be habitat-limited if there is a notable increase in the abundance of the species after several generations.

Lac Bay, located toward the southeast corner of Bonaire, was once a popular site for queen conch harvesting. They consequently became over-harvested, which led to its endangered species status (Dutch Caribbean Nature Alliance 2011). On Bonaire, it is illegal to commercially, recreationally, and subsistence fish queen conch. Even though harvesting them is illegal, poaching is still a prevalent issue. Bonaire's non-profit Stichting Nationale Parken Bonaire (STINAPA) launched a queen conch restoration project that had a multi-faceted approach. The three components of the project involved a media outreach campaign, scientific research, and legal enforcement. Since 2010 when the project first began, the population of queen conch in Lac Bay has increased as shown through anecdotal evidence by local fishermen, and scientifically through monitoring (Dutch Caribbean Nature Alliance 2011). The Dutch Caribbean Nature Alliance claims that awareness about the protection of queen conch is high and there is large community support for the cause, a promising and hopeful claim for the return of its populations.

Little research has studied the use of individual empty shells as refuge in sand flats and on the reef slope. Brendle (2010) found a natural community succession over time from juvenile fish, to adult fish, and then to crabs using empty queen conch shells as refuge in the sand flats of Lac Bay, Bonaire. Marine species in Bonaire find value in empty queen conch shells as refuge and interspecific competition exists for the habitat (Brendle 2010). In an effort to supplement previous research, this study put empty, sun bleached conch shells from the shores of Lac Bay on the reef to investigate fish and invertebrate use of queen conch shells on the reef slope and in the sand flats. Not only could this research advocate for the re-use of the empty shells as refuge, but it could also provide new information that could be used in the protection of other endangered species that would benefit from the use of

supplemental habitat throughout the Caribbean. The research tested the following hypotheses:

H_1: Around shells: Sand flats will have the greatest number of fish and invertebrates around the shells because rugosity and refuge availability is lower

H_2: Inside shells: Sand flats will have the greatest number of fish and invertebrates inside the shells because rugosity and refuge availability is lower

Materials and methods

Study site

The study was conducted on a fringing reef off the coast of Kralendijk, Bonaire, Dutch Caribbean (12°9'36"N 68°16'55'W; Fig. 1). The shells were placed in the sand flats between 3-6 m and on the reef slope between 9-12 m at Yellow Sub dive site. Research was conducted over a five-week period in September and October 2016.

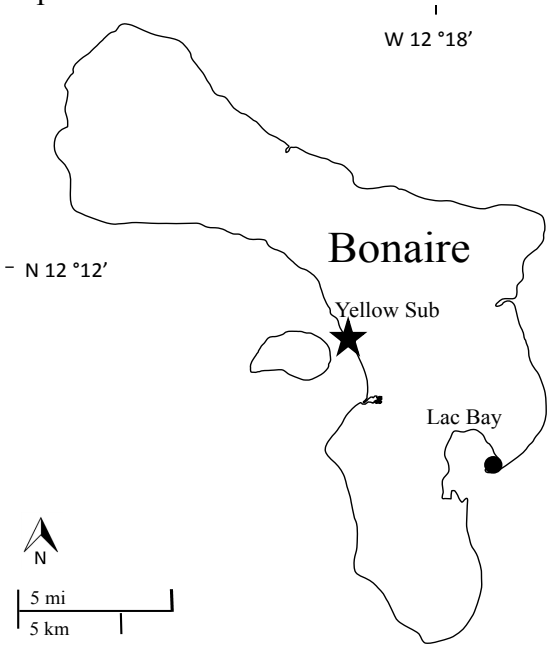

Fig. 1 Map of Bonaire, located in Dutch Caribbean (overview of Caribbean in top right corner), showing (⋆) Yellow Sub sampling site and (•) source of empty *Lobatus gigas* shells

Shell selection

Thirty *L. gigas* shells were collected from the same large mound of sun bleached, discarded queen conch shells near Lac Bay (12°5'18"N, 68°14'6"W; Fig.1). For the purposes of this experiment, shells were selected in such a way that minimized differences in shell characteristics because shell morphologies affect their occupation by marine organisms (Martín-Mora et al. 1995). The shells available for this study measured between 20-25 cm, had a relatively small non-natural hole due to conch extraction, and intact lips.

Shell placement

The shells were numbered from one to thirty with a permanent marker, had floating corks, labels with designated numbers, and colored flagging tape attached for ease of locating them on data collection dives (Fig. 2). Fifteen of the empty shells were placed at 9-12 m on the reef slope and the other fifteen empty shells were placed between 3-6 m in the sand flats. Ten shells at each habitat type, placed in two rows of five, were used for 'around shell' observational data collections. Five shells at each habitat type, scattered south of the last row of shells, were used for the 'inside shell' data collection. Shells were placed aperture up in the sand flats and in valley-shaped sandy patches on the reef slope (Fig. 2). All shells were at least 2 m from any other shell in any given direction as shown in Fig. 3 to avoid psuedoreplication.

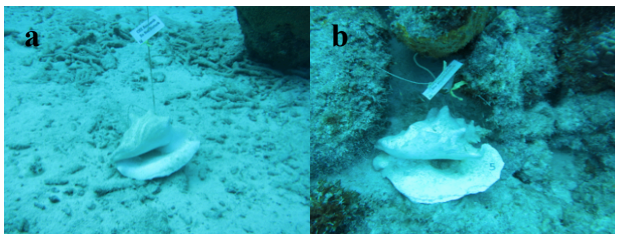

Fig. 2 Photographs of *Lobatus gigas* shell set-up underwater in (a) sand flats and (b) reef slope

Data collection

Around shells assessment

Species counts were taken between 0900-1100 hrs for six data collection days over the span of five weeks. Observations were recorded from a distance of 2 m for two minutes after a one-minute acclimation period. The number and species of fish and invertebrates within a 1 m^3 area of the shells was observed and recorded. Juveniles were recorded for four species: *Stegastes partitus* (bicolor damselfish), *Chromis multilineata* (brown chromis), *Halichoeres bivitattus* (slippery dick), *and Cantherhines pullus* (orangespotted filefish). Direct and indirect interactions were also recorded for each fish or invertebrate. Direct interactions included touching the shell in any way while indirect interactions included fish or invertebrates that passed through the 1 m^3 area or remained in the immediate area of the shell but did not go inside the shell or eat algae from the top of it.

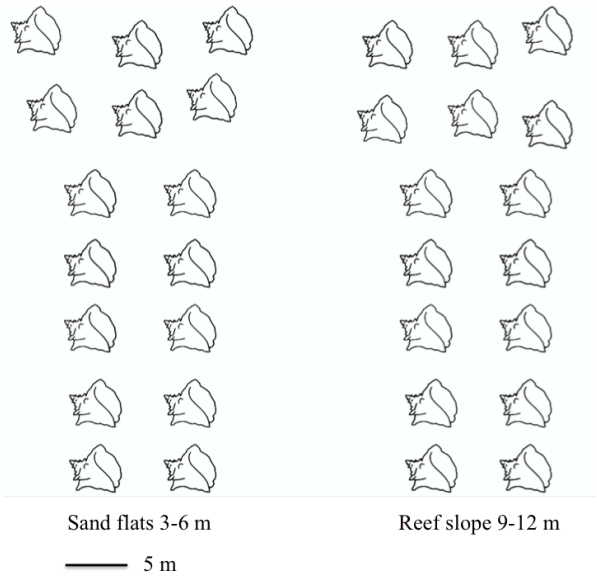

Fig. 3 Placement of empty *Lobatus gigas* shells at Yellow Submarine dive site (pictures of shells not to scale)

Inside shells assessment

At each depth, one of the five shells deployed for inside shell assessment was randomly selected by the roll of a dice each week to be collected and crushed for data on the fish and invertebrate species composition inside of the shells. To collect these shells, a dry bag was placed over each shell, sealed, and carefully brought to the surface so no organisms could escape. Shells were assessed one at a time to not mix up shell contents from different locations. The shell and the water collected with it were placed in a bucket on the shore and a sledgehammer was used to crush the shell. The species, quantity, and size of each organism visible to the naked eye were recorded and then the animals were quickly returned to the water. The bucket was thoroughly rinsed between each shell crushing.

Algae cover assessment

Algae cover was recorded three times during the course of the study. The first algae recording occurred 11 days after the shells were placed in the water, the second recording happened eight days after the first recording, and the last recording occurred six days after the second recording. Algae was recorded per shell as a percent cover (%) by visual observation.

Data analysis

This study looks at species inside and around the empty conch shells in two different locations. Comparisons were drawn between species abundance and species interactions between the sand flats and the reef slope. Two one-way ANOVA analyses were run in JMP to compare total individuals found (1) around the shells and (2) inside the shells between the reef slope and the sand flats.

Results

Around shells

A total of 1494 fish and invertebrates individuals were recorded within a 1 m^3 area of the shells on the reef slope and 892 in the sand flats after 60 total observations per location.

Table 1 Total amount of fish and invertebrates observed in a 1 m^3 area around single *Lobatus gigas* shells. Ten shells on reef slope and ten shells in sand flats were surveyed six times over three weeks (60 observations per location). Total number of individuals (Ind.) was summed per species over all observations and percent direct interaction (% DI) was calculated

		Reef Slope		Sand Flats	
Common name	**Scientific name**	**Ind.**	**% DI**	**Ind.**	**% DI**
Angelfish					
French	*Pomacanthus paru*	2	0		
Boxfishes					
Smooth Trunkfish	*Rhinesomus triqueter*	1	0	1	0
Damselfishes					
Bicolor	*Stegastes partitus*	96	23.96	50	82
Juv. Bicolor	*Stegastes partitus*	49	20.41	100	88
Blue Chromis	*Chromis cyanea*	2	0		
Brown Chromis	*Chromis multilineata*	12	8.33		
Juv. Brown Chromis	*Chromis multilineata*	7	0		
Cocoa	*Stegastes variabilis*	2	100		
Sergeant-major	*Abudefduf saxatilis*	1	0		
Dusky	*Stegastes adustus*	21	19.05		
Yellowtail	*Microspathodon chrysurus*	1	0		
Gobies					
Bridled	*Coryphopterus glaucofraen*	138	10.14	577	27.94
Goatfishes					
Spotted	*Pseudupeneus maculatus*			1	100
Yellow	*Mulloidichthys martinicus*	1	0	2	50
Grunts					
French	*Haemulon flavolineatum*	8	0		
Jacks					
Bar Jack	*Caranx ruber*	2	0	5	20
Parrotfish					
Princess	*Scarus taeniopterus*	1	0	1	0
Queen	*Scarus vetula*	1	100		
Redband	*Sparisoma aurofrenatum*			1	0
Stoplight	*Sparisoma viride*	2	0	2	0
Striped	*Scarus iseri*	3	33.33		
Grouper/Seabasses					
Bantam Bass	*Parasphyraenops incisus*	1097	0.18		
Graysby	*Cephalopholis cruentata*	6	0		
Snapper					
Schoolmaster	*Lutjanus apodus*	2	0		
Surgeonfish					
Ocean	*Acanthurus bahianus*	2	0	7	71.43
Soldierfish					
Blackbar	*Myripristis jacobus*	5	0		
Wrasse					
Bluehead	*Thalassoma bifasciatum*	11	27.27	3	66.67
Slippery Dick	*Halichoeres bivitattus*	1	0	2	100
Juv. Slippery Dick	*Halichoeres bivitattus*	8	0	112	25.89
Filefish/Puffers					
Juv. Orangespotted Filefish	*Cantherhines pullus*	7	0	2	0
Sharpnose Puffer	*Canthigaster rostrata*			5	20
Invertebrates					
Bearded Fireworm	*Hermodice carunculata*			5	60
Hermit Crab	Diogenidae	1	0	4	100
Sally Lightfoot Crab	*Percnon gibbesi*			2	100
Snail	Turbinidae/Cerithiidae	3	100	7	100
Spotted Cleaner Shrimp	*Periclimenes yucatanicus*	1	100	3	100

Thirty-one species were found on the reef slope while only 21 species were found in the sand flats. On the reef slope, four out of 31 species interacted more frequently directly with the shells, while 11 out of 21 species found interacting with the shells on the sand flats had a higher percentage of direct interactions (Table 1). The total number of fish and invertebrates interacting with the shell was significantly higher on the reef slope than in the sand flats
(ANOVA; $F = 5.83$, $df = 1$, $p = 0.017$).

Inside shells

A total of 24 fish and invertebrates individuals were found inside the shells on the reef slope and 18 were found in the sand flats after four total observations per location. Ten species were found on the reef slope while only seven species were found in the sand flats (Table 2). The total number of fish and invertebrates inside shells was not significantly different on the reef slope than in the sand flats (ANOVA; $F = 0.44$, $df = 1$, $p = 0.530$).

Algae cover

Mean percent algae cover increased between 12 October 2016 and 20 October 2016 for both sites. Mean percent algae cover also increased minimally between 20 October 2016 and 26 October 2016 on the reef slope, but decreased in the sand flats (Fig 4).

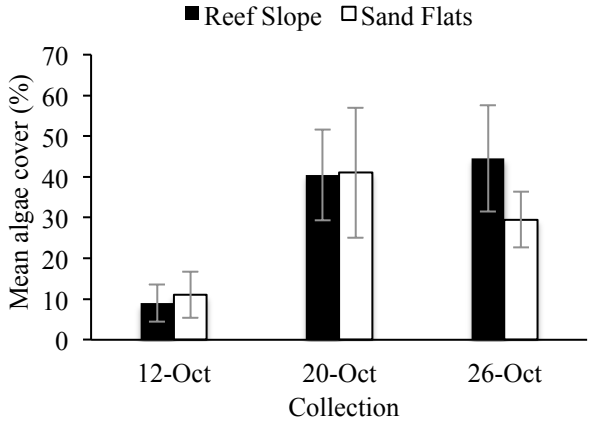

Fig. 4 Mean algae cover (%) on *Lobatus gigas* shells for each collection date in 2016 on the reef slope (n = 10) and in the sand flats (n = 10). Data presented as means ± SD

Discussion

Neither of the hypotheses was supported by the results, but interesting biological trends were found. Both the number of individuals recorded around the shells and inside the shells was significantly higher on the reef slope than the sand flats.

The hypothesis stating that the sand flats would have the greatest number of fish and invertebrates around the shells was rejected because more species and individuals were recorded on the reef slope than in the sand flats. According to observations made throughout this experiment, more fish may live on the reef slope than in the sand flats. The results stating that a higher number of individuals around shells on the reef slope could be attributed to the overall difference in abundance of fish between the two locations, which probably increased the likelihood of a fish passing through the 1 m^3 area surveyed on the reef slope.

Both direct and indirect interactions were observed on the reef slope and in the sand flats. When considering those interactions, the sand flats had more observed incidences of direct interaction than the reef slope. Since direct interactions involved predation of algae on shells, swimming into shells, and or resting on shells, one can infer that the habitat was still valuable in the sand flats despite fewer fish passing through the area around the shells.

Additionally, it is interesting to note that more juveniles were found in the sand flats, which might suggest that juveniles seek or need additional microhabitat to survive in the sand flats. This could have to do with the size and shape of the queen conch shells since their spiraling inside can help smaller sized organisms successfully hide (Claydon et al. 2011).

The hypothesis stating that the sand flats would have the greatest number of fish and invertebrates inside the shells was rejected since more species and individuals were recorded on the reef slope than in the sand flats. This could be attributed to there being more species overall on the reef slope that look

Table 2 Fish and invertebrates found inside *Lobatus gigas* shells. Table shows abundance of species per shell. Four shells were collected on the reef slope (RS_x) and four shells collected in the sand flats (SF_x)

Order	Common Name	Species	RS_1	RS_2	RS_3	RS_4	SF_1	SF_2	SF_3	SF_4	Total
Amphinomida											
	Bearded Fireworm	*Hermodice carunculata*				1				6	7
Decapoda	Hermit Crab	Unknown			8	5					13
	Spotted Cleaner Shrimp	*Periclimenes yucatanicus*			1	2					3
	Sally Lightfoot	*Percnon gibbesi*				1					1
	Shrimp	Unknown								1	1
Perciformes											
	Belted Cardinalfish	*Apogon townsendi*		1				1		1	3
	Bridled Goby	*Coryphopterus glaucofraenum*					6				6
	Glasseye Squirrelfish	*Heteropriacanthus cruentatus*				1					1
	Bicolor Damselfish	*Stegastes partitus*	1						1		2
	Dusky Damselfish	*Stegastes adustus*								1	1
	Threespot Damselfish	*Stegastes planifrons*						1			1
	Bantam Bass	*Parasphyraenops incisus*			1						1
Tetraodontiforme	Juv Scrawled Filefish	*Aluterus scriptus*		1							1
Gastropoda	Snail	Unknown				1					1
		Total number of organisms		24				18			42
		Total number of species		10				7			

for rugose environments, with holes and crevices that are limited in the sand flats. Having the empty queen conch shells serve as niche habitats for numerous organisms would positively serve the reef environment (Wilson et al. 2005). This could also point to a higher need for this type of supplemental habitat on the reef slope in order to maintain local biodiversity (Gratwicke and Speight 2005).

Mean algae cover increased on the reef slope making the shells even more camouflaged on the reef over time. Mean algae in the sand flats increased and then decreased. This difference could be attributed to predation by herbivores or perhaps abiotic sources of mortality like the aftermath of Hurricane Matthew between 1-7 October 2016 just before the first data collection or the rainstorm on 19 October 2016, which occurred the day before the second data collection. Shells in the exposed sand flats were not protected by the reef and were more vulnerable to turbulence since some moved around during the duration of the experiment.

Availability of shelter on reefs promotes species diversity and abundance (Munday et al. 1998; Gratwicke and Speight 2005). This study's findings contradict a previous study by Wilson et al. (2005) that shows shells in the sand flats were used more than shells on coral reefs. This study found that queen conch shells act as both a substrate for algal growth and refuge from predators, suggesting that empty shells serve an important purpose for reef creatures regardless of their location in the marine environment (Gratwicke and Speight 2005; Wilson et al. 2005). Shells were used by numerous species in both locations. The added rugosity and shelter provided valuable habitat for several species both around and inside the shells. With more observation or a longer study, important niches for species' interactions with the shells might be identified, but due to limitations imposed by the short duration of the program, continued research was not feasible.

Using empty queen conch shells might be an effective means of providing shelter on the sand flats for juveniles and species that need more shelter. Another study in the Caribbean by Briones-Fourzàn and Lozano-Álvarez (2001) provided evidence that empty queen conch shells were used by juveniles of multiple endangered species as habitat in a refuge-limited environment. While none of the species recorded during the duration of this experiment are considered endangered, further research may find that empty queen conch shells add to the matrix of the reef in a way that benefits threatened species over time.

Much like Brendle (2011), this study found that fish and invertebrates on Bonaire find value in empty queen conch shells as refuge and interspecific competition exists for the habitat. Humans are the sole reason for large piles of empty conch shells on the shores of islands throughout the Caribbean. Returning empty queen conch shells to the reef and sand flat communities would allow the widely coveted gastropod to fulfill its post-mortem ecological function by providing essential habitat for other marine organisms.

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REPORT

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Fish species diversity and interactions at varying classes of *Acropora palmata* and *Acropora cervicornis* in Bonaire

Abstract One structurally important genus of coral, *Acropora*, has declined roughly by 80-98% in the Caribbean alone. In the recent decade, active reef restoration efforts have been implemented through the Coral Restoration Foundation to help lessen declining patterns and enhance recovery for two important species of *Acropora: A. palmata* and *A. cervicornis.* Previous studies have examined its role as a well-established habitat provider; however, previous research has not addressed fish species diversity at varying morphological classes of *Acropora* spp. This *Acropora* spp. restoration has provided a unique platform to investigate fish species community dynamics at various classes including Coral Tree Nursery® fragments (class 1), bar method fragments (class 2), natural crops of *Acropora* spp. (class 3), and dead *Acropora* spp. piles (class 4). To address this, visual snorkeling surveys were conducted over a five-week study period assessing fish species diversity and interaction time and level. Fish species diversity was greatest among smaller, less complex corals (class 1), which was contradictory to past studies. The highest interaction time and amount of indirect interaction occurred among larger, more complex corals (class 3), where the highest amount of direct interaction level occurred among smaller, less complex corals (class 1). These results demonstrate and provide merit for continued active *Acropora* spp. restoration projects to improve the declining coral reef ecosystem. Further, dead *Acropora* spp. piles (class 4) had a high fish abundance (n = 1820) that mainly exhibited high interaction time (> 95%), which greatly demonstrates and supports *Acropora* spp. as indispensable reef habitat providers.

Keywords Fish abundance • interaction level • interaction time

Introduction

The coral reef ecosystem is one of the most biologically diverse marine ecosystems known today (Odum and Odum 1955; Connell 1978). While only occupying approximately 255,000 km^2 of the ocean floor (Spalding and Grenfell 1997), they provide habitats to 25% of all marine species (Thornhill 2012). Not only do marine species rely heavily on coral reefs, but so do people around the world. Coral reefs provide food, tourism jobs, shoreline protection, and even medicine, with the values of goods and services provided by coral reefs estimated to be 172 billion US dollars per year (Martinez et al. 2006). However, in the recent decade, coral reef species have been declining due to anthropogenic and natural causes. Since the 1980s, coral reef bleaching and mortality events have occurred almost annually in one or more of the world's tropical or subtropical seas (Baker 2008).

One structurally important genus of coral, *Acropora*, has declined roughly by 80-98% in the Caribbean and up to 95% in some areas since the 1980s (Porter and Meier 1992; Bruckner 2002). This decline may be due to white-band disease (Aronson and Precht 2001) and other anthropogenic and natural causes such as hurricane damage, increased predation

pressure, reduced water quality associated with terrestrial runoff, overgrowth by macroalgae, boat groundings, and anchor damage (Precht et al. 2002). Subsequently two species of this genus, *Acropora palmata* and *Acropora cervicornis,* have been listed as threatened under the Endangered Species Act since 2006 (Hogarth 2006) and are listed as critically endangered on the IUCN redlist. *Acropora* spp. that once dominated the reefs at shallow depths (< 10 m) in historical studies in Bonaire, a southern island in the Caribbean, (Van Duyl 1985) were almost absent by 2008 (Stokes et al. 2010).

Acropora palmata, or elkhorn coral, is a species of branching coral that has extensive thick and sturdy branches that resemble the anterlers of an elk. *Acropora palmata* is a fast growing coral, growing up to 9 cm per year (Bak 1976) and reaching maturity within 10-12 years (CRF Bonaire 2016). *Acropora cervicornis,* or staghorn coral, is a species of branching coral that can grow to over 2 m in length and has intertwining cylindrical branches that resemble a male deer's or stag's antlers (Van Duyl 1985).

Acropora palmata and *A. cervicornis,* which will henceforth be referred to as *Acropora* spp.*,* provide habitat, shelter, and food for a wide range of fish species due to their high structural complexity, leading to healthy and productive coral reef ecosystems (Precht et al. 2002; Young et al. 2012). Furthermore, *Acropora* spp. are important for reef growth, coastal buffering, and fisheries habitats (Young et al. 2012). Natural recovery processes alone may be ineffective in preserving and restoring the biodiversity and long-term integrity of coral reefs (Goreau and Hilbertz 2005). Therefore, other alternatives, such as active reef restoration, have been developed to help lessen declining patterns and enhance recovery (Guzman 1991; Rinkevich 2005).

In February 2012, the Coral Restoration Foundation Bonaire established nursery projects on the sandy flats of Bonaire to promote restoration and conservation of *Acropora* spp. The founder of the Coral Restoration Foundation developed the Coral Tree Nursery® design (Fig. 1).

This consists of a vertical 2 m PVC pipe, pierced with 10 horizontal 1.5 m fiberglass rods containing 10 to 16 small holes (Boomstra 2014). The small holes are used to attach monofilament line to the fiberglass rods via aluminum crimp sleeves, which keep the monofilament line in place (Boomstra 2014). The end of the monofilament line is tied to a fragment of *Acropora* spp. (~10 cm) enabling it to grow in the water column (Nedimyer 2011; Boomstra 2014). This suspension design allows for a multitude of advantages including increased coral growth rates facilitated by higher water flow and lower coral mortality from disease and benthic predators (Nedimyer 2011). After approximately eight months when corals have grown to ~30 cm (*A. cervicornis*) or the size of a basketball (*A. palmata*), they are transplanted from the Coral Tree Nursery® to squares of rebar or bamboo (bar method) or onto pre-designated reef sites (Boomstra 2014;

Fig. 1 A single Coral Tree Nursery® filled with multiple fragments of *Acropora cervicornis* off the West coast of Bonaire (Picture taken by CRF Bonaire 2016)

CRF Bonaire 2016). These two methods aid in stabilizing *Acropora* spp. The bar method is conducted by attaching the fragments with plastic tie-wraps to structures of approximately 1 m^2 rebar or bamboo, 40-50 cm from the bottom of the ocean floor (Fig. 2a), or through the use of marine epoxy on natural rocky substrate (Boomstra 2014) (Fig. 2b). The bar method is quicker and requires less labor than the marine epoxy method and also results in a higher survival rate due to less predation from bottom dwelling organisms such as fireworms (Boomstra 2014).

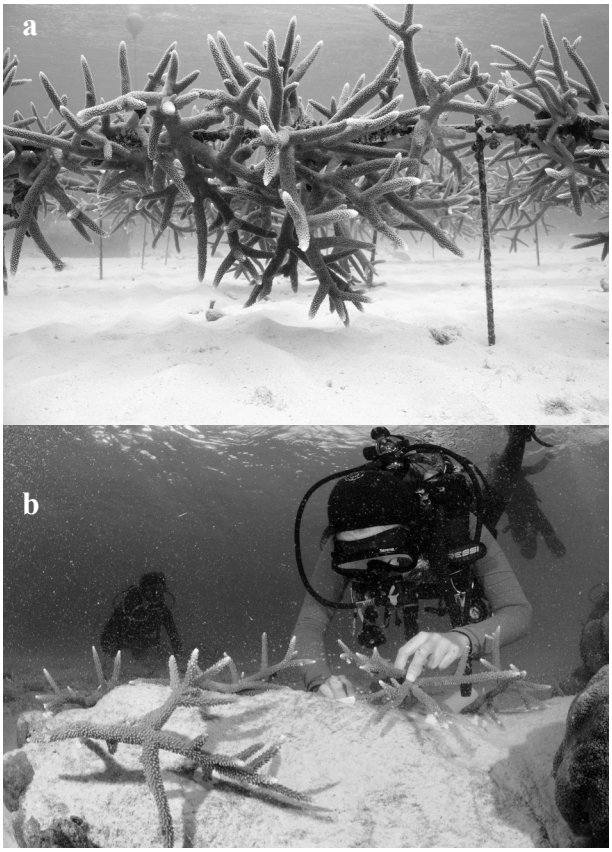

Fig. 2 Coral Restoration Foundation transplantation methods: (a) bar method (tying fragments of *Acropora cervicornis* to rebar with tie wraps) (CRF Bonaire 2016) (b) marine epoxy method (gluing fragments of *A. cervicornis* to stable substrate with marine epoxy) (CRF Bonaire 2016)

The Coral Restoration Foundation Bonaire's *Acropora* spp. restoration method has yielded positive results, as the nursery increased from ~250 coral fragments to ~8,500 coral fragments just after two years from when they were established in 2012 (CRF Bonaire 2016). This method has provided a unique platform to investigate fish species community dynamics at various *Acropora* spp. morphological classes including Coral Tree Nursery® fragments (class 1), bar method fragments (class 2), natural crops of *Acropora* spp. (class 3), and dead *Acropora* spp. piles (class 4), here after refered to as classes.

The purpose of this study was to provide quantitative data regarding differences among fish species richness, evenness, and diversity among classes of *Acropora* spp. Species diversity is comprised of two components: species richness, defined as the number of species in a given area, and species evenness, defined as how well distributed abundance or biomass is among species within a community (Wilsey and Potvin 2000). Previous studies suggested *Acropora* spp.'s role as a well-established habitat provider (Precht et al. 2002); however, research has not addressed fish species diversity at varying classes of *Acropora* spp.

This study further aimed to provide novel insight into the interspecific dynamics of fish within separate classes of *Acropora* spp., with implications for the progression of *Acropora* spp. restoration. The study not only evaluated fish species diversity, but also assessed fish interaction levels (direct or indirect) and time (low, moderate, or high) among separate classes of *Acropora* spp. Defining the fish species communities associated with different classes of *Acropora* spp. and which, if any, classes of *Acropora* spp. supported the most diverse fish communities could show an additional benefit derived from restoration. For instance, larger corals in later classes may support significantly higher fish species abundance or diversity than smaller corals in earlier classes, providing merit for continued active *Acropora* spp. restoration projects.

Past studies have shown that dead branching *Acropora* are ideal hábitat for fish by facilitating the growth of turf algae and providing food and predator protection (Garpe and Öhman 2003). Therefore, this study included a dead coral class, which is a common substrate in the sand flats of Bonaire, to provide further support that *Acropora* spp. is a

continued habitat provider without its additional role as a food source itself. Due to the greater size and hábitat complexity of corals in class 2 and 3, it was hypothesized that:

- H_1: Corals in classes 2 and 3 will have greater fish abundance than corals in class 1
- H_2: Corals in classes 2 and 3 will have greater fish species diversity than corals in class 1
- H_3: Interaction time will be greatest in coral classes 2 and 3
- H_4: The amount of direct in teraction level will be greatest in coral classes 2 and 3

Materials and methods

Study site

This study was conducted in Bonaire, Dutch Caribbean (Fig. 3a) between Buddy Dive House Reef (12°10'15"N 68°17'14"W) and Bonaire Dive and Adventure (12°10'08"N 68°17'14"W) on the shallow (< 10 m) sand flat (Fig. 3b). This site was chosen as it included all classes of *Acropora* spp. within a feasible snorkeling distance from one another. Surveying took place between September 2016 and October 2016.

Classes of *Acropora* spp.

Acropora spp. were categorized into four classes (Table 1). Class 1 consisted of the Coral Tree Nursery® fragments and occurred at depths of 3.5-5.5 m (Boomstra 2014). Class 2 consisted of the rebar structures and occurred at depths of 4-4.5 m (Boomstra 2014). Class 3 consisted of the *Acropora* spp. natural crops and occurred at depths of 1.5-2 m. Class 4 consisted of the dead pile fragments of *Acropora* spp., and occurred at depths of 3-4 m. Five replicates were sampled in classes 1, 2, and 3. Four replicates were sampled in class 4.

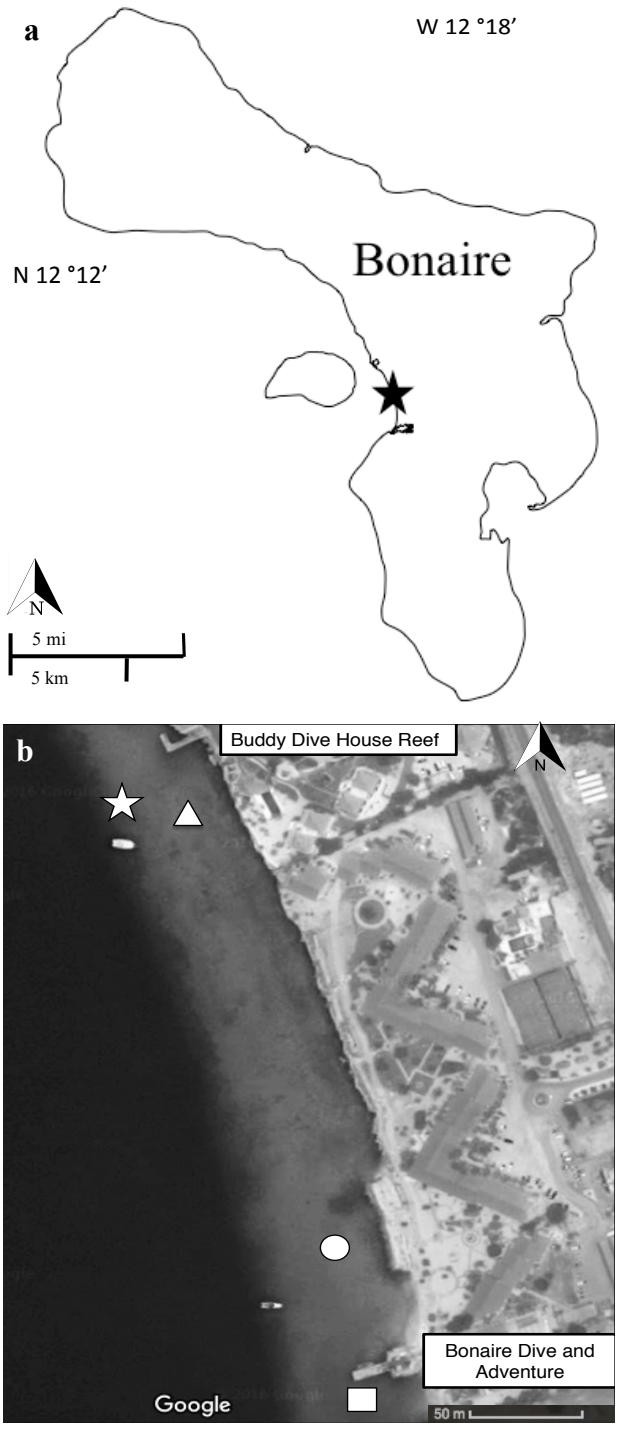

Fig. 3 (a) Map of Bonaire, Dutch Caribbean, star indicating study site. (b) Google satellite image of study site between Buddy Dive House Reef (top) and Bonaire Dive and Adventure (bottom) (Google 2016). Star represents *Acropora* spp. class 1. Triangle represents *Acropora* spp. class 2. Circle represents *Acropora* spp. class 3. Square represents *Acropora* spp. class 4

Table 1 Four classes of *Acropora* spp. characteristics including type, depth, and sample size

Class	Type	Depth (m)	Sample Size
1	Coral Tree Nursery®	3.5–5.5	n = 5
2	Rebar Structure	4.0-4.5	n = 5
3	Natural Crop	1.5–2.0	n = 5
4	Dead Pile	3.0-4.0	n = 4

Interaction time and level

Interaction levels between fish species and *Acropora* spp. were distinguished by time and level of interaction. Interaction time between fish and coral species was grouped into three categories:

1. Limited interaction: fish swims by coral to move from point A to point B with little to no hesitation (< 30 s).
2. Moderate interaction: fish stops and interacts with coral for a short period of time (0.5-4.5 min) and then leaves.
3. High interaction: throughout the study period (> 4.5 min) fish does not leave coral.

Level of interaction between fish and coral species was grouped into two categories:

1. Direct interaction: fish is touching, settling on, or eating coral polyps.
2. Indirect interaction: fish is within the coral study site without direct contact.

Survey protocol

A 30 s acclimation period took place after arrival to each study site. Once completed, surveying took place for five minutes. In that time, fish species abundance, interaction level, and interaction time was recorded. Specifically, each fish in the study site area, that was recorded during the five minutes, was given a "D/I" for direct or indirect interaction level, and a "L/M/H" for low, moderate, or high interaction time. All surveys were conducted during similar weather conditions and time of day to decrease the amount of variability between replicate surveys.

Data analysis

A two-way ANOVA with Tukey-Kramer HSD test was used to compare differences in total fish abundance surveyed in *Acropora* spp. class 1, 2 and 3. Fish surveyed were then grouped into their respective families and represented as a total percent of fish surveyed with *Acropora* spp. class 1, 2, and 3. Fish species diversity, which includes species richness and evenness, among *Acropora* spp. classes 1, 2, and 3 was calculated using the Simpson's Diversity Index. Results from the Simpson's Diversity Index were compared among *Acropora* spp. classes 1, 2, and 3. A two-way ANOVA with Tukey HSD post hoc test was used to compare the mean frequency of low, medium, or high interaction time and direct or indirect interaction level of fish with *Acropora* spp. and between *Acropora* spp. classes 1, 2, and 3. Average fish species richness, evenness, and diversity for class 4 were evaluated separately using the Simpson's Diversity Index. The mean frequency of direct or indirect interaction level and low, medium, or high interaction time of fish with *Acropora* spp. class 4 was further evaluated separately from class 1, 2, and 3 using a proportion of total interaction level and time frequencies.

Results

Total fish abundance

A total of 2,525 fish were surveyed in *Acropora* spp. class 1 (n = 128), class 2 (n = 577), and class 3 (n = 1820) throughout the five-week study period (Fig. 4). An analysis of variance showed that there was a significant difference in the total fish abundance surveyed between *Acropora* spp. classes 1, 2, and 3 ($F = 6.822$, $df = 2$, $p = 0.002$; Fig. 4) Further, the Tukey-Kramer HSD test revealed that there was a significant difference in the total number

of fish surveyed between class 3 and 1, and classes 3 and 2, but no significant difference between classes 1 and 2 (Fig. 4).

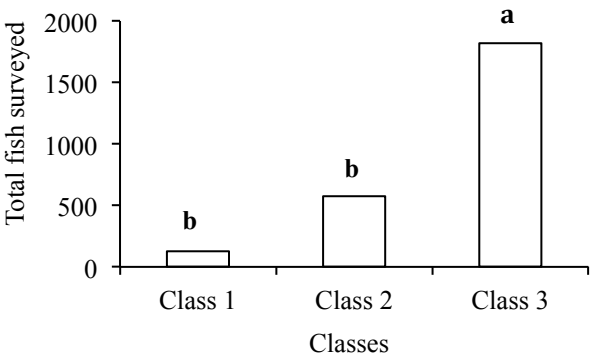

Fig. 4 Total amount of fish surveyed at three different classes of *Acropora* spp.: class 1 (n = 128), class 2 (n = 577), and class 3 (n = 1820). Groups that do not share a letter are significantly different from one another (two-way ANOVA with Tukey HSD post hoc test)

Fish species diversity

Fish surveyed were grouped into their respective families and represented as a percentage of the total fish surveyed during the five-week study period (Fig. 5). The Simpson's Diversity Index revealed that class 1 had the greatest species diversity (D = 0.906), followed by class 3 (D = 0.895), and then class 2 (D = 0.831) (Table 2).

Table 2 Simpson's Diversity Index among separate classes of *Acropora* spp.

Class	Type	Simpson's Diversity Index (D)
1	Coral Tree Nursery®	0.91
2	Rebar Structure	0.83
3	Natural Crop	0.89

Interaction time

Fish in class 1 (n = 128) exhibited mainly low interaction times (53.13%) with *Acropora* spp., followed by moderate (
35.94%), and lastly high (10.93%) (Fig. 6). Fish in class 2 (n = 577) exhibited mainly low interaction times (50.43%) with *Acropora* spp., followed by moderate (41.25%), and lastly high (8.32%) (Fig. 6). In class 3 (n = 1820), fish exhibited mostly low interaction times (49.78%) with *Acropora* spp., followed by high (33.08%), and lastly moderate (17.14%) (Fig. 6).

An analysis of variance showed that there was a significant difference in the interaction time (low, moderate, or high) between *Acropora* spp. classes 1, 2, and 3 (class × low/moderate/high, $F = 12.060$, $df = 4$, $p < 0.0001$; Fig. 6). The Tukey HSD post hoc test

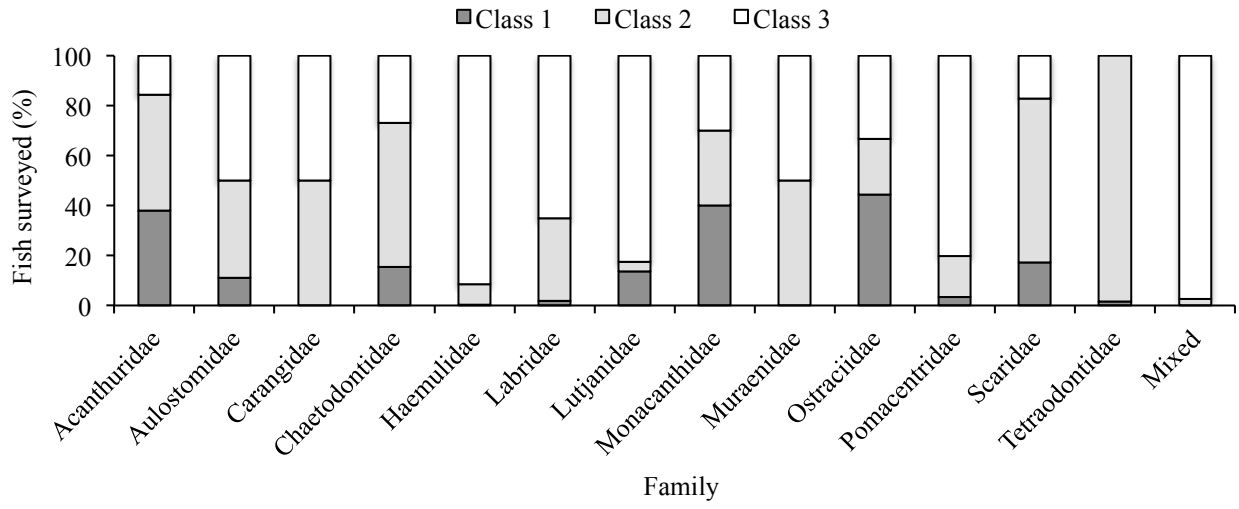

Fig. 5 Percent of fish species surveyed (%) grouped into families among *Acropora* spp. class 1 (n = 128), class 2 (n = 577) and class 3 (n = 1820). Mixed family group represents Actinopterygii, Epinephelidae, Gobiidae, Grammatidae, Mullidae, and Serranidae

revealed that the amount of low interaction time was significantly different between classes 1 and 3, but not classes 2 and 3 or classes 2 and 1 (Fig. 6). Further, results showed no significant difference among moderate interaction time between classes 1, 2, or 3 (Tukey HSD post hoc test; Fig. 6). Lastly, results revealed a significant difference in high interaction time between classes 1 and 3, and classes 2 and 3, but not classes 1 and 2 (Tukey HSD post hoc test; Fig. 6).

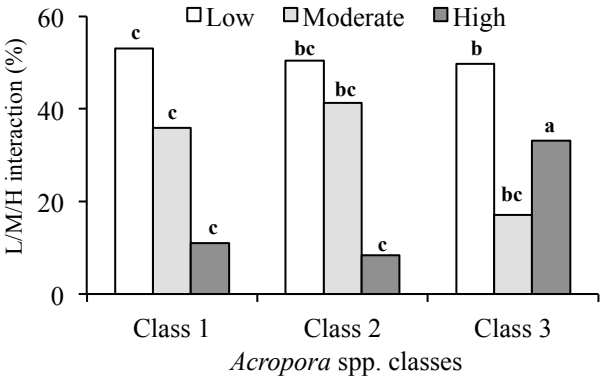

Fig. 6 Percent of limited (L), moderate (M), or high (H) interaction (%) among *Acropora* spp. class 1 (n = 128), class 2 (n = 577) and class 3 (n = 1820). Groups that do not share a letter are significantly different from one another (two-way ANOVA with Tukey HSD post hoc test)

Interaction level

In class 1, fish exhibited mainly direct interaction levels (56.25%) with *Acropora* spp., rather than indirect (43.75%) (Fig. 7). In class 2, fish mainly exhibited indirect interaction levels (64.8%) with *Acropora* spp., instead of direct (35.18%) (Fig. 7). In class 3, fish exhibited mainly indirect interaction levels (80.5%) with *Acropora* spp., rather than direct (19.51%) (Fig. 7).

An analysis of variance indicated a significant difference in the interaction level (direct or indirect) between *Acropora* spp. classes 1, 2, and 3 (class × direct/indirect, $F = 31.848$ $df = 2$, $p < 0.0001$; Fig. 7). The Tukey HSD post hoc test revealed no significant difference in the amount of direct interaction levels between classes 1, 2, or 3. However, there was a significant difference in indirect interaction levels between classes 1 and 3 and classes 2 and 3, but not classes 1 and 2 (Tukey HSD post hoc test; Fig. 7).

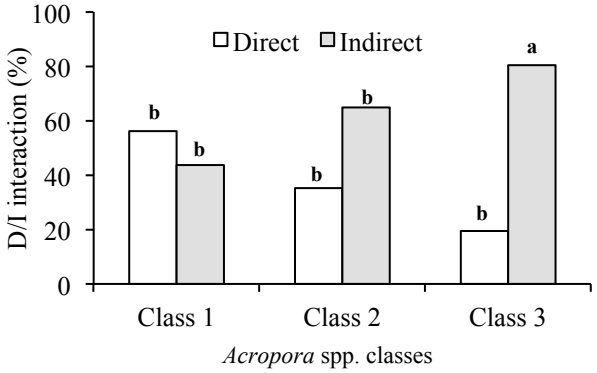

Fig. 7 The percent of direct (D) or indirect (I) interaction (%) among *Acropora* spp. class 1 (n = 128), class 2 (n = 577) and class 3 (n = 1820). Groups that do not share a letter are significantly different from one another (two-way ANOVA with Tukey HSD post hoc test)

Acropora spp. class 4

A total of 2,996 fish were surveyed in *Acropora* spp. class 4 throughout the five-week study period. Of those fish surveyed, 59.21% were in the family Pomacentridae, while each of the other families represented < 7% of the total fish surveyed (Fig. 8). The Simpson's Diversity Index revealed that species diversity in *Acropora* spp. class 4 was lower than any of the other classes (D = 0.558). In class 4, fish exhibited mainly high interaction time (95.16%) with *Acropora* spp., followed by low (3.38%), and lastly moderate (1.50%) (Fig. 9). Lastly, total fish surveyed in class 4 exhibited mainly direct interaction levels (56.67 %) with *Acropora* spp., followed by indirect interaction levels (47.33%).

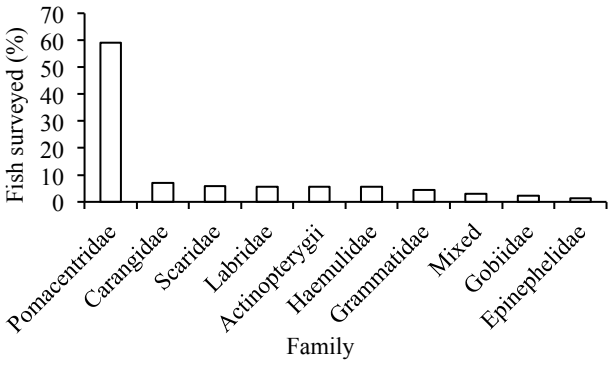

Fig. 8 Percent of fish species surveyed (%) (n = 2996) among *Acropora* spp. class 4 (grouped into families)

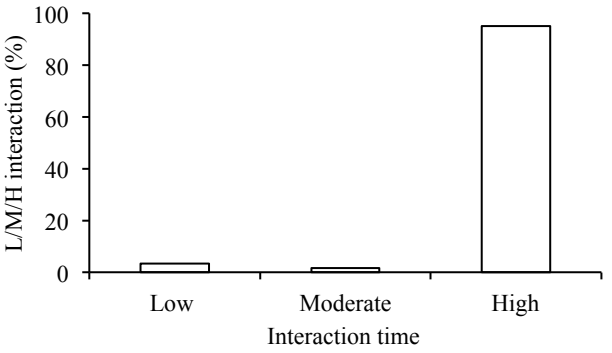

Fig. 9 The percent of low (L), moderate (M), or high (H) interaction time (%) of fish surveyed (n = 2996) among *Acropora* spp. class 4

Discussion

Results from this study showed many interesting and surprising trends regarding the fish species community dynamics among separate classes of *Acropora* spp. corals. First, the greatest fish abundance was seen in classes 2 and 3. This may be due to greater coral size, which leads to higher rugosity and increased habitat availability. This finding is consistent with Gratewick and Speight (2004) who revealed that the most important predictor of fish abundance was coral height. As *Acropora* spp. branches grow bigger, both vertically and horizontally, the amount of overhang and crevices provided by the coral increases, resulting in a greater amount of habitat and shelter for fish.

Interestingly, the majority of fish surveyed in *Acropora* spp. class 3 were schools of fish (~100) in the family Haemulidae, which were not present in class 1 or class 2 corals. These fish are known for their resting and protective daytime schooling aggregations (McFarland and Hillis 1982), which are largely supported by larger, more complex corals. These findings revealed that only certain classes can support large schools of fish, which may have affected trends in total fish abundance.

Furthermore, results regarding species diversity were contradictory with findings from Chabanet et al. (1996) and Barabult (1992), who both showed that species diversity and fish assemblages were correlated with branching coral cover and a highly complex environment. However, this study found that there was greater species diversity among smaller, less complex corals compared to larger, more complex corals. One cause for this may be because the less complex corals surveyed in this study contained no or few territorial fish, which may have resulted in a greater variety of fish species and amount of fish that have the opportunity to utilize these corals.

Additionally, the greater amount of high interaction time seen in class 3, may also be due to the increased size and habitats they provide. Similar to findings by McFarland and Hillis (1982), this study showed that fish in the family Haemulidae predominately utilize larger, more complex *Acropora* spp. corals for longer periods of the day, accounting for the dominance in high interaction time among corals in class 3.

Explanations regarding interaction level were similar to findings regarding interaction time. The increase in indirect, and decrease in direct, interaction levels as the size and complexity of corals increased, may be due to varying fish utilization strategies of corals at different sizes. This study observed greater amounts of fish utilizing larger branches of *Acropora* spp. for shelter (indirectly aggregating underneath the corals), whereas greater amounts of fish were utilizing smaller corals directly. Comparable findings by Gratwicke and Speight (2005) found that the two most important predictors of observed species richness were rugosity and variety of

growth forms. Therefore, since smaller branches in class 1 provide little to no shelter, this may account for the differences among interaction levels and utilization strategies between *Acropora* spp. classes.

Lastly, similar to a study conducted by Garpe and Öhman (2003), results from this study supported the finding that dead branching *Acropora* spp. is an ideal habitat provider for fish by providing food (algae) and predator protection. This study showed that even when the *Acropora* spp. fragments were dead, they continued providing habitat.

There were some potential sources of error throughout this study including disturbances from Hurricane Matthew, fish identification error, and surveyor bias which resulted in differences in interaction timing and levels. After the hurricane, which occurred in week one of the study period, one replicate from class 1 and class 3 were destroyed. Fortunately, through the Coral Restoration Foundation Bonaire, a replicate from class 1 was replaced in week 3, however it only contained corals on the bottom row of the Coral Tree Nursery®, which may have resulted in less fish being observed in class 1. Unfortunately, the natural crop replicate in class 3 never recovered, which may have led towards a decrease in abundance of fish observed in class 3.

Furthermore, the dead *Acropora* spp. piles were not of a similar size or a comparable depth to class 1, 2, or 3 and were therefore excluded from direct comparisons with the other classes. Future studies may want to design a study that will be able to analyze the relationship of fish community dynamics and dead *Acropora* spp. piles in more detail. Also, it would be interesting to look into comparing separate classes of *Acropora* spp. at different depths and locations (a less human impacted area instead of a house reef with high diver density), to see if there are any differences in fish abundance at different depths and between the two sites.

Results and conclusions from this study demonstrate and provide merit for continued active *Acropora* spp. restoration projects to improve the declining coral reef ecosystem. Larger corals in later classes provide shelter for a wide range and amount of fish that will lead to healthier coral reef ecosystems. Active restoration projects that aim at restoring corals should continue to be supported and implemented to aid in the recovery of one of the most biologically diverse marine ecosystems known today.

Acknowledgements I would like to thank the CIEE Bonaire Research Station and Dr. Rita Peachey for all of the equipment and support in making this study possible. In addition, I would like to thank Instructor Fadilah Ali and Emily Dawson for their encouraging support and advice throughout this entire process. Further, I would like to thank Instructor Kelly Hannan for her help with data analysis regarding statistics. Savannah D'Orazio was also an essential part of this project in order to make field work run smoothly. She was a very encouraging research partner throughout the entire project development and procedure.

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REPORT

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Algal consumption preference between *Diadema antillarum* and *Tripneustes ventricosus*

Abstract Sea urchins are an important herbivore in coral reefs ecosystems because they consume macroalgae that compete with corals. One important urchin found on the reefs is *Diadema antillarum*, the long-spined sea urchin. Due to a die off caused by an unknown disease in 1983-1984, their populations have yet to fully recover, and macroalgae has taken over some parts of the coral reefs around the island of Bonaire in the Dutch Caribbean, causing a phase shift from coral dominated to algal dominated reefs. Though, *Tripneustes ventricosus*, another Caribbean Sea urchin, was present and unaffected during the *D. antillarum* die-off, this phase shift still took place. To determine the reason behind the phase shift with another urchin present, a choice experiment was set up in the laboratory. The two algae that were studied were *Padina* spp. and *Dictyota* spp. because they are commonly found on coral reefs around Bonaire. To test algal preference, each species of urchin was given 2.5 g each of *Padina* spp. and *Dictyota* spp., and the mass of each algae was weighed before and after a 24 h feeding period to determine the amount consumed. There was a greater amount of algae consumed by *T. ventricosus* than *D. antillarum*. However, no significant difference was found in the preference for *Padina* spp. or *Dictyota* spp. for either species of urchin. Understanding the relationship between urchin species and their algal preferences can help create a better understanding of the connection between urchins and algae.

Keywords Urchin • Bonaire • feeding

Introduction

Coral reefs are highly productive and provide a multitude of ecosystem services that benefit humans including coastal protection, nutrient cycling, tourism, raw materials as well as cultural and aesthetic benefits while also providing habitat for many marine organisms (Moberg and Folke 1999; Harley et al. 2006; Barbier et al. 2011). Although coral reefs are a crucial part of our environment, there are many things that threaten their existence. Reef destruction has been caused by both anthropogenic and natural sources, such as severe weather, climate change, water pollution, and disease (Lirman 2001; Harley et al. 2006; Barbier et al. 2011).

Another significant threat to coral reefs is increased macroalgal growth, which has been associated with declines in coral cover (Lirman 2001). Although some direct effects of macroalgae on corals are unknown, increased competition for light and space make survival of corals more difficult (Lirman 2001). Interaction between algae and corals can cause reduced growth and fecundity and an increase tissue mortality in corals; these ailments can also be caused by increased competition from algae growing on the colony edges as well as completely covering smaller coral colonies (Lirman 2001). Herbivorous organisms, such as fish and invertebrates, are crucial for the survival of corals as they reduce algae cover through consumption (Lirman 2001). In places that have experienced major reductions in herbivorous fish through overfishing, invertebrates can become the top herbivores on the reef (Moses and Bonem 2001). One of the

most imporatant herbivorous invertebrates is the sea urchin (Echinodermata: Echinoidea) (Moses and Bonem 2001). Research has shown that urchin presence is directly related to greater coral cover and greater diversity of coral species (Sammarco and Williams 1982; Edmunds and Carpenter 2001). Although most urchins are thought to be generalists, there are studies where urchins show preference for certain algae species (Lilly 1975; Solandt and Campbell 2001; Tuya et al. 2001; Stimson et al. 2007).

Two common urchins found in Bonaire, an island in the Dutch Caribbean, are the long-spined sea urchin (*Diadema antillarum*) and the West Indian sea egg (*Tripneustes ventricosus*). Both species can be found in the shallows and on the reef consuming algae on corals (Haley and Solandt 2001; Moses and Bonem 2001). *T. ventricosus* and *D. antillarum* have the potential to remove macroalgae from corals allowing for more coral recruitment sites to be available (Macia and Robinson, 2008).

Before 1983, *D. antillarum* was the most dominant grazer on the reef throughout the Caribbean (Haley and Solandt 2001, Ruiz-Ramon et al. 2011). During 1983-1984, however, an unknown disease reduced many of the *D. antillarum* populations all throughout the Caribbean down to a small portion of what they were before the die-off (Haley and Solandt 2001; Moses and Bonem 2001; Ruiz-Ramos et al. 2011). Since then, populations have only recovered by a small fraction to the population sizes beforehand (Haley and Solandt 2001; Moses and Bonem 2001; Ruiz-Ramos et al. 2011). Currently, in the Caribbean, populations are roughly 12% of the size they were prior to the die-off (Lessios 2016). *Tripneustes ventricosus* was still present on the reefs in the absence of *D. antillarum*, but in Jamaica, they had shifted location from the shallows to the reef creating more spatial competition with the recovering *D. antillarum* populations (Haley and Solandt 2001). Though *T.* ventricosus shifted to the reefs during the loss of *D. antillarum*, the ecosystem still experienced coral death and a phase shift from coral-dominated to algae-dominated reefs (Macia et al. 2007; Lessios 2016).

Although these two urchin species can be found in the same location (Haley and Solandt 2001), they may show differences in algal preference and consumption rates, which could be an important factor affecting the overgrowth of algae during the *D. antillarum* die-off. *Tripneustes ventricosus* has been observed to prefer more mature macroalgae allowing for other macroalgae preferred by *D. antillarum* to settle and grow (Bodmer et al. 2015). When comparing previous experiments looking at algal consumption in the two species independently, slight differences were found in algal preferences for each urchin (Lilly 1975; Solandt and Campbell 2001; Tuya et al. 2001). However, none of the experiments had side-by-side comparisons of *D. antillarum* and *T. ventricosus* and the species of the algae varied between experiments. Thus, this study will look at the algal consumption rates (g d^{-1}) of both *T. ventricosus* and *D. antillarum* using two different species of algae that can commonly be found on the reef: *Dictyota spp.* and *Padina spp.* (Lirman 2001; Tuya et al. 2001). *Padina spp*. and *Dictyota spp*. have been observed on the reef interacting with corals (Lirman 2001).

The comparison of the algal consumption of *D. antillarum* and *T. ventricosus* seeks to further our understanding of how top herbivores have the potential to decrease algal biomass allowing for increased coral growth and overall health of the reef. Preserving the health of coral reefs is important because many organisms, including humans, depend on the ecosystem services they provide, such as habitat, food, shelter, nutrient cycling, etc. (Barbier et al. 2011). Looking at both species of urchin will provide a better understanding as to why algal growth during the die-off was increased even in the presence of *T. ventricosus*. This study will address the following hypotheses:

H_1: *Diadema antillarum* will consume more *Dictyota spp.* compared to *T.*

ventricosus who will consume more *Padina spp.*

H_2: *Diadema antillarum* will consume larger amounts of algae overall than *T. ventricosus*

Materials and methods

Collection site

Diadema antillarum (n = 7), *T. ventricosus* (n = 7), *Padina spp.*, *Dictyota spp.* and water were collected at Yellow Sub dive site (12˚9'36.2"N, 68˚16'55.2"W) located on the west side of Bonaire, an island in the Caribbean (Fig. 1). The site consists of a sandy flat leading up to a fringing reef with high coral cover. *Dictyota spp.* is prominent on the reef commonly found growing on the underside of corals or on dead corals. Both urchin species were collected in the shallows at 1 m with the use of a net and a spatula. Sea water was collected in five-gallon jugs and brought back to the lab from this site.

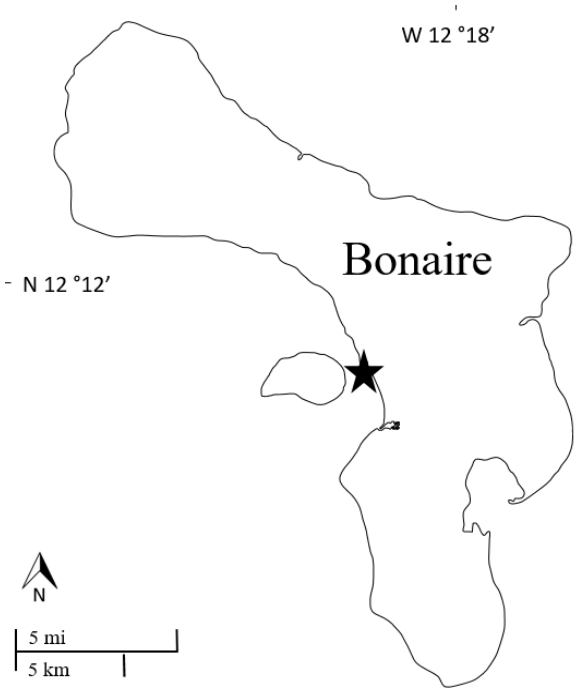

Fig. 1 Map of the island Bonaire, Dutch Caribbean. The star marks the study site at Yellow Submarine dive site (12˚09'36.2"N 68˚16'55.2"W)

Aquaria preparation

Before collection, four separate 19 L (40 × 20 × 25 cm) aquaria were washed, dried and filled with filtered sea water. The tanks were aerated to ensure water oxygen levels stay as close to natural a possible. A label was placed on each tank identifying each urchin. The tanks remained in the shade and covered to reduce temperature fluctuations and evaporation while the urchins were being tested.

Sea water was filtered using vacuum filtration with 0.7 µm filters (GE Healthcare Life Sciences Whatman). However, due to availability of resources, this was changed to using Basic® coffee filters. These filters were placed in funnels and placed in 60 L containers. The water was siphoned using Tygon silicone tubing (3/16 x 5/16) into the filters. Coffee filters were changed after half of the container was empty, approximately 30 L. Water quality, dissolved oxygen, pH, temperature and salinity, was measured daily using a 556 MDS YSI water quality probe.

Urchin collection

Diadema antillarum (n = 7) and *Tripneustes vetricosus* (n = 7) were collected south of Yellow Sub dock along the shore in approximately one meter of water. Both species of urchins were collected using a net and spatula along the shore and then placed in a bucket for transportation back to the laboratory. Four urchins (*D. antillarum* n = 2 and *T. vetricosus* n = 2) were collected at a time. Once in the laboratory, the urchins were measured across the middle of the underside using a 50-cm ruler. The urchins were then placed into individual tanks.

Algae collection

Algae was also collected from Yellow Sub dive site. The two alga's that were collected for experimentation were *Dictyota spp.* and *Padina spp*. *Dictyota spp.* was collected on SCUBA at approximately 14 m. Due to inclement weather removing algae at Yellow Sub, *Padina spp.* was collected along the westward side of Bonaire in various locations along the shoreline in 1 m of water using benthic grab. Once > 10

g of each species of algae was collected alga's were put into 1 gallon Ziploc bags. In the laboratory, exact measurements were taken of the semi-dry weight for each sample. Paper towels were used to remove excess water before placing the algae on the scale.

Experimental procedures

Once the urchins were placed in their respective tanks, they were starved for 48 h. After the 48-h starvation period, 2.5 g of both *Dictyota spp.* and *Padina spp.* were weighed using a glass bowl and a 400 g scale (Ohaus Scout Pro), and placed on the bottom of each tank using small sieves (50 μm mesh, 9 cm diameter) to insure they remained on the bottom. The urchins were then allowed to feed for 24-h. Once the 24-h grazing period was complete, the water from the tanks was poured through a sieve (50 μm mesh, 21 cm diameter) to catch all the algae pieces. Those pieces were sorted and collected using tweezers. This process was repeated for each urchin. The semi-dry weights, post-grazing period, were then re-measured. After the algae was reweighed, the amounts were recorded and urchins were returned to the ocean. Urchins were released north of the dock at Yellow Sub to ensure that urchins were not recaptured.

Data analysis

Data was analyzed using a two-way ANOVA to compare algae consumption (*Dictyota* spp. and *Padina* spp.) between the sea urchin species (*D. antillarum* and *T. ventricosus*) and their interaction (algae × urchin). If at least one of the main effects in the ANOVA model was significant, or it the interaction term was significant, a Tukey-Kramer honestly significant difference (HSD) *post hoc* test was applied to separate means. A t-test was used to compare size between urchin species. T-tests were also used to compare water quality measurements (pH, temperature and salinity) between the species to ensure they did not differ for two urchin species. A t-test was also used to compare the overall algae consumption (*Dictyota* spp. and *Padina* spp.) between each species. All data is represented as mean ± SD.

Results

Water quality

Measures of water quality (pH, temperature and salinity) did not vary between *D. antillarum* and *T. ventricosus* tanks during the sampling period (pH: $t = 0.39$, $df = 9$, $p = 0.353$; temperature: $t = 0.13$, $df = 9$; $p=0.448$; and salinity $t = 1.33$, $df = 9$, $p = 0.108$; Table 1). Though dissolved oxygen was measured, due to a malfunctioning probe, these values were not taken into account.

Algae consumption

Test size was not significantly different between urchin species ($t = 0.36$, $df = 12$, $p = 0.363$; *D. antillarum:* 9.21 ± 0.49; *T. ventricosus:* 9.07 ± 0.62). Neither *D. antillarum* nor *T. ventricosus* showed a preference for *Padina spp*. or *Dictyota spp*. (ANOVA: $F = 0.31$, $df = 1$, $p = 0.582$; Fig. 2a). *Diadema antillarum* consumed 0.73 ± 0.75 g of *Padina spp*. and 1.09 ± 0.50 g of *Dictyota spp*., whereas *T. ventricosus* consumed 1.91 ± 0.72 g of *Padina spp*. and 1.83 ± 0.59 g of *Dictyota spp*. (Fig. 2a). There was a significant difference between the total amount of algae consumed between *D. antillarum* and *T. ventricosus* ($t = -2.99$, $df = 12$, $p = 0.006$; Fig. 2b), where *Diadema antillarum* consumed less algae overall than *T. ventricosus.*

Table 1 Water quality measurements (pH, temperature (˚C), and salinity (ppt)) among separate tanks of *Diadema antillarum* and *Tripneustes ventricosus* taken over the study period (t-test). Dashes represent trials that were done before water quality testing started. Data are presented as means ± SD

Species	ID	pH	Temperature (˚C)	Salinity (ppt)
D. antillarum	Ursula	-	-	-
	Kaiju	7.59 ± 0.11	29.18 ± 0.16	36.90 ± 0.59
	Striker	7.52 ± 0.14	29.00 ± 0.19	36.64 ± 0.47
	Akela	7.58 ± 0.07	27.75 ± 0.87	36.83 ± 0.98
	Bagheera	7.65 ± 0.10	27.79 ± 0.97	36.54 ± 0.69
	Tantor	7.60 ± 0.05	28.51 ± 1.47	36.12 ± 0.64
	Kerchak	7.62 ± 0.03	28.32 ± 1.35	36.31 ± 0.76
T. ventricosus	Ariel	-	-	-
	Sebastian	-	-	-
	Gypsy	7.57 ± 0.12	29.27 ± 0.07	36.80 ± 0.64
	Baloo	7.60 ± 0.06	27.90 ± 1.13	36.45 ± 0.59
	Mowgli	7.58 ± 0.09	27.99 ± 1.20	36.41 ± 0.53
	Jane	7.59 ± 0.05	28.24 ± 1.20	35.95 ± 0.51
	Tarzan	7.58 ± 0.10	28.48 ± 1.15	35.70 ± 0.49

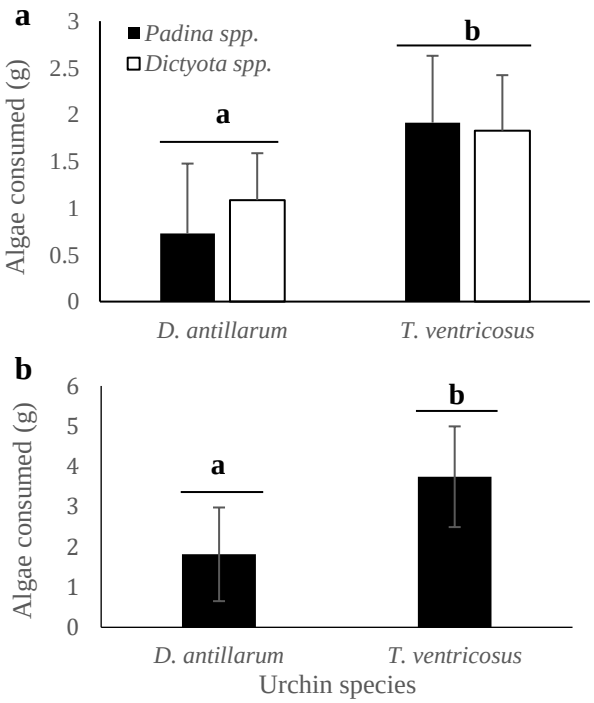

Fig. 2 (a) A comparison of the amount of algae (*Padina spp*. or *Dictyota spp*.) consumed between *Diadema antillarum* (n = 7) and *Tripneustes ventricosus* (n = 7) (two-way ANOVA). (b) A comparison of the total amount of algae consumed between *D. antillarum* (n=7) and *T. ventricosus* (n = 7) (t-test). Bars that do not share a letter are significantly different from one another Data presented as means ± SD

Discussion

Although in previous studies differences in preference for species of algae for both *D. antillarum* and *T.* ventricosus have been seen (Lilly 1975; Solandt and Campbell 2001; Tuya et al. 2001), in the current study there was no significant difference in the amounts of *Dictyota* spp. nor *Padina* spp. algae consumed by *D. antillarum* or *T. ventricosus.* Thus, leading to the conclusion that there is no preference of one algae over the other for either urchin species rejecting the first hypothesis. There have been, however, many studies showing that there is a higher degree of selectivity in many urchin species, including *D. antillarum* and *T. ventricosus,* for different algae types (Tuya 2001). Tuya (2001) also found that *Dictyota* spp. was a preferred alga for *D. antillarum* and *Padina* spp. was only an intermediately preferred alga. Further research by Lilly (1975) found that *T. ventricosus* preferred *Padina* spp. when compared to *Dictyota* spp. It is a possibility that the urchin species studied are more generalist feeders than originally thought leading to the lack in preference observed for either urchin species.

The second hypothesis was also rejected because the opposite of what was hypothesized was found; *T. ventricosus* consumed

significantly higher amounts of algae than *D. antillarum* during the 24 h feeding period.

Although *T. ventricosus* consumed more algae than *D. antillarum* in this experiment, there was a phase shift from coral dominated to algal dominated reefs when *D. antillarum* experienced the die off in 1983-84. Some possible reasons why this could have happened are that the *T. ventricosus* did not shift to different parts of the reef fast enough to stop the algal growth before it could damage the existing corals. Although it has been seen that *T. ventricosus* has shifted locations on the reef after the disappearance of *D. antillarum*, this was only observed in one location, Jamaica (Haley and Solandt 2001). *Tripneustes ventricosus* are not as prominent on the reefs of Bonaire as compared to other locations in the Caribbean. This could be a reason this shift to algal dominated reefs took place.

These results might be related to unnatural conditions of the aquarium changing the feeding behavior of the urchins or leading to slightly stressed behavior. There also might not have been enough trials to truly show a preference to one algae.

Based on the findings of this study, continued research is required to improve the understanding of the phase shift that occurred throughout the Caribbean from coral dominated reefs to algae dominated reefs during the *D. antillarum* die-off. Future research could test different urchin species, different types of algae and more than two types at a time. Another potential experiment could look at testing urchins under stresses to see if stress impacts their feeding behaviors. It is essential to consider all possible effects sea urchin populations will have on algal growth that threaten the health of the coral reefs.

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REPORT

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The effect of colony size on the frequency of intraspecific and interspecific aggressive behaviors in the tropical damselfish *Abudefduf saxatilis*

Abstract Parental care is a reproductive strategy that increases the survivorship of offspring, but costs more energy which could affect future reproduction. Sergeant majors, *Abudefduf saxatilis*, are a species of damselfish that exhibit parental care. Males build nests alone (solo nesters) or near other nest sites (colony nesters). They defend their nests by chasing other fishes, including conspecifics, consequently expending large amounts of energy that could otherwise be allocated to future fecundity. There is currently a lack of research concerning differences in aggressive behaviors exhibited by solo and colony nesting *A. saxatilis* and implications for energy expenditure. This study examined the advantages, in terms of energy expenditure, of nesting in a colony or in solitude by comparing the number of intraspecific, interspecific, and total chases displayed by each nesting type. The aggressive behaviors of both solo and colony nesting *A. saxatilis* were observed by filming nesting males. The footage was analyzed to determine the number of interspecific, intraspecific, and total chases displayed during a 5 min period. Results showed that solo nesters displayed more interspecific and total chases than colony nesters (ANOVA, $F = 9.2$, $df = 1.0$, $p < 0.01$). When the number of chases was used as a proxy for energy expenditure, results suggested that colony nesters exhibit a more advantageous nesting type as they must allocate less energy toward defense than solo nesters. These findings increase our understanding of energy conservation seen in colonial nesting as opposed to solo nesting in a variety of territorial nesting species.

Keywords Parental care • territoriality • energy expenditure

Introduction

A variety of reproductive strategies exist in the animal kingdom, each with its own set of trade-offs. Providing parental care is one strategy that has the benefit of significantly increasing the survivorship of offspring, thus passing more genes on to the next generation (Bessa and Sabino 2012). However, parental care requires energy expenditure, which leads to mating costs, adult survivorship costs, and future fecundity costs (Gross and Sargent 1985). Mating costs occur when the time spent caring for offspring reduces the total number of reproductive events the parent is capable of participating in during a breeding season (Gross and Sargent 1985). Survival cost is the reduced ability of the parent to survive to the next breeding season due to the energy expended while caring for offspring (Gross and Sargent 1985). Animals often fast while caring for their offspring, which increases their exhaustion of energy reserves and decreases growth rate, restricting their future ability to reproduce (i.e. future fertility costs) (Gross and Sargent 1985). The benefits of increasing offspring survival rates must outweigh the costs associated with parental care for this behavior to be selected for.

In species of fish that exhibit parental care 61% do so through male care of offspring, making male parents the most common caregivers (Gross and Sargent 1985). This likely occurs because the costs of parental care are diminished for males compared to females

(Gross and Sargent 1985). For instance, in many fish species, male fecundity (amount of young they can produce) remains relatively constant, whereas fecundity of female fishes increases with body size; thus expending energy on parental care would affect a female's future fecundity more so than a male's (Gross and Sargent 1985). Additionally, over 90% of fish species that have male parental care had multiple spawning events per male, which reduces the mating costs of parental care for territorial male fish because they can provide care for their offspring by guarding nest territory while still attracting new mates to their nest site (Gross and Sargent 1985). However, it is often beneficial for females to mate with multiple males as well. This reduces their chance of mating solely with a fish who is infertile, incapable of caring for offspring, or who has non-advantageous genetic combinations (Byrne and Keogh 2009). Therefore, the benefits of male parental care in many fish species may outweigh the costs.

Sargent majors, *Abudefduf saxatilis*, are a type of damselfish that are abundantly found throughout Caribbean coral reefs. They fill an important trophic role on the reef as planktivores and are commonly predated on by larger piscivorous fishes such as bar jack (*Caranx ruber*) and barracuda (*Sphyraena barracuda*) (Frédérich et al. 2009). Like many damselfish, *A. saxatilis* have the reproductive strategy of promiscuity and male paternal care for their offspring (Fishelson 1970; 1998). During mating, which occurs continuously throughout the year, males clear a nest site and then entice a female to lay her eggs at the site (Foster 1987). Once a female is attracted to the nest site, she drags her body along the substrate depositing her eggs, closely followed by the male who externally fertilizes them (Mar 2008). Males continuously care for their nest sites as the eggs develop, fanning the eggs to ensure adequate water flow and aggressively defending them from predators (Bessa and Sabino 2012). Nesting in close proximity to other *A. saxatilis* is a common practice, termed colonial nesting, and nests are typically found on smooth surfaces such as shipwrecks, pilings, and reef outcroppings (Bessa and Sabino 2012). One benefit that *A. saxatilis* males may gain from colonial nesting is greater protection from nest predators. With multiple *A. saxatilis* present, there could be an increase in the number of defenders, leading to a decrease in individual acts of aggression necessary for each fish because they would only be responsible for chasing away a portion of the intruding predatory species. Males nesting alone are responsible for all the defensive acts, thus potentially expending more energy than males that nest in colonies.

Continuously chasing away other fishes requires large amounts of energy, suggesting that there must be a selective advantage to this behavior that outweighs its costs (Myrberg and Thresher 1974). Nesting *A. saxatilis* must defend their egg patches and territories from a variety of fish species, including conspecifics (Mossler 2012). Intraspecific aggressive behaviors, attacks on members of the same species, are commonly seen when the availability of nesting sites is low, creating the need for fish to defend this scarce resource from members of their own species (Bessa and Sabino 2012). Interspecific aggressive behaviors, attacks on members of different species, are typically directed at predatory species that pose a threat to the egg patch of a nesting *A. saxatilis* (Bessa and Sabino 2012).

There has been a lack of research looking into the benefits to fish nesting in colonies as opposed to nesting alone in terms of number and type of attack (i.e. interspecific and intraspecific). Thus, this study aimed to discover more about the advantages, in terms of energy expenditure, to *A. saxatilis* nesting in a larger colony versus having solitary nests. Comparisons were made between the two nest types, examining the number of intraspecific, interspecific, and total aggressive chases displayed, with aggressive chases acting as a proxy for energy expended. The close proximity of colony nesters to other *A. saxatilis* has the potential to reduce the number of defensive acts necessary; however, it may increase the amount of intraspecific interactions. Solo nesters are spatially separated from other *A. saxatilis*,

which may increase total defensive behaviors, but decrease the frequency of encounters with members of their own species and lead to a reduction in the number of intraspecific acts of aggression needed to defend the nest site from competitors. Therefore, the following hypotheses were tested:

- H_1: *Abudefduf saxatilis* nesting in colonies will display more intraspecific than interspecific aggressive behaviors
- H_2: Solitary *A. saxatilis* nesters will have more interspecific than intraspecific aggressive behaviors
- H_3: *Abudefduf saxatilis* nesting alone will display more total aggressive chases than those nesting in a colony

The aim of this study was to help establish a better understanding of *A. saxatilis* nesting behaviors and the advantages to different nesting types. This greater knowledge of *A. saxatilis* nesting behaviors could provide new insights on territorial nesting behaviors observed in a variety of animal species as well as the relationship between colony size and interspecific or intraspecific interactions.

Materials and methods

Study site

Data on the territoriality of nesting *A. saxatilis* (N = 32) was collected in Bonaire, Dutch Caribbean, an island near the northern coast of Venezuela (Fig. 1). The study site is located on the leeward west coast of the island at Yellow Submarine dive site (12°09'36. 2" N, 68°16'55. 2" W) in Kralendijk, Bonaire. Due to its location in Bonaire's capital, Yellow Submarine dive site is frequented by many recreational divers and boaters. To prevent boat anchors from damaging corals, there are large cement mooring blocks (80 × 80 × 80 cm) with attached buoys placed in clusters of three, parallel to the shoreline. The mooring blocks are located in 5-6 m of water on the sand flats near the reef crest. The smooth surfaces of these mooring blocks makes them a common nesting site for *A. saxatilis*.

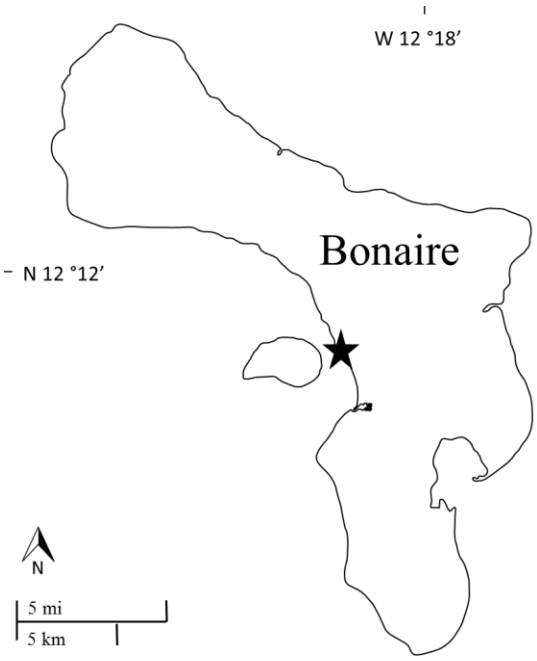

Fig. 1 Map of Bonaire, Dutch Caribbean, in the Caribbean Sea. The star represents the study location at Yellow Sub Dive Site (12°09'36.2"N, 68°16'55.2"W)

Nest selection

Mooring blocks were examined to determine if they possessed a nest site that met the required variables for this study. Colony (n = 18) and solo (n = 14) nesters were categorized by the presence or absence of other *A. saxatilis* nest sites on the same mooring block. *Abudefduf saxatilis* were classified as colony nesters when one or more nest sites were present on the same or adjacent faces of the mooring block. If there was a single nest site on one face of the mooring block, with no other nests on adjacent faces of the block, this *A. saxatilis* was considered a solo nester.

Data collection

Data was collected on SCUBA over a five-week period in September and October, 2016. Nests were observed on Wednesdays and Saturdays between 1330 and 1530 hrs. *Abudefduf saxatilis* eggs typically hatch within 4-5 days (Foster 1987), so it was assumed that new male fish were present at the mooring blocks each time

data was collected allowing nest site locations to be studied multiple times. During each research dive 2-6 nest sites were observed. Upon selection of a nest, measurements were taken for the height and width of the egg patch to the nearest cm, *A. saxatilis* were categorized as a solo or colony nester, and the size of its colony was recorded. Additionally, the total length of the nesting *A. saxatilis* was estimated from the video footage by comparing the length of the fish to the size of the egg patch. A GoPro camera attached to either a clip mount or a 30 cm high PVC pipe stand was positioned in front of the egg patch. The camera was left to record footage for 10 min without the presence of divers.

Video analysis

The first and last 2.5 min of footage were designated as acclimation periods to account for disturbances to fish behavior from the divers' presence. The remaining 5 min of footage were analyzed to determine the number of defensive behaviors (chases) that occurred and the species that were attacked (intraspecific and interspecific for each nesting fish). Aggressive chases were recorded anytime an *A. saxatilis* suddenly and rapidly darted at another fish, scaring them away from the egg patch.

Data analysis

To determine the influence of possible co-factors on *A. saxatilis' p*reference for nest type (solo or colony), Students t-tests were run to compare nest type to fish length and nest size (width and height). The number of intraspecific and interspecific chases displayed by *A. saxatilis* nesting in colonies and alone, were compared using a two-way ANOVA with nest type (solo or colony) and chase type (intraspecific or interspecific) as the main factors. A Tukey-Kramer honestly significant difference (HSD) *post hoc* test was applied to separate means if the interaction term or at least one of the main effects in the ANOVA model proved to be significant. Additionally, the total number of chases displayed by *A. saxatilis* were compared to nesting types (solo or colony) with a Students t-test. A two-way ANOVA was also run to compare the total number of chases with the variations in colony size (number of nests). All data are presented as means ± SD where appropriate, and all tests were performed using R (version 3.2.2). Differences were considered significant if p was less than 0.05.

Results

There was not a difference between nest sizes (height and width) for the different nest types (solo and colony), eliminating nest size as a confounding factor (width; $t = -0.24$, $df = 25$, $p = 0.812$ and height; $t = 1.1$, $df = 30$, $p = 0.278$, Table 1). Additionally, the total length from tip to tail of nesting *A. saxatilis* (N = 32) did not vary between the different nest types, likewise eliminating fish length as a possible confounding factor ($t = -0.57$, $df = 27$ $p = 0.575$, Table 1).

Nest and chase types

There was a significant interaction between nesting type (solo or colony) and chase type (interspecific or intraspecific) on number of chases (ANOVA, $F = 9.2$, $df = 1.0$, $p < 0.01$, Fig. 2A). Both solo and colony nesters displayed more interspecific (solo: 4.6 ± 2.5 and colony: 2.3 ± 1.8) than intraspecific chases (solo: 0.14 ± 0.36 and colony: 0.11 ± 0.32, Fig. 2A). Additionally, there were more interspecific chases displayed by solo nesters than by colony nesters in the 5 min observation period (Fig. 2A). However, there was not a significant

Table 1 Fish length, nest size (width and height), and colony size for solo (n = 14) and colony (n = 18) nesting *Abudefduf saxatillis.* Data reported as means ± SD

Nest type	Fish length (cm)	Nest width (cm)	Nest height (cm)	Colony size
Colony	10.72 ± 1.32	23.00 ± 7.50	23.67 ± 6.91	3.50 ± 1.50
Solo	11.00 ± 1.41	24.64 ± 8.97	22.27 ± 5.66	1.00 ± 0.00

difference between the numbers of intraspecific chases displayed by solo and colony nesters (Fig. 2A).

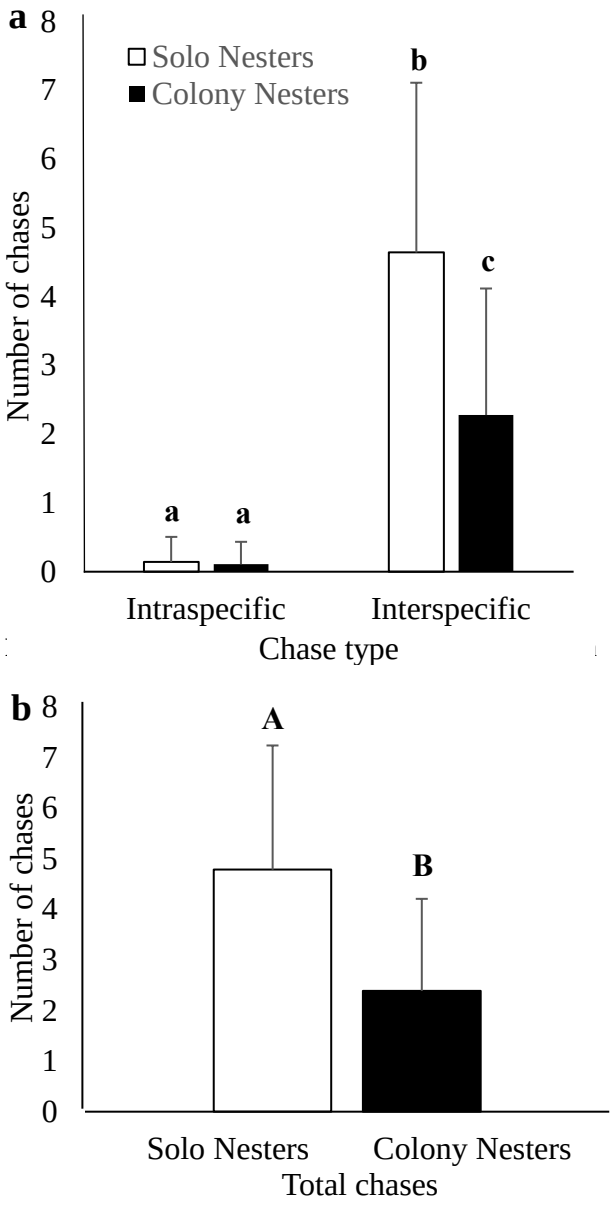

Fig. 2 (a) Comparison of the number of chases between both chase types (intraspecific and interspecific) for colony (black, n = 18) and solo nesters (white, n = 14). (b) Comparison of the total number of chases (intraspecific and interspecific combined) for colony (black, n = 18) and solo nesters (white, n = 14). Groups that do not share a letter are significantly different from each other (two-way ANOVA and Students t-test, respectively). Data reported as means ± SD

Colony size and total chases

Solo nesting *A. saxatilis* displayed more total chases (intraspecific and interspecific combined) than those nesting in a colony (t-test, t = -3.1, df = 23, p = 0.006, Fig. 2B). However, the total number of aggressive chases displayed by *A. saxatilis* did not differ when compared across the various colony sizes (i.e. number of adjacent nests) (ANOVA, F = 3.2, df = 1.0, p = 0.086, Fig. 3).

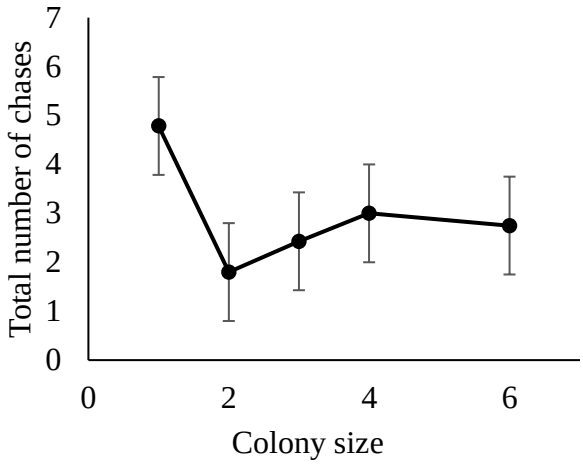

Fig. **3** A comparison of colony size (number of adjacent nests 1: n = 14, 2: n = 5, 3: n = 7, 4: n = 2. 6: n = 4) versus the total number of chases (combined intraspecific and interspecific). No *Abudefduf saxatilis* were observed with a colony size of five. Data reported as means ± SD

Discussion

Nest size (height and width) did not differ between the nest types (solo or colony) of *A. saxatilis*. Likewise, the size of the nesting fish did not differ between the two nesting types. This suggests that nest and fish sizes are not factors that influence nesting in solitude or in a colony. Therefore, they do not interfere with variables measured against nesting type, allowing for clear comparisons to be made between chase types and nest types.

Additionally, *A. saxatilis* nesting in a colony displayed more interspecific than intraspecific chases, contradicting the first hypothesis. Intraspecific aggressive behaviors most commonly occur when the availability of nest sites is low, requiring fish to guard this limited resource from conspecifics (Bessa and Sabino 2012). Colony nesters may have displayed less intraspecific chases due to an abundance of

suitable nesting sites. The mooring blocks at Yellow Submarine dive site in Bonaire may have provided an ample amount of suitable nesting substrate for the *A. saxatilis* population size, removing the need to compete over this resource and reducing the number of intraspecific chases necessary to defend nest sites.

Solo nesting *A. saxatilis* displayed more interspecific than intraspecific aggressive chases, supporting the second hypothesis. The lack of intraspecific chases could be explained by the spatial separation of solo nesters to other *A. saxatilis*. This separation would decrease the frequency of their encounters with other *A. saxatilis*, limiting their opportunities to display aggressive behaviors to conspecifics. Additionally, interspecific aggressive behaviors are typically directed toward predatory species that threaten egg patches (Bessa and Sabino 2012). A greater abundance of predatory species than other *A. saxatilis* around the nest site would lead to more interactions with predatory species, accounting for more interspecific than intraspecific chases displayed by solo nesters. Additionally, continuously chasing away predators would require the expenditure of a large amount of energy to protect the eggs from predation.

Solo nesting *A. saxatilis* displayed a greater number of total chases (intraspecific and interspecific combined) than those nesting in a colony, supporting the third hypothesis. However, the total number of chases did not vary between colony sizes, suggesting that the number of nests in a colony do not have an effect on the number of chases individuals display. Colony nesters may have displayed fewer chases than solo nesters because in a colony, there are multiple adults present to attack intruders, decreasing the individual acts of aggression necessary for each fish. Having just one more *A. saxatilis* present may be enough to significantly minimize the acts of aggression individuals are responsible for, explaining why there was not a difference in total number of chases displayed between small or large colony sizes. Solo nesters do not have this benefit of multiple defenders, and are thus responsible for all of the necessary defensive acts. Continuously chasing away other fishes requires large amounts of energy (Myrberg and Thresher 1974). Since males nesting alone are responsible for performing all the defensive acts needed to protect the nest site, they potentially expend a greater amount of energy protecting their egg patch than colony nesters. This suggests that nesting in a colony is more advantageous than nesting alone, as it would require less energy to defend the nest site.

Although the results of this study are supported by previous research, several assumptions remain that could lead to possible sources of error. It was assumed that nesting *A. saxatilis* that were at least one face of the mooring block away from other nest sites were not affected by the presence of these other nesting fish, and thus termed solo nesters. The possibility remains that this distance between nest sites was not enough separation, and the presence of *A. saxatilis* on the opposite side of the mooring block could still have had an effect on the number of intruders to the general area. Additionally, fish sizes were estimated while reviewing footage. This decreases the accuracy of the fish lengths recorded, allowing for the possibility that a trend could actually exist between fish length and nesting type. Finally, a number of the videos recorded had poor visibility due to sediments suspended in the water or shadows cast by the mooring blocks. It is possible that some fish behaviors were hidden, resulting in fewer chases recorded than were actually performed. While each of these potential sources of error may have affected the results of this study, they were each uniform across colony and solo nesters, suggesting that the data provides an accurate representation of *A. saxatilis* aggressive behaviors.

Since solo nesters exhibited more interspecific chases and more total chases than colony nesters, this suggests that nesting in a colony has the advantage of an increased number of defenders against intruders, decreasing the number of chases each individual must display. Furthermore, since large amounts of energy are required to relentlessly chase other fishes, the total number of chases observed acts

as a proxy for the amount of energy exerted by the fish (Myrberg and Thresher 1974). Therefore, solo nesters exerted much more energy defending their egg patches than colony nesters. Colony nesters would thus have more energy left to devote to parental care and future fecundity, making colony nesting the more advantageous nesting type. Colonial nesting should be the favored nesting type because it is the more advantageous strategy in terms of energy exertion, however solo nesters are still common. Solo nesting may occur as a result of limited nest sites within a colony's territory, forcing some *A. saxatilis* to build their nests away from established colonies. The results of this study not only contribute a greater understanding about the nesting behaviors of *A. saxatilis*, but can also relate to a variety of other animal species that exhibit territorial nesting behaviors. It is possible that saving energy by increasing the number of territorial guarders is a ubiquitous advantage for all colonial nesting species.

Future studies should be done to observe the effect of nest location on the number of chases displayed. Nests located at various points on the mooring blocks appeared to differ in their exposure to fish traffic. It is possible that more sheltered egg patches near the sandflats need less defense than more exposed nests at the top of the mooring blocks. Research could also be done to examine which species and or functional groups are most commonly chased away by nesting *A. saxatilis* to uncover more about their interactions with fishes from various ecological roles. The continuation of research on the aggressive behaviors of *A. saxatilis* will further our understanding of aggressive territorial behaviors and the advantages of different nesting types for species that exhibit parental care.

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REPORT

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The role of habitat structure and topographic complexity in species diversity and abundance of fish and invertebrate communities, and how it is affected by algae communities

Abstract Coral reefs are an important ecosystem providing a wide array of ecosystem services that benefit society and the environment. There are many factors, such as habitat structure, topographic complexity, fish and invertebrate species diversity that are interconnected, contributing to the success of coral reefs. It is essential to understand the variety of relationships that are occurring among habitat structure, topographic complexity, and species diversity because they are influential on the stability and resilience of coral reefs. In this study, habitat structure and topographic complexity were measured to determine their influence on fish and invertebrate species diversity, in addition to the effects that algae communities have on habitat structure and topographic complexity. Habitat structure and topographic complexity were determined by measuring the rugosity at the study sites while species diversity information was collected using roving diver surveys and photos. The rugosity was positively correlated with percent cover of benthic communities. Rugosity had a weak correlation with algae cover. Rugosity did not influence fish or invertebrate communities. However, there was a significant difference in fish species composition at different times of day and by date, whereas, invertebrate species composition differed significantly only for different times of day. The similarities of rugosity and species diversity among fish and invertebrates likely led to differences not being observed. There were complex interactions occurring among habitat structure, topographic complexity, fish and invertebrate species diversity making it difficult to fully understand the relationships that exist. Further studies are needed to understand how species diversity changes temporally.

Keywords Rugosity • coral cover • algae cover

Introduction

Climate change affects many ecosystems and coral reefs are no exception, especially since this ecosystem is sensitive to environmental and anthropogenic stresses (Graham et al. 2006). Coral reefs are being negatively impacted due to high mortality of corals from ocean acidification and bleaching that was caused by climate change (Graham et al. 2006). Ocean acidification does not directly lead to mortality but reduces coral growth by decreasing the concentration of carbonate ions available for use (Hoegh-Guldberg et al. 2007). A reduction in coral growth can indirectly lead to mortality because corals have less chance of recovering from bleaching or disease resulting from climate change. As corals die, habitats for marine life are lost, and consequently, species diversity is reduced, especially in fish communities (Graham et al. 2006).

Species diversity plays an important role in ecosystems (Peterson et al. 1998). Ecosystems are impacted by natural and anthropogenic disturbances, and every ecosystem has a different resilience level (Roff and Mumby 2012). Resilience is the ability of an ecosystem to endure a certain amount of disturbance

before a phase shift to an alternative stable state occurs (Holling 1973). Phase shifts are changes in the community composition (i.e. coral-dominated to algal-dominated coral reefs) (Nyström et al. 2008). Therefore, higher resilience is important for coral reefs because if they are maintained, then the preservation of coral reefs is more feasible (Hoegh-Guldberg et al. 2007). Species diversity impacts resilience by having a variety of functional groups. In a diverse community, functional groups are made up of multiple individual species creating functional redundancy within a system (Nyström et al. 2008). This redundancy increases resilience because if certain species disappear during a disturbance, their function would not be completely lost allowing the system to recover. Species diversity plays a critical role in maintaining ecosystems. It is therefore important to understand how ecological interactions, habitats, environment, anthropogenic factors, and climate factors influence diversity (Obura and Grimsditch 2009). Some ecological interactions that influence diversity are microbial interactions, suppression, and herbivory (Obura and Grimsditch 2009). Habitats such as connectivity, substrate, and topographic complexity also influence diversity (Obura and Grimsditch 2009). Environmental factors such as water and substrate quality along with anthropogenic factors like coastal development, fishing, nutrients, pollution, and land use influence diversity as well (Obura and Grimsditch 2009). A reduction in either habitat structure or topographic complexity would negatively affect species diversity by reducing the resilience of the ecosystem (Peterson et al. 1998; Bellwood and Hughes 2001; Newman et al. 2015).

Fish and invertebrate communities depend on differences in habitat structure and topographic complexity to support a wide array of species. Increased habitat structure and topographic complexity provide different niches. Different niches provide different resources for organisms; therefore, more species can be supported in a specific habitat. Different types of habitats provide various habitat structure and topographic complexity. Large spatial scale habitat types (e.g. mangroves, seagrass meadows, coral reefs) support diverse communities (Chittaro et al. 2005; Wilson et al. 2007). Within these large spatial scale habitats there are small spatial scale habitats nested within. These microhabitats such as individual coral heads, promote specialist species performing specific roles (Munday et al. 1997; Wilson et al. 2007).

Habitat complexity positively correlates with species diversity and abundance in fish communities (Luckhurst and Luckhurst 1978; Sano et al. 1984; Caley and John 1996; Friedlander and Parrish 1998; Gratwicke and Speight 2005a, 2005b; Wilson et al. 2007). A similar relationship between habitat structure, topographic complexity, and invertebrate communities is expected to occur; however, there is less research in this area. In freshwater ecosystems, increased habitat structure and topographic complexity increases invertebrate species diversity (Downes et al. 1998). A similar pattern has been shown with predatory gastropods; however, it was in relation to different habitat types (e.g. areas with turf algae or areas that were smooth and bare) (Kohn and Leviten 1976). This study provides more information on the relationships amongst invertebrate species diversity, habitat structure, and topographic complexity in similar habitat types.

Topographic complexity on coral reefs can be influenced by a variety of natural and anthropogenic disturbances such as coral bleaching, destructive fishing techniques (Graham et al. 2011), *Acanthaster planci* population explosions (Colgan 1987) and El Niño-Southern Oscillation (ENSO) events (Guzman and Cortés 2007). Disturbances can cause immediate loss of topographic complexity or loss over a longer time frame. Immediate loss can occur from storms and high wave action breaking coral branches (Ball et al. 1967). Losses occurring over time can be from disturbances that result in coral mortality by pollution, temperature fluctuations, diseases, or organism outbreaks (Wilson et al. 2006). Coral mortality impacts topographic complexity

because corals are no longer able to grow and produce new coral recruits. The combination of no new growth, bioerosion, and damages from disturbances slowly reduces topographic complexity. Disturbances also create the potential for phase shifts altering community composition. One potential phase shift is from coral dominated systems to algae dominated systems. This phase shift could occur due to a variety of disturbances, such as *A. planci* population explosions and ENSO events providing algae a competitive advantage over corals. When a system has low resilience, algae species cover often increases after a disturbance (Guzman and Cortés 2007) lowering the available space for corals to successfully recruit (Colgan 1987; Guzman and Cortés 2007). There is evidence that suggests that low coral cover reefs have more algae cover than high coral cover reefs because herbivorous fish reach an algae consumption threshold (Williams et al. 2001). In general, it can be implied that reefs with low coral cover have reduced habitat structure and topographic complexity. Though this evidence exists, there is still the question of the influence algae cover has on species diversity, habitat structure and topographic complexity.

This research investigated how habitat structure and topographic complexity influences species diversity and abundance, as well as, explored how the cover of algae communities influenced habitat structure and topographic complexity. Algae cover and diversity were assessed. If the cover and composition of the algae community is relatively stable over time, then the impacts algae have on coral growth and settlement will influence rugosity overtime. The following hypotheses were developed:

H_1: Higher algae cover will reduce habitat structure and topographic complexity
H_2: Fish species diversity will be higher when rugosity is high
H_3: Non-coral invertebrate species diversity will be higher when rugosity is high

It is important to understand the relationship among habitat structure, topographic complexity, fish and invertebrate communities (i.e. species diversity) because of the influences these have on coral reef ecosystems. Coral reefs are important for economies because of the benefits that are provided to society and the environment through a diverse array of ecosystem services (Hoegh-Guldberg et al. 2007). Relationships between habitat structure, topographic complexity, and species diversity can be identified through studies on natural and artificial reefs. Artificial reefs have been used to restore damaged coral reef systems, and through this study, guidance can be provided on how topographic complexity can influence fish and invertebrate communities.

Materials and methods

Study site

This research was conducted from September 2016 to October 2016 at Yellow Submarine dive site (12°09’36.20”N, 68°16’55.2”W) located just north of downtown Kralendijk, Bonaire, Dutch Caribbean located in the Caribbean Sea north of Venezuela (Fig. 1).

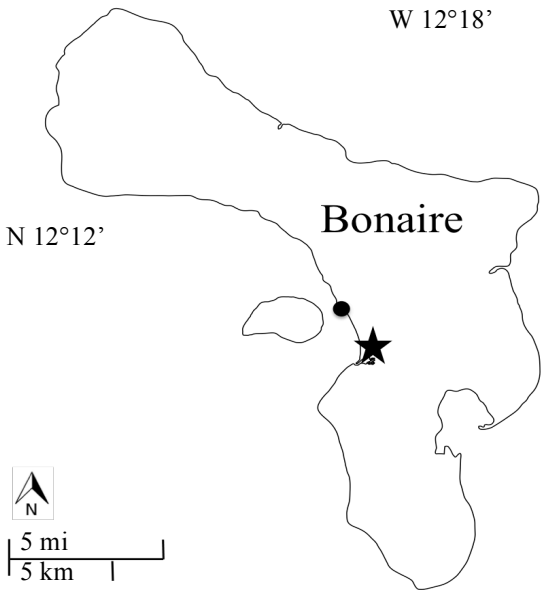

Fig. 1 Map of Bonaire, Dutch Caribbean. The black circle is the location of the study site at Yellow Submarine dive site (12°9’36.20”N, 68°16’55.25”W) and the star is Kralendijk

At this dive site, offshore mooring blocks located adjacent to the reef crest were used to conduct research. Mooring blocks sized ~0.8 m x ~0.8 m x ~0.8 m, were positioned in groups of three for a total of four groups. All blocks were placed between the years 1994 and 1996, allowing for colonization of sessile invertebrates to occur within a similar timeframe (Feighery 2014). However, individual sessile invertebrates have colonized the blocks at different times making their ages different. These groups of mooring blocks are situated north and south from the dive site between 50 m and 60 m offshore (Fig. 2) (Feighery 2014). The depth at which the mooring blocks were placed is at ~6 m, which required SCUBA divers to survey the blocks (Feighery 2014).

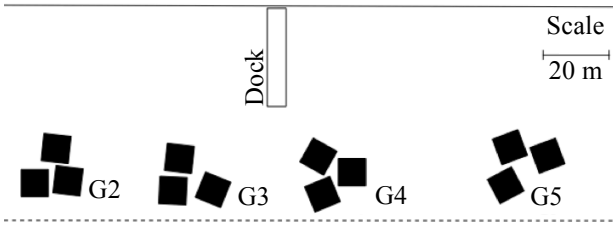

Fig. 2 Location of the four groups of mooring blocks offshore at Yellow Submarine. Black squares are individual blocks, dashed line represents reef crest, and solid line represents shoreline. Note: Mooring blocks are not to scale (~10 times larger)

Data collection

Rugosity

Rugosity of the mooring blocks was measured using a modified chain (weighted rope) intercept method (Hill and Wilkinson 2004). The weighted rope was laid to measure all accessible faces of the mooring blocks. Two divers laid out the rope to make sure lines were straight (Feighery 2014). Two parallel transects spaced a distance of ~0.25 m apart were laid over three faces of the block. Depending on how the blocks were positioned and accessibility of the faces of the block, one or two more faces were measured (Fig. 3). The linear distance (i.e. length of the block face that was bare) was measured using a transect tape. An average linear distance was taken by measuring multiple faces from different blocks that were free of species influencing rugosity.

Coral and algae cover

To determine coral and algae cover, a photo was taken of the exposed faces of each individual mooring block to analyze in an image processing software (Feighery 2014). Four to five photos were taken of each block depending on the placement of the blocks within the groups. This data was collected once, at the beginning of the project from 29th September 2016 to 8th October 2016. The reason for collecting the data only once was that these species were assumed to not change drastically over the course of the study impacting the outcome of the results.

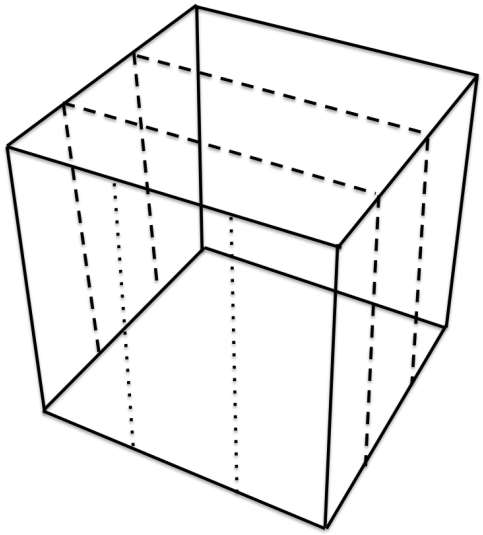

Fig. 3 Diagram of the rugosity weight rope layout. Two transects (dashed lines) were laid a distance of ~0.25 meters apart to measure three sides of the block. Two more transects (dotted line) were laid to measure the remaining exposed side of the block

Assessment of fish communities

To survey fish communities, similar methods employed by Jaco (2012) and Feighery (2014) were used to identify individual species (Humann and DeLoach 1989; Lang et al. 2010). Initial survey of fish used the roving diver method, staying a distance of ~2 m from the mooring blocks to prevent disturbance to fish (Jaco 2012), and recording the abundance of different fish species for 2 min. After the

initial survey, the divers approached the blocks surveying the smaller fish species that swam around the blocks as well as the ones that hid in holes and crevices. During these different assessments, each diver was responsible for recording different types of fish to avoid recording individuals more than once as well as differentiating between juvenile and adults (Jaco 2012). Surveys were conducted for five weeks for a total of four daytime surveys (29th September 2016 to 15th October 2016) and three nighttime surveys (19th October 2016 to 26th October 2016).

Assessment of invertebrate communities

Sessile invertebrates were identified (Humann and DeLoach 1992) and counted once during the study for each of the mooring block groups (G2 & G3: 8th October 2016; G4 & G5: 6th October 2016) because it was assumed that the abundance would not change over the course of the study. Mobile invertebrates were recorded five times throughout the entire study.

The abundance of nocturnal, mobile invertebrate species were identified and recorded during night dives. During the night surveys, flashlights, blue lights, and yellow filters were used. For the ease of counting, the use of fluorescence was used. Blue lights and yellow filters make the fluorescence of invertebrates, such as fireworms, visible to the human eye. A regular flashlight was used to identify invertebrate species that do not fluoresce. Night surveys were conducted for two weeks for a total of three surveys from 19th October 2016 to 26th October 2016.

Data analysis

The rugosity (R) was determined for each individual block by first summing the weighted rope measurements collected for each block, then they were summed together. The length of the weighted rope (L_A) was divided by the linear distance (L_B) of the block face (Hill and Wilkinson 2004; Friedman et al. 2012).

$$R = L_A \div L_B$$

The rugosity for the mooring block group was then calculated by averaging the rugosity of each individual block within the group.

Fish and invertebrate species diversity was determined by using the Shannon index (H). Using the Shannon index takes into account species richness and evenness (Dejong 1975). An important assumption using this index was that within a sample all species are represented and randomly sampled putting more weight on species richness (Dejong 1975). More diverse communities were denoted by higher H values while communities with an H value of zero only have one species within the community.

For coral and algae cover, ImageJ computer software was used to identify and measure the area of each individual coral species (Humann 1993) as well as algae present on the blocks. Once measurements were completed and combined amongst species, the percent cover was determined by dividing the area covered by each individual species by the area of the block face.

Multivariate analysis was conducted using PRIMER software running PERMANOVA tests to examine relationships amongst rugosity, fish species composition, invertebrate species composition, algae cover, and coral cover (Clarke and Warwick 2001).

Results

The mooring block groups were similar amongst each other when looking at the average rugosity and the Shannon index for fish (Table 1). However, there was more variation among live coral cover, algae cover and the invertebrate Shannon index (Table 1).

Table 1 Comparison of species diversity (Shannon index), rugosity, and benthic substrate cover of mooring block groups

	G2	G3	G4	G5
Fish Shannon index	2.78	2.83	2.74	2.73
Invertebrate Shannon index	0.88	1.05	0.83	0.72
Average rugosity	1.34	1.30	1.29	1.24
Live coral cover	46.1%	30.0%	43.4%	16.5%
Algae cover	14.0%	11.8%	10.9%	7.1%

Coral and algae cover

Rugosity was positively correlated with the benthic community structure (percent live coral cover & percent algae cover) of the mooring blocks (Fig. 4). The rugosity increased as the percent cover increased for both algae (Fig. 4A; $R^2 = 0.02$; $y = 1.3e^{0.0007x}$) and live coral cover (Fig. 4B; $R^2 = 0.28$; $y = 1.22e^{0.0018x}$). The mooring blocks were not completely covered by benthic communities; however, live coral cover had the highest cover of all the different benthic communities (Fig. 5). A total of 12 different coral species were present on the mooring blocks (Table 2) with *Diploria labyrinthiformis* (16.43% ± 0.11) and *Diploria strigosa* (10.94% ± 0.09) being the most abundant species.

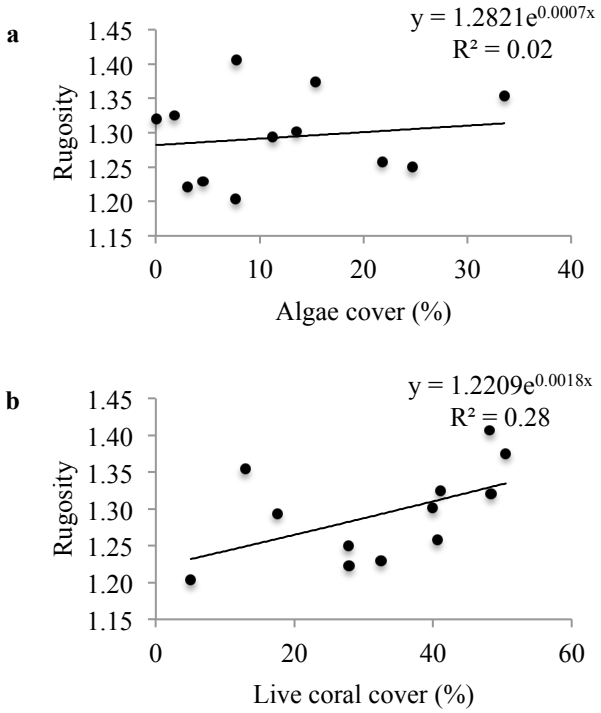

Fig. 4 The relationship between percent cover of different benthic substrate ((a) algae, (b) live coral) and rugosity. Each circle represents one mooring block (N = 12)

Assessment of fish communities

During the course of the study, 52 fish species were observed around the mooring blocks (Table 3). Rugosity and percent cover of benthic communities did not have an influence

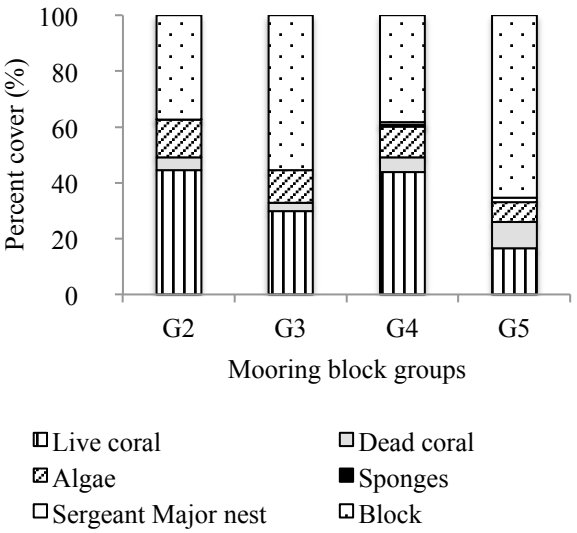

Fig. 5 Percent cover of benthic community assemblages for the four different mooring block groups (N = 4). Benthic community assemblages observed were live coral (vertical lines), dead coral (gray), algae (diagonal lines), sponges (black), Sergeant Major nest (white), and the rest was bare (block, dots)

Table 2 Benthic communities on mooring blocks. Coral and non-coral species observed on all mooring blocks during the study

Corals	
Scientific name	**Code**
Diploria strigosa	DSTR
Diploria labyrinthiformis	DLAB
Porites astreoides	PAST
Favia fragum	FFRA
Millepora complanata	MCOM
Orbicella faveolata	OFAV
Colpophyllia natans	CNAT
Millepora alcicornis	MALC
Orbicella annularis	OANN
Undaria agaricites	UAGA
Meandrina meandrites	MMEA
Montrastraea cavernosa	MCAV
Non-coral substrate	
Name	**Code**
Turf algae	TA
Sponge	SPO
Crustose coralline algae	CCA
Cyanobacteria	CYAN
Sergeant Major nest	SMN

Table 3 Fish species observed around the mooring block groups throughout the study

Fish species					
Common name	**Scientific name**	**Functional group**	**Common name**	**Scientific name**	**Functional group**
Angelfishes (Pomacanthidae)			**Surgeonfishes (Acanthuridae)**		
French Angelfish	*Pomacanthus paru*	Invertivore, Herbivore"	Blue Tang	*Acanthurus coeruleus*	Herbivore"
Queen Angelfish	*Holacanthus ciliaris*	Invertivore, Herbivore"	Ocean Surgeonfish	*Acanthurus tractus*	Herbivore"
Butterflyfishes (Chaetodontidae)			**Wrasses (Labridae)**		
Banded Butterflyfish	*Chaetodon striatus*	Invertivore"	Blackear Wrasse	*Halichoeres poeyi*	Invertivore"
Foureye Butterflyfish	*Chaetodon capistratus*	Invertivore"	Blueheaded Wrasse	*Thalassoma bifasciatum*	Invertivore"
Spotfin Butterflyfish	*Chaetodon ocellatus*	Invertivore"	Puddingwife	*Halichoeres radiatus*	Invertivore"
Damselfishes (Pomacentridae)			Rainbow Wrasse	*Halichoeres pictus*	Invertivore"
Bicolor Damselfish	*Stegastes partitus*	Herbivore^	Slippery Dick	*Halichoeres bivittatus*	Invertivore"
Cocoa Damselfish	*Stegastes variabilis*	Herbivore^	Yellowhead Wrasse	*Halichoeres garnoti*	Invertivore"
Dusky Damselfish	*Stegastes adustus*	Herbivore^	**Others**		
Sergeant Major	*Abudefduf saxatilis*	Invertivore, Herbivore^	Bar Jack	*Caranx ruber*	Invertivore, Piscivore"
Threespot Damselfish	*Stegastes planifrons*	Invertivore, Herbivore^	Barred Cardinalfish	*Apogon binotatus*	Invertivore^
Yellowtail Damselfish	*Microspathodon chrysurus*	Herbivore"	Brown Chromis	*Chromis multilineata*	Invertivore^
Gobies (Gobiidae)			Coney	*Cephalopholis fulva*	Piscivore, Invertivore"
Bridled Goby	*Coryphopterus glaucofraenum*	Herbivore^	Creolefish	*Paranthias furcifer*	Invertivore^
Glass Goby	*Coryphopterus personatus/hyalinus*	Invertivore, Herbivore^	Fairy Basslet	*Gramma loreto*	Invertivore^
Sharknose Goby	*Elacatinus evelynae*	Invertivore^	Blackbar Soldierfish	*Myripristis jacobus*	Invertivore^
Yellownose Goby	*Elacatinus randalli*	Invertivore^	Orangespotted Filefish	*Cantherhines pullus*	Invertivore"
Grunts (Haemulidae)			Redlip Blenny	*Ophioblennius macclurei*	Invertivore^
Bluestriped Grunt	*Haemulon sciurus*	Invertivore"	Sharpnose Pufferfish	*Canthigaster rostrata*	Invertivore, Herbivore^
Caesar Grunt	*Haemulon carbonarium*	Invertivore"	Smooth Trunkfish	*Lactophrys triqueter*	Invertivore^
French Grunt	*Haemulon flavolineatum*	Invertivore"	Spotted Goatfish	*Pseudupeneus maculatus*	Invertivore^
Smallmouth Grunt	*Haemulon chrysargyreum*	Invertivore"	Spotted Moray	*Gymnothorax moringa*	Piscivore, Invertivore"
Parrotfishes (Scaridae)			Spotted Trunkfish	*Lactophrys bicaudalis*	Invertivore"
Princess Parrotfish	*Scarus taeniopterus*	Herbivore"	Trumpetfish	*Aulostomus maculatus*	Invertivore, Piscivore^
Queen Parrotfish	*Scarus vetula*	Herbivore"	Whitespotted Filefish	*Cantherhines macrocerus*	Invertivore"
Redband Parrotfish	*Sparisoma aurofrenatum*	Herbivore"	Yellow Goatfish	*Mulloidichthys martinicus*	Invertivore^
Redtail Parrotfish	*Sparisoma chrysopterum*	Herbivore"	Yellowtail Snapper	*Ocyurus chrysurus*	Piscivore, Invertivore"
Stoplight Parrotfish	*Sparisoma viride*	Herbivore"	Unknown Blenny		
Striped Parrotfish	*Scarus iseri*	Herbivore"			

Functional groups determined using:

" Lang JC, Marks KW, Kramer PA, Kramer PR, Ginsburg RN (2010) AGRRA protocols version 5.4.

^ Froese R, Pauly D (2016) FishBase.

on fish species diversity (Fig. 6A; Pearson's correlation). However, there was a significant difference between fish species diversity at different times of day (Fig. 6A; PERMANOVA; df = 1; Pseudo-F = 29.31; p = < 0.001) and among dates (Fig. 6B; PERMANOVA; df = 8; Pseudo-F = 10.69; p = < 0.001). Analysis of MDS plot showed that most fish species were more abundant during the day and many were more abundant at earlier dates in the study period.

Assessment of invertebrate communities

There were 11 invertebrate species identified on the mooring blocks during the duration of the study (Table 4). There was a correlation between invertebrate species diversity and percent cover of different benthic communities (Fig. 7; Pearson's correlation). There was a significant difference between invertebrate species diversity during different times of day (Fig. 7; PERMANOVA; df = 1; Pseudo-F = 72.32; p = < 0.001) and dates (PERMANOVA; df = 4; Pseudo-F = 21.42; p = 0.001) when analyzed separately. However, when the dates were nested within the time of day of the survey, there was not a significant difference (PERMANOVA; df = 3; Pseudo-F = 0.44; p = 0.90).

Table 4 Invertebrate species observed on mooring blocks

Invertebrate species	
Common name	**Scientific name**
Long-spined urchin	*Diadema antillarum*
Christmas tree worms	*Spirobranchus giganteua*
Bearded fireworm	*Hermodice carunculata*
Dark mantis shrimp	*Neogonodactylus curacaoensis*
Spotted cleaner shrimp	*Periclimenes yucatanicus*
Banded coral shrimp	*Stenopus hispidus*
Nimble spray crab	*Percnon gibbesi*
Nodose clinging crab	*Mithrax coryphe*
Paved clinging crab	*Mithrax verrucosus*
Yellowline arrow crab	*Stenorhynchus seticornis*
Hairy clinging crab	*Mithrax pilosus*

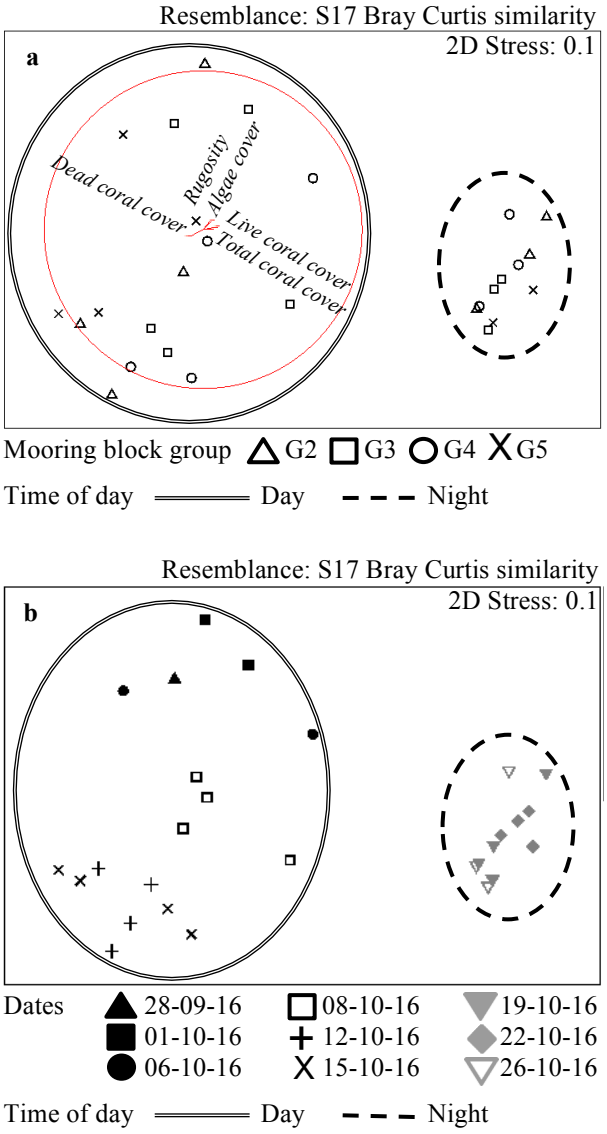

Fig. 6 Multidimensional scaling ordination (MDS) using the Bray Curtis similarity to show the correlation among rugosity, different benthic substrate (i.e. live coral, dead coral, & algae) and fish species composition. Each point is a mooring block group. (a) Red lines starting in the middle of the red circle show Pearson's correlation between the fish species composition and factors listed next to the lines. Longer lines display stronger relationships and the direction shows which samples have higher values in that factor (i.e. higher values are located further from the center). (b) Fish species diversity measurement grouped by date (black solid = before tropical storm; black open = right after tropical storm; crosses and gray = 5+ days after tropical storm)

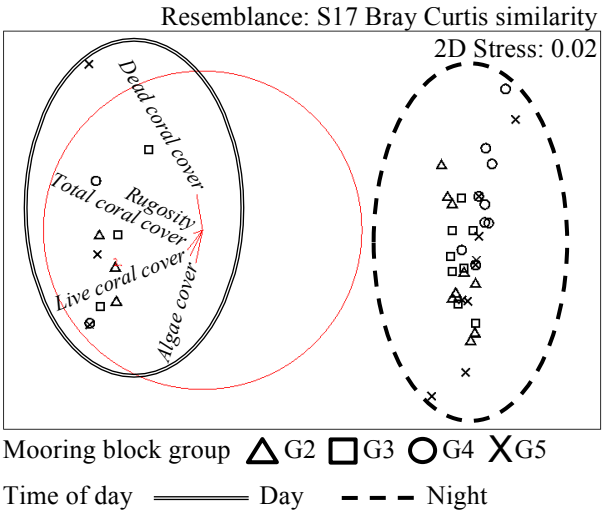

Fig. 7 Multidimensional scaling ordination (MDS) using the Bray Curtis similarity to show the correlation among rugosity, different benthic substrate (i.e. live coral, dead coral, & algae) and invertebrate species composition. Red lines starting in the middle of the red circle show Pearson's correlation between the invertebrate species composition and factors listed next to the lines. Longer lines display stronger relationships and the direction shows, which samples have higher values in the factor (i.e. higher values are located further from the center). Each point is a mooring block group

Discussion

The mooring block groups were similar to each other in regards to rugosity, fish and invertebrate species diversity; thus, the tests led to the rejection of all hypotheses. The positive correlation between algae cover and rugosity suggested that an increase in algae cover correlates with increased rugosity. The relationship shown between algae cover and rugosity does not support the hypothesis that algae cover will reduce habitat structure and topographic complexity.

Algae cover did not likely influence coral recruitment because the mooring blocks had ~40-65% bare area. Given the large amount of available space on the mooring blocks, other factors such as abundance of coral planulae or high mortality of coral recruits (Colgan 1987) were influencing the establishment of corals contributing to habitat structure and topographic complexity. If coral recruitment is reduced then habitat structure and topographic complexity will decline. Live coral cover had a stronger correlation with rugosity than algae cover supporting the finding by Feighery (2014) between coral cover and rugosity. Feighery (2014) found that mooring blocks with *D. labyrinthiformis* had higher live coral cover, which increased habitat structure and topographic complexity. The most abundant brain corals observed during this survey on the mooring blocks were *D. labyrinthiformis* and *D. strigosa*.

The rugosity of the mooring block groups were similar to each other, which may be a reason why there was no difference observed in species diversity. However, findings by Feighery (2014) showed that there was a significant positive correlation between rugosity and fish species diversity. Two years ago, on the same mooring blocks, Feighery (2014) used an average rugosity and average fish species diversity on the mooring block groups while this study assessed individual mooring block by groups. This could be a possible explanation for why a difference was not observed in this study.

Fish species composition was less impacted by rugosity but more by the benthic community. However, these correlations were not strong for any of these factors (i.e. algae cover, live coral cover, & dead coral cover), thus, did not fully support the hypothesis (H_2) that fish species diversity was influenced by the benthic community or rugosity. Generally, fish species diversity and abundance was higher when there was more topographic complexity (Wilson et al. 2007). Benthic community cover has been found to be an important factor when looking at fish species composition (Luckhurst and Luckhurst 1978). Large fish tend to be influenced more by habitat structure and topographic complexity (Luckhurst and Luckhurst 1978). This could explain why species composition varied among mooring block groups even though rugosity and fish species diversity were similar among groups. Dead coral cover had the strongest relationship with species composition. Coral, whether living or dead,

provided habitat structure and topographic complexity influencing fish diversity (Sano et al. 1984).

Additionally, there was a difference in the species composition depending on the time of day. Fish species composition was more influenced by the time of day than by rugosity or different benthic communities. The fish species composition was more diverse during the day than during the night, which was in opposition of the study conducted by Munday et al. (1997), where fish species diversity was higher at night. In this study, it was observed that the fish species seen during the day were more active than the fish species seen at night. Nocturnal activity varies among species. Some species were more abundant at night because these species were able to avoid daytime predators (Mattila et al. 1999). The fish species observed at night were located in between the mooring blocks or close to the block faces for protection. During the day, fish species were observed swimming all around the 2 m radius of the blocks and in between the blocks.

The species composition during the daytime varied by dates. A tropical storm passed by bringing nutrients from other locations, which could be a contributing factor in the variation observed in fish species composition. Similarities among species composition for the mooring block groups formed discrete groups in relation to the tropical storm. Fish species composition was the most varied among the mooring block groups before the storm (29th September 2016, 1st October 2016, 6th October 2016). Right after the storm (8th October 2016), the composition was similar among mooring block groups and a few days to weeks after the storm (12th October 2016, 15th October 2016) the composition started to vary more.

The relationship between rugosity and species diversity had a similar pattern when comparing the difference between invertebrate and fish species. There was more variation of invertebrate species composition among the mooring block groups. Since rugosity did not influence the invertebrate species composition, the hypothesis (H_3) that rugosity will increase invertebrate species diversity was not supported. However, benthic community composition influenced species composition. Blocks with high amounts of dead coral cover supported a different invertebrate species composition than blocks with high amounts of live coral and algae cover. The difference in benthic communities between blocks provided different areas for invertebrates to use as shown by Kohn and Leviten (1976), who found that invertebrate densities were higher when there was more habitat structure. Downes et al. (1998) also found that there were differences in invertebrate species diversity when there was a change in habitat structure and topographic complexity.

A more significant relationship between species composition and time of day was observed, further reinforced by Mattila et al.'s (1999) finding that species diversity was significantly higher during night than day. Invertebrate species composition varied more at night because invertebrates become more active at night (Mattila et al. 1999). Species observed were highly cryptic, so during the day these species blend into their surroundings when not active (Mattila et al. 1999). Since invertebrate species are less mobile, species composition will be less influenced by date than time of day. Low mobility reduced the likelihood that there will be major differences between dates. This could also account for the difference observed in invertebrate and fish species diversity. Fish species were mobile allowing for the shift in species composition observed overtime among mooring block groups. However, a change in invertebrate species composition was not observed due to the tropical storm that occurred during the study.

All the relationships observed throughout this study amongst habitat structure, topographic complexity, fish and invertebrate species diversity help to provide insight on coral reef ecosystems. Rugosity for the mooring block groups were similar amongst each other, possibly explaining why there was

not a significant difference seen in fish and invertebrate species diversity. Habitat availability supports different fish and invertebrate species (Munday et al. 1997) explaining why there was some variation seen in species composition. However, the fish species composition varied due to the tropical storm suggesting that storms can influence species composition. Another factor, such as shelter (Sano et al. 1984; Friedlander and Parrish 1998) can influence species composition as well. There are many contributing factors, such as natural and anthropogenic disturbances, that influence habitat structure, topographic complexity, and species diversity; therefore, understanding the connectivity among these factors is essential to begin the process of restoring damaged coral reefs.

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REPORT

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Use of fluorescence to determine if the polychaete annelid, *Hermodice carunculata* will be affected by future pH levels in the year 2100 as a result of ocean acidification

Abstract Marine ecosystems play an important role in regulating the climate. The ecosystem services that the ocean provides benefit human society. The beginning of the 18th century marked a time when large quantities of carbon dioxide (CO_2) were released into the atmosphere and taken up by the ocean. This uptake has caused pH levels to drop in a process known as ocean acidification. If CO_2 emissions do not begin to decrease, then the projected partial pressure of CO_2 (pCO_2) in the year 2100 (1000 µatm) will have deleterious effects on the marine calcifiers that marine ecosystems depend on. *Hermodice carunculata*, an invertebrate annelid, presents a unique opportunity – they fluoresce, which makes observing how pH affects them a fairly simple process. The purpose of this experiment was to use *H. carunculata*'s ability to fluoresce to determine how they, and other marine ectotherms, could be affected by future levels of CO_2. Abiotic data taken on the date of data collection was used to calculate pH values projected for 2100, and this information was used to simulate that environment and observe changes in fluorescence. Results showed that in the short term, corrected total cell fluorescence (CTCF) of *H. carunculata* may not be affected by future CO_2 values projected for 2100. These results open up a window of opportunity to study how metabolic changes caused by ocean acidification can be monitored using fluorescence.

Keywords Metabolic downregulation • CO_2 • marine calcifiers

Introduction

In marine ecosystems, rising anthropogenic levels of CO_2 and climate change are associated with shifts in temperature, nutrient input, and ocean acidification which have potentially wide-ranging and harmful biological effects (Doney et al. 2012). Covering 70-71% of the Earth's surface, the ocean has been, and continues to have an important role in regulating the climate (Hoegh-Guldberg and Bruno 2010). The ecosystem services that the ocean provides (e.g. detoxification, carbon sequestration, pollination, habitat provision, etc.) are both directly and indirectly related to biological interactions between organisms, and provide the natural benefits that our society depends on (Hoegh-Guldberg and Bruno 2010; Doney et al. 2012).

The beginning of the industrial revolution in the late 18th century marked a time when large quantities of CO_2 were released into the atmosphere as a result of fossil fuel burning and land use practices such as deforestation (Sabine et al. 2004); both of which increased atmospheric inputs of strong acids and bases that decreased the alkalinity and pH of the ocean (Doney et al. 2009). This decrease and its subsequent consequence is referred to as ocean acidification (Doney et al. 2009). Dissolved CO_2 reacts with water to form carbonic acid (H_2CO_3) which dissociates to bicarbonate (HCO_3^-), carbonate ions (CO_3^{2-}), and protons (H^+); increased atmospheric CO_2 shifts the equilibrium in the ocean in favor of higher CO_2 and HCO_3^-, and lower CO_3^{2-}. This lowers the pH of the water, which over time has disastrous consequences for both calcifying marine organisms and the ecosystems that depend on them (Cigliano et al. 2010).

If CO_2 emissions continue to rise at the rate that they are now, atmospheric CO_2 is expected to rise from current 380 ppm (pCO_2 = 380 µatm) to more than 1000 ppm (pCO_2 = 1000 µatm) by the year 2100 (Pörtner 2008). The reduction of pH that is expected to accompany increased levels of CO_2 in the ocean has dire implications for the physiological processes of marine organisms (Harley et al. 2006). Experimental elevation of CO_2 has resulted in the reduction of certain subcellular processes such as protein synthesis and ion exchange, and the effects were more pronounced in invertebrates than they were in fish (Pörtner et al. 2005). These processes should not be life-threatening for the individual, but are expected to impede already slow processes like growth and reproduction on longer timescales (Pörtner et al. 2005).

Calcifying marine invertebrates are those that produce shells and skeletons made out of calcium carbonate ($CaCO_3$) (e.g. echinoderms, scleractinian corals, crustose coralline algae, and coccolithophores) (Andersson et al. 2008; Hofmann et al. 2008; Cigliano et al. 2010). Ocean acidification poses a major threat to marine calcifiers because it decreases the amount of carbonate ions available for skeletogenesis – an important process in echinoderms that facilitates the formation of skeletal ossicles (any small bony or chitonous structure found in various skeletal parts of animals (Oxford dictionary of biology)) and induces physiological hypercapnia (excessive carbon dioxide in a system (Michaelidis et al. 2005)) which has a narcotic effect that suppresses metabolism (Sarashina and Endo 2006; Byrne 2011). Although invertebrate gametes can tolerate both ocean warming and acidification values projected for 2100, early stage larvae and juveniles can succumb to skeletal dissolution which has deleterious effects for adult populations and marine communities (Byrne 2011). The invertebrate species that broadcast-spawn their gametes and have pelagic larvae that spend up to a few months in the water column will be less likely to live to the next generation (Byrne 2011). Nonetheless, there are some invertebrate species that are more resilient than others.

As a group, polychaete worms have an incredible ability to adapt to different environmental conditions. Cigliano et al. (2010) found 12 different polychaete taxa at varying abundances at a volcanic CO_2 vent running parallel to shore with a pH gradient from 8.17 down to 6.57. The ability of these polychaetes to acclimatize to elevated pCO_2 environments and the physiological processes involved is a near unexplored topic (Calosi et al. 2013). Typically, when exposed to elevated levels of CO_2, ectotherms demonstrate downregulation of their metabolic rate which is a process they may have evolved in order to maintain a balance between their energy supply and how much their body may demand due to environmental stresses (Calosi et al. 2013).

Hermodice carunculata, a polychaete annelid, presents a unique opportunity – they produce proteins which react to UV light and allow them to fluoresce, which makes it easy to observe the effects of decreased pH and increased pCO_2. *Hermodice carunculata* are facultative corallivores and their main diet consists of decaying corals and fish, suggesting that they are omnivorous scavengers (Wolf et al. 2014). Sites of anaerobic decomposition have more concentrated levels of CO_2 which are attractants to marine scavengers such as *H. carunculata* (Riemann and Schrage 1988). Given this information, it is expected that *H. carunculata* will be tolerant to varying levels of CO_2.

Since the discovery of the green fluorescent protein (GFP) found in the hydromedusa *Aequorea victoria* approximately 40 years ago, the scientific community has been gifted with a protein capable of visible-spectrum fluorescence which has allowed it to be used as an *in situ* and *in vivo* protein marker (Evdokimov et al. 2006). There is not much known about fluorescence in annelids, but divergent evolution doctrine suggests that all other fluorescent organisms other than hydromedusae likely possess GFP homologues (Evdokimov et al. 2006). Additionally, research done on fluorescence in Anthozoa has shown that each fluorescent color is determined by a sequence of a single protein molecule (Labas et al. 2002).

The aim of this study was to use the fluorescence of *H. carunculata* as a tool to determine how they could be affected by future levels of CO_2, specifically, predicted levels likely reached by the year 2100, and make inferences about how these levels will affect their metabolic processes (i.e. growth and reproduction). *Hermodice carunculata* with various lengths between 15-22 mm collected on the sand flats of the Caribbean island of Bonaire were used for this study.

H_1: There will be no significant difference in the fluorescence between worms immersed in water with pCO_2 = 300-500 µatm and pCO_2 = 1000-1200 µatm

Materials and methods

Study site

Yellow Submarine dive site (12°09'36.47"N, 68°16'55.16"W) located in Kralendijk, Bonaire, Dutch Caribbean was the chosen study site for this project (Fig. 1). Specimens of *H. carunculata* were collected in the sand flat at a depth of 3-7 m where there was little to no coral cover. Partial pressure of CO_2 (pCO_2) at Yellow Submarine dive site ranged from 290-500 ppm.

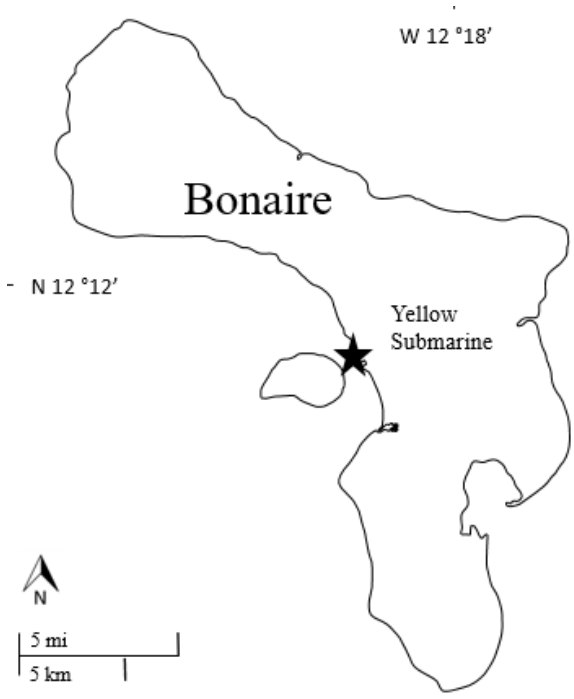


Fig. 1 Yellow Submarine dive site in Kralendijk, Bonaire in the Dutch Caribbean (12°09'36.47"N, 68°16'55.16"W). The star marks the location of Yellow submarine

Study organism

H. carunculata, also known as the bearded fireworm, is segmented with a reddish-brown tint, however, under UV light, they fluoresce bright green. Fluorescence is concentrated in the outer edges of the body with lowest fluorescence intensity in the mid-body. They are typically found in the coral reefs of the Caribbean, but have been found in the West Indies, Mediterranean Sea, and Ambon (WoRMs). They are facultative corallivores, but WoRMs lists them as omnivores, predators, and scavengers as well (Wolf et al. 2014).

Specimen collection and treatment set-up

Prior to each data collection day, 20 worms were collected at night using SCUBA, and were left in a plastic container (lightly capped) for 3 days without food. Only 16 worms were needed per trial, but four extra were collected in the event that one or more of them died. On the day of data collection, newly obtained seawater was poured into two glass tanks (dimensions: 40 cm x 20 cm), and a 556 MDS

YSI meter and La Motte saltwater aquaculture test kit (model: AQ-4) were used to obtain the pH, temperature (°C), salinity, and alkalinity of each tank. Tank 1 contained the CO_2 treatment and Tank 2 was the control. The pH, temperature, salinity, and alkalinity values obtained on each day of data collection were then input into USGS CO_2 calculator to calculate pCO_2 and the pH necessary to simulate acidic conditions projected for 2100 (pCO_2 = 1000 µatm). Then, CO_2 was bubbled into Tank 1 until the target pH was reached. The YSI meter stayed inside of the tank during this period so that the pH could be monitored throughout. Once the target pH was reached, four glass jars were set up labeled T_1G_1 (Tank 1 Group 1), T_1G_2 (Tank 1 Group 2), T_2G_1, and T_2G_2. Each jar was filled with water from its subsequent tank, four worms were added, and the jars were subsequently capped. The same steps were done to the jars labeled T_2. The worms were left in the treatment for two hours.

Microscope set-up

A dissecting microscope with an AmScope digital camera attachment was used to take pictures of the bristle worms. A rig made of PVC piping was used to hold the GoBe NightSea UV flashlights in place (Fig. 2). A yellow filter was also attached to the microscope in order to see the fluorescence.

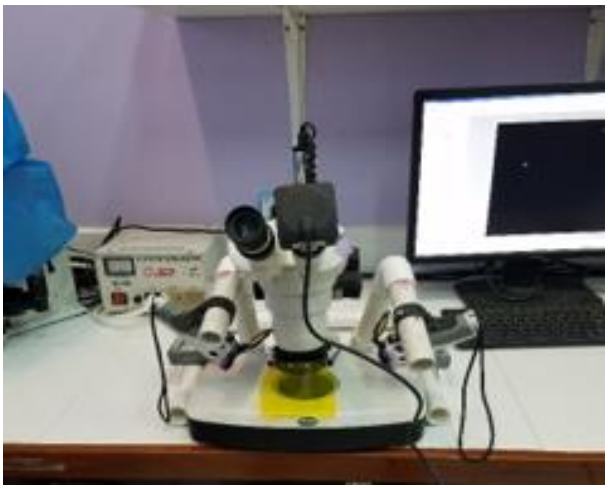


Fig. 2 Microscope set-up for fluorescence photography. PVC pipe rig fitted with GoBe NightSea UV lights and a dissecting microscope fitted with an AmScope microscope digital camera and yellow filter

Image collection

The worms from each of the T_1 jars were placed into petri dishes (36 mm diameter) filled with T_1 salt water and 15 drops of 9% $MgCl_2$. The YSI and La Motte test kit were then used to obtain the pH, temperature, salinity, and alkalinity of both jars. These steps were then repeated with the worms from treatment 2. Between 5-10 photos of each worm were then taken using the dissecting microscope and AmScope digital camera.

Image analysis

One image per worm was selected based on clarity of the image and analyzed using ImageJ software. The area, mean gray value, and integrated density were obtained using the measuring tools provided by the program (Fig. 3). Those values were then used to calculate the corrected total cell fluorescence (CTCF: integrated density – area).

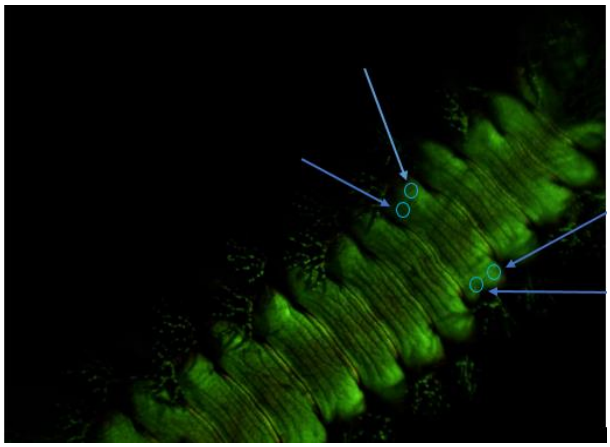


Fig. 3 Image of *Hermodice carunculata* taken using an AmScope microscope digital camera. Each blue circle represents the areas in which corrected total cell fluorescence (CTCF) was calculated. ImageJ software calculated the 'mean gray value' and 'integrated density' (integrated density = area * mean gray value). Each circular area amounted to 2828 pixels (x = 60, y = 60). All four circles were placed within the same segment per worm. Segments were determined based on clarity and evenness of fluorescence. CTCF = integrated density - area

Statistical tests

A two-way ANOVA comparing the dates of data collection and treatment type was used to determine if there was a significant difference in CTCF of the worms. Once a significant difference was identified, a Tukey post hoc test was used to determine between which dates CTCF of the worms were significantly different, and a subsequent t-test was later used to determine if there was a significant difference between treatments on each day of data collection.

Results

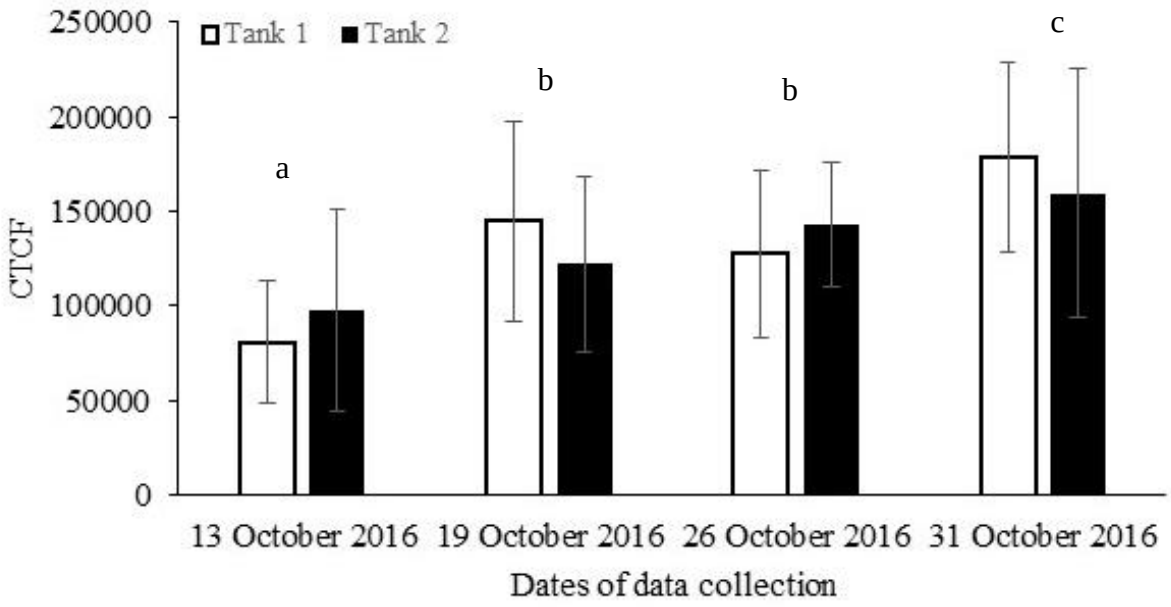
Throughout the course of the study, the pH in the Tank 1 treatment varied from 7.3 to 7.45 and the pH in the Tank 2 treatment varied from 7.65 to 7.83 (Table 1). The temperature in the Tank 1 treatment varied from 27.5°C to 28.96°C and the temperature in the Tank 2 treatment varied from 26.06°C to 29.04°C (Table 1). The salinity in the Tank 1 treatment varied from 35.53 ppt to 37.03 ppt and the salinity in the Tank 2 treatment varied from 35.38 ppt to 37.02 ppt (Table 1). The alkalinity in the Tank 1 treatment varied from 96 ppm to 144 ppm and the alkalinity in the Tank 2 treatment varied from 92 ppm to 106 ppm (Table 1).

A two-way ANOVA revealed that date played a significant role in the corrected total cell fluorescence (CTCF) of the worms exposed to each treatment ($F=29.51$, $df=3$, $p<0.001$). CTCF of the worms was significantly lower on 13 October 2016 compared to all other dates (Fig. 4). The CTCF of the worms was significantly higher on 31 October 2016 compared to all other dates (Fig. 4). Average CTCF of the worms on both 19 October 2016 and 26 October 2016 were not significantly different from each other, but were significantly different than on 13 October 2016 and on 31 October 2016 (Fig. 4). The type of treatment did not play a significant role in the CTCF of the worms over the four dates of data collection ($F=0.1493$, $df=1$, $p = 0.700$). The interaction of both factors (date and treatment) lead to a significant difference in CTCF values

Table 1 Abiotic data taken on each day ofata collection. 'Preliminary' rows show the data used to obtain the target pH while 'trial' rows show the resulting data obtained after each treatment. n=4. TA = Total Alkalinity, pCO_2 = partial pressure CO_2, SW = salt water.

Category	Date:	Time:	Tank	Group	Temp (°C)	Salinity (ppt)	pH	Alkalinity (ppm)	TA (µmol kg^{-1} SW)	pCO_2 (µatm)	Target pH (using CO_2 calc)
Preliminary	13/10/2016	11:04	1 (CO_2) 2	-	32.42	36.53	7.86	124	1238.884	322.949	7.4
Preliminary	13/10/2016	11:09	(Control)	-	32.45	36.43	7.8	112	1118.992	344.089	-
Trial	13/10/2016	21:12	1	1	28.16	37.03	7.45	144	1438.704	1152.92	-
Trial	13/10/2016	21:45	1	2	27.5	36.78	7.44	128	1278.848	1049.052	-
Trial	13/10/2016	22:15	2	1	27.89	36.95	7.83	104	1039.064	298.803	-
Trial	13/10/2016	23:07	2	2	26.06	37.02	7.76	104	1039.064	365.745	-
Preliminary	19/10/2016	13:23	1	-	29.39	35.71	7.73	104	1039.064	395.32	7.37
Preliminary	19/10/2016	13:27	2	-	29.71	35.84	7.8	104	1039.064	324.635	-
Trial	19/10/2016	17:16	1	1	28.82	35.94	7.34	108	1079.028	1135.444	-
Trial	19/10/2016	17:29	1	2	28.96	36.06	7.31	100	999.1	1130.133	-
Trial	19/10/2016	17:53	2	1	28.19	35.99	7.69	106	1059.046	450.953	-
Trial	19/10/2016	18:10	2	2	27.92	36.05	7.7	104	1039.064	430.412	-
Preliminary	26/10/2016	13:34	1	-	29.02	35.53	7.68	108	1079.028	472.549	7.39
Preliminary	26/10/2016	13:37	2	-	29.35	35.66	7.74	106	1059.046	392.691	-
Trial	26/10/2016	17:08	1	1	28.41	35.79	7.34	104	1039.064	1093.006	-
Trial	26/10/2016	17:18	1	2	28.44	35.53	7.37	96	959.136	934.799	-
Trial	26/10/2016	18:00	2	1	29.04	35.77	7.73	96	959.136	363.364	-
Trial	26/10/2016	18:13	2	2	28.88	35.82	7.7	96	959.136	394.586	-
Preliminary	31/10/2016	16:11	1	-	31.26	35.58	7.75	92	919.172	325.174	7.32
Preliminary	31/10/2016	16:25	2	-	30.7	35.54	7.77	92	919.172	308.487	-
Trial	31/10/2016	19:22	1	1	28.6	35.54	7.3	100	999.1	1104.57	-
Trial	31/10/2016	19:25	1	2	28.38	35.68	7.33	104	1039.064	1121.057	-
Trial	31/10/2016	19:52	2	1	28.53	35.38	7.65	92	919.172	433.47	-
Trial	31/10/2016	20:02	2	2	28.43	35.56	7.73	94	939.154	356.706	-

Table 2: Results of t-test run against the two different treatments (treatment 1: CO_2 and treatment 2: control) and the dates of data collection. n = 64



Date	t	df	p
13 Oct. 2016	-1.547	62	0.127
19 Oct. 2016	1.835	62	0.071
26 Oct. 2016	-1.550	62	0.126
31 Oct. 2016	1.293	62	0.201

Fig. 4 Fluorescence of *Hermodice carunculata*. Average CTCF (corrected total cell fluorescence) shown with ± standard deviations. Throughout each week, tank 1 had pH levels between 7.3-7.4 (pCO_2 = 900-1200 ppm), and tank 2 had pH levels between 7.7-7.9 (pCO_2 = 290-500 ppm). Days of data collection are shown (n=4). Dates on which the CTCF value obtained from *H. carunculata* were not significantly different according to Tukey HSD Post hoc test share the same letter

(F=3.139, df=3, p = 0.026). A t-test comparing the treatments between dates showed that there was no significant difference in CTCF of the worms between tank 1 and tank 2 (Table 2).

Discussion

Ocean acidification has dire consequences for calcifying marine organisms and the ecosystems that depend on them (Cigliano et al. 2010). The purpose of this experiment was to use fluorescence to test the resilience of *H. carunculata* to oceanic pH levels projected for the year 2100 (pCO_2 = 1000 µatm). Based on a past study on polychaete worms found at a volcanic vent with a pH gradient between 6.57-8.17 (Cigliano et al. 2010) it was hypothesized that there would be no significant difference in the fluorescence between worms immersed in water with pCO_2 = 300-500 µatm and pCO_2 = 1000-1200 µatm. The results of this experiment support this hypothesis.

Results revealed that average CTCF was significantly different between the different dates of data collection, but that there was no significant difference in the CTCF of the worms between treatments implying that fluorescence of *H. carunculata* may not be affected by pH values projected for 2100. It is difficult to extrapolate with any degree of certainty if *H. carunculata* will actually be capable of tolerating future oceanic pH values since the treatments in this study only lasted two hours. However, it would be interesting to study polychaete tolerance and response to decreasing pH levels in future, long term

experiments. Within an organism, changes in CO_2, pH, and bicarbonate affect cellular, molecular, and whole organism body functions (Calosi et al. 2005); so the continued use of fluorescence as an indicator of whole body system health would still be a good track to follow. Additionally, *H. carunculata* are scavengers (Riemann and Schrage 1988; Wolf et al. 2014) which over time could have made them more tolerant to varying levels of CO_2, a posible explanation for the results seen. Preliminary data used to calculate the target pH necessary to simulate the oceanic environment predicted for 2100 showed that on October 13th 2016, abiotic values were the highest in all categories. Regardless of the change of temperature, salinity, and alkalinity between the preliminary and trial period, the target pH was reached. It is worth noting that on this date, the average CTCF of the worms had the lowest values. Based on this alone, there must be some driving force, independent of pH, that influences fluorescence. Continued statistical tests run on the abiotic data obtained in this study would be beneficial in identifying this driving force.

GFP and its homologues are capable of visible-spectrum fluorescence and have been used as *in situ* and *in vivo* protein markers (Evdokimov et al. 2006). Given that *H. carunculata* is expected to have a GFP homologue (Evdokimov et al. 2006), they would need to metabolize this protein in order to emit light. If fluorescence in these worms is a metabolic process, then any change to the pH of their external environment that affects other metabolic processes (e.g. growth and reproduction) would have a visible effect in fluorescence, which was not observed in this experiment. It's possible that *H. carunculata* is a resilient species unaffected by variations in pH, or it is possible that fluorescence is not a metabolic process at all which could also explain the results.

Typically, marine ectotherms demonstrate downregulation of their metabolic processes when exposed to elevated levels of CO_2 which may have been a process they evolved in order to maintain a balance between energy supply, and the demand for this energy (Calosi et al. 2013). Given their varying habitats and eating habits, different species of polychaete worms may have evolved the ability to downregulate their metabolic processes faster than other marine ectotherms. In his paper, Pörtner et al. (2005) explained that existing information showed that the most important adaptive strategy of invertebrates living in variegated CO_2 habitats is their ability to suppress aerobic energy turnover rates (metabolic depression) in response to environmental stressors. The main environmental stressor that they talked about is hypercapnia, which when studied in *Sipunculus* nudus, a marine worm, found that there was an increased production of bicarbonate in their systems to compensate for the intracellular acidosis caused by the metabolic imbalance due to a lack of oxygen consumption (Pörtner et al. 2005). However, long-term extracellular acidosis induced by hypercapnia may in extreme cases arrest cellular transcription and translocation and possibly other cellular processes as well. In conclusion, this information implies that metabolic depression in marine invertebrates can be beneficial in the short term – as can be seen in the success of 12 different polychaete taxa found living next to a volcanic CO_2 vent (Cigliano et al. 2010), but that long term exposure to decreased levels of CO_2 has detrimental effects as well. More studies and experiments are necessary to make any conclusive statements on how marine calcifiers will be affected by conditions predicted for both 2100 and beyond.

Acknowledgements

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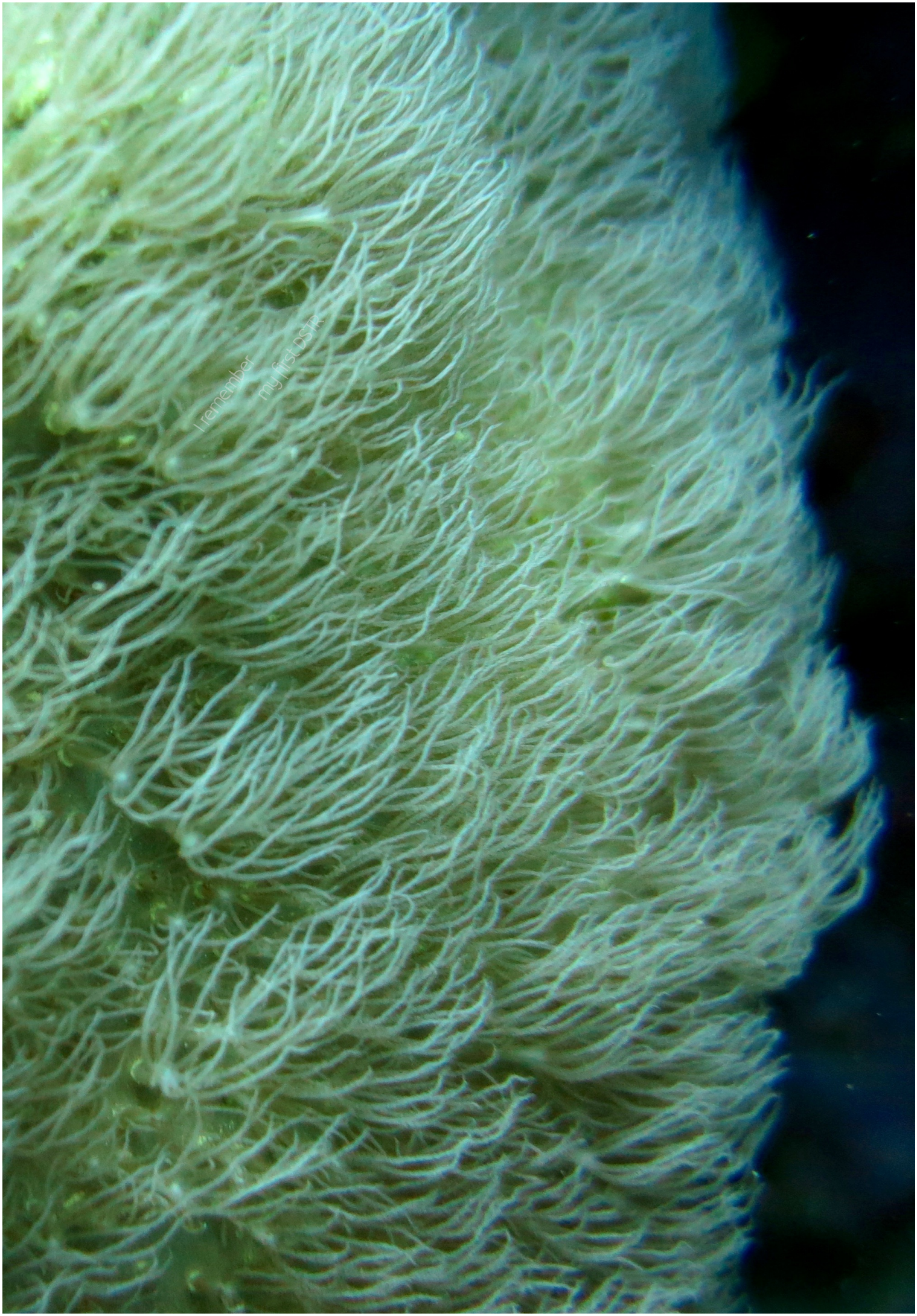
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