

PHYSIS

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Physis

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Journal of Marine Science

CIEE Research Station Bonaire

Tropical Marine Ecology and Conservation Program

Volume XI Spring 2012

Aristotle claimed physis was nature, Homer used physis as growth,
We use physis differently, with admiration for them both,
Since that time a species has developed, from civilizations they arose,
Humans have come to fight the world, but to themselves they inflict the blows.

The Earth is our planet, the land is our home,
But nature is where we truly live, and our imaginations roam.
Nature is our giver, but from nature we have taken,
Our greed has made a nightmare, of which we must awaken.

But how to wake and stop the loss of nature's giving soul?
Fourteen of us chose to study, with education as the goal.
With readings, papers and public events, we invested hours,
Conscious that feeding education, the tree of knowledge flowers.

We chose these months to grow and learn in a place like no other,
Trips to mangroves, beaches, protected areas, one after another.
Topics were chosen, measurements taken, surveys now completed,
All in hopes that with more knowledge the oceans won't be mistreated.

Our recent contribution to the world may be the first step to change,
We've educated ourselves and you, on what others consider strange.
Here we present our final work, showing all we've done,
We've worked towards bettering the word, though our work has just begun.

The meaning of physis has changed with time,
No longer is it only nature, or a natural sublime.
Now, it stands a symbol, of work we all must do,
Work towards a better Earth, one we can start anew.

Amelie Jensen
Max Mossler



FOREWORD

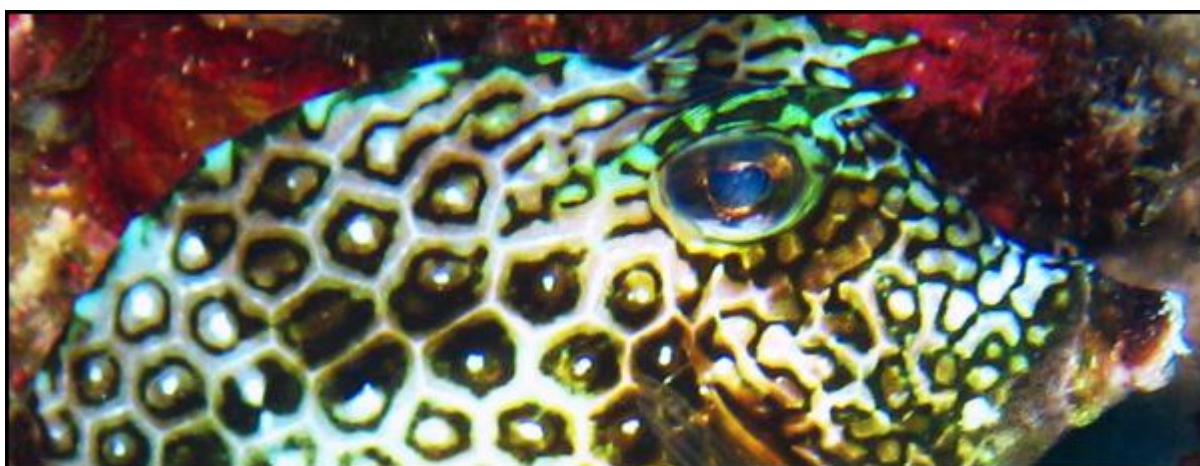
The Council on International Educational Exchange (CIEE) is an American non-profit organization with over 150 study abroad programs in 40+ countries around the world. Since 1947, CIEE has been guided by its mission:

“To help people gain understanding, acquire knowledge, and develop skills for living in a globally interdependent and culturally diverse world.”

The Tropical Marine Ecology and Conservation program in Bonaire is a one-of-a-kind program that is designed for upper level undergraduates majoring in Biology. The goal of the CIEE Research Station Bonaire is to provide a world-class learning experience in Marine Ecology and Conservation. The field-based science program is designed to prepare students for graduate programs in Marine Science or for jobs in Natural Resource Management and Conservation. Student participants enroll in six courses: Coral Reef Ecology, Marine Ecology Field Research Methods, Advanced Scuba, Tropical Marine Conservation Biology, Independent Research and Cultural & Environmental History of Bonaire. In addition to a full program of study, this program provides dive training that prepares students for certification with the American Academy of Underwater Scientists, a leader in the scientific dive industry.

The student research reported herein was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Dutch Caribbean. The research this semester was conducted on the leeward side of Bonaire where most of the population of Bonaire is concentrated. Students presented their findings in a public forum on the 18th and 19th of April, 2012 at the research station for the general public.

The proceedings of this journal are the result of each student’s Independent Research project. The advisors for the projects published in this journal were Rita B.J. Peachey, PhD and John A.B. Claydon, PhD. In addition to faculty advisors, each student had CIEE Interns that were directly involved in logistics, weekly meetings and editing student papers.



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Dr. Rita Peachey is the Resident director in Bonaire. She received her B.S. in Biology and M.S. in Zoology from the University of South Florida and her Ph.D. in Marine Sciences from the University of South Alabama. Dr. Peachey's research focuses on ultraviolet radiation and its effects on marine invertebrate larvae and is particularly interested in issues of global change and conservation biology. Rita teaches Independent Research and Cultural and Environmental History of Bonaire.



Dr. John Claydon is the Tropical Marine Conservation Faculty. He received a B.S. in Marine and Environmental Biology from St. Andrews University in Scotland and a M.S. and Ph. D. degree in Tropical Marine and Fisheries Ecology from James Cook University in Australia. His research interests include spawning aggregations of coral reef fishes, the red lionfish invasion, migration of reef fishes and reef fish fisheries. John teaches Tropical Marine Conservation Biology and Independent Research.



Professor Caren Eckrich is the Coral Reef Ecology Faculty and the Dive Safety Officer. She holds a B.S. in Wildlife and Fisheries Management from Texas A&M University and a M.S. in Biological Oceanography from the University of Puerto Rico in Mayaguez. Caren is the instructor for Marine Ecology Field Research Methods and Advanced SCUBA and her research interests include fish behavior, seagrass and algal ecology, and coral disease.

FACULTY



Anouschka van de Ven is the Assistant Resident Director for CIEE. She is a PADI dive instructor and underwater videographer. She assists with Advanced SCUBA and Cultural and Environmental History of Bonaire courses. She has a BA First Class Honors degree in communications studies from the London Metropolitan University and worked in television and advertising in Amsterdam before moving to Bonaire. Anouschka is responsible for the website and public relations.



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Marta Calosso is the Educational Specialist & Research Associate at CIEE. She has a Master's in Applied Fish Biology from the University of Plymouth, UK, and a Master of Arts in Humanities & Philosophy from the University of Milan, Italy. Her research interests include ecology of sharks, turtles, and reef fishes. Marta has been working with local schools and after school programs in Bonaire. She is also conducting research on habitat preference of rainbow parrotfish around the island.

INTERNS



Jason Flower assisted Dr. Claydon with Independent Research and Prof. Eckrich with Coral Reef Ecology and Advanced Scuba. He has an MSc in Tropical Coastal Management from Newcastle University in the UK and a BSc in Chemistry and Molecular Physics. Previously he has worked as a diving instructor and assisted marine conservation projects in Honduras, Grand Cayman, Greece and Tobago.



Lisa Young is the intern for Tropical Marine Conservation Biology and Independent Research. She is a native Floridian with an A.A. in Business from Valencia College, and a B.S. and M.S. in Marine Biology from Florida Institute of Technology. Lisa's research interests involve coral reef fish ecology.



Christina Wickman is the Marine Ecology Field Research Methods and Independent Research Intern. She recently received her Bachelor's degree in Marine Biology from the University of Oregon. In the fall of 2008 she was a student at CIEE Bonaire, where she looked at the possibilities of predicting coral bleaching around the island. Her research interests include coral reef ecology, coral reef preservation and public education of tropical reef ecosystems.

INTERNS



Graham Epstein assisted with Marine Ecology Field Research, Advanced Scuba, Cultural & Environmental History of Bonaire and Independent Research. He has a background in Genetics with a BSc in Biological Sciences from University of Edinburgh and a MSc in Marine Ecology & Environmental Management from Queen Mary, University of London. He is a PADI and BSAC dive instructor and his specific research interest is biogenic reef systems, with research projects on Scottish coralline algae beds and tropical coral reefs.



Fadilah Ali assisted with Independent Research and Prof. Eckrich with Coral Reef Ecology. Originally from Trinidad and Tobago, she has a Masters in Environmental Science from University of Southampton and is currently enrolled there, pursuing a PhD in Ocean and Earth Science. She has spent the last two years researching the lionfish invasion in Bonaire and has now expanded her research to the wider Caribbean region. Her research interests include invasive species biology, tropical ecology and conservation biology.



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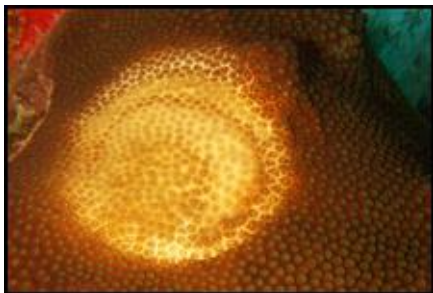
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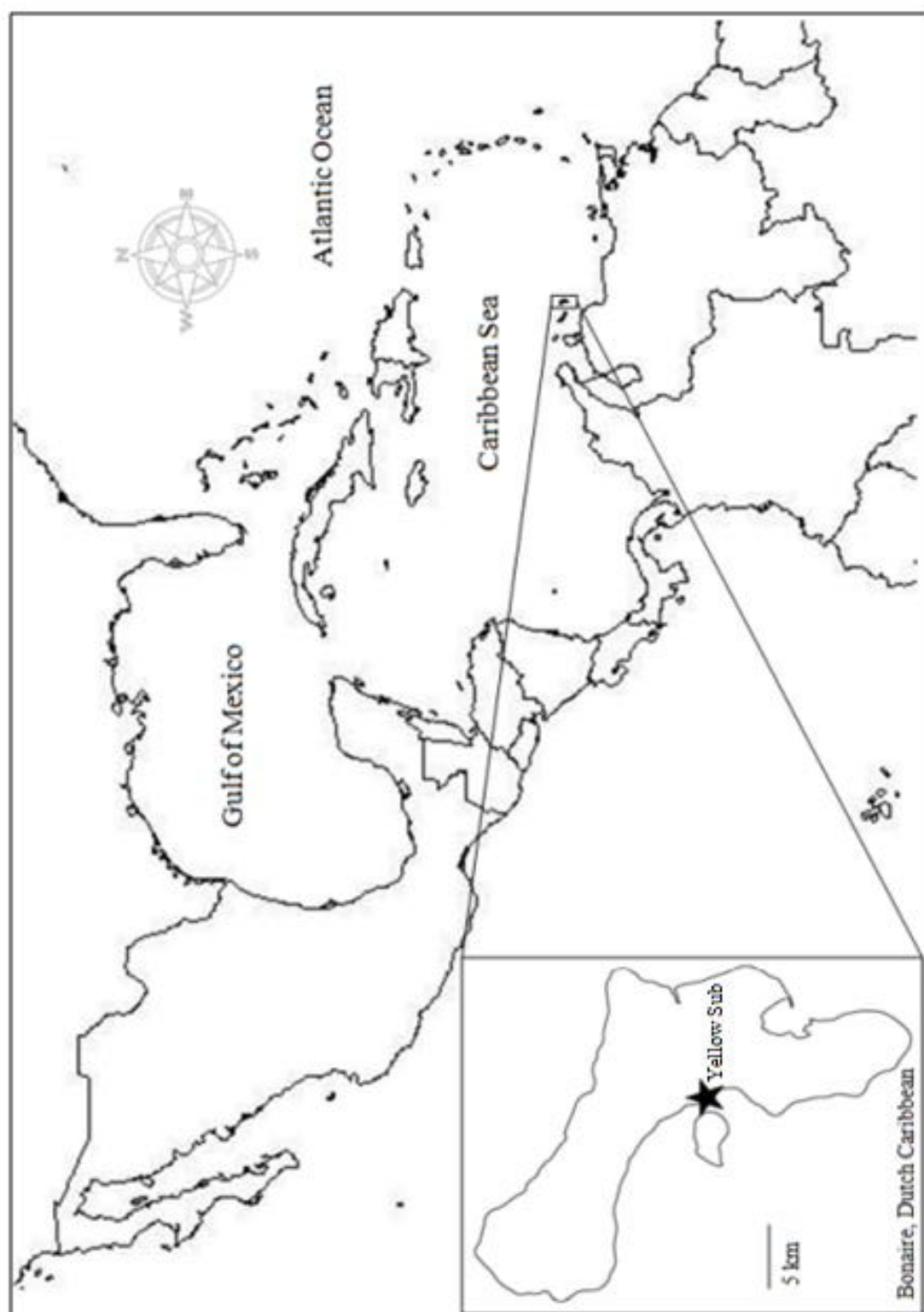
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Examining the effect of different grazers on algal biomass

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Abstract

Herbivory drives ecosystem dynamics in both terrestrial and marine habitats, controlling type and biomass of vegetation. In tropical coral reefs, herbivorous fishes and invertebrates feed on benthic macroalgae, resulting in decreased algal biomass and increased hard substratum available for coral growth and recruitment, providing for increased levels of biodiversity. In 1983, the long-spined sea urchin, *Diadema antillarum*, suffered mass mortality in the Caribbean, resulting in dramatic changes to ecosystem dynamics such as decreased coral cover and increased macroalgal cover. This study aimed to examine the impact of various grazers on algal biomass in areas with and without *D. antillarum* in Bonaire, Dutch Caribbean, from late February to early April, 2012, using herbivore exclusion cages with varying levels of exclusion. Grazer categories were established based on cage type and proximity to *D. antillarum*. It was hypothesized that algal biomass would decrease with increased herbivore access. At locations with *D. antillarum*, there was a general increase in algal biomass with increased exclusion, whereas at locations without *D. antillarum*, the opposite trend was observed. Algal biomass generally decreased with increased grazer access; however, differences were not statistically significant. Herbivorous fishes removed the highest amount of algae, followed by *D. antillarum*, and large invertebrates. This study shows the importance of multiple herbivores in maintaining low algal biomass in Bonaire.

Introduction

In terrestrial and marine habitats, herbivory is a driver of ecosystem dynamics, controlling the type and biomass of vegetation (Cyr and Pace 1993). In terrestrial ecosystems, primarily on grasslands and savannas, the dominant herbivores are mammals (Carpenter 1986), while the dominant herbivores in aquatic environments, such as coral reefs, are teleost fish (Choat and Clements 1998) and sea urchins (Ogden 1976; Carpenter 1986). In hard-bottom marine ecosystems, many herbivores feed by scraping or taking whole bites of the substrate, usually calcium carbonate or sand, along with plant or other organic material growing on the substrate (Ogden 1976; Bak et al. 1984; Huntley 1991).

In marine environments, such as coral reefs, herbivorous organisms, including fish from the Scaridae (parrotfishes) and Acanthuridae (surgeonfishes) families as well as invertebrates from the Echinoidea

family (sea urchins) graze on benthic macroalgae resulting in decreased algal biomass and more exposed hard substratum (Ogden 1976; Carpenter 1986). This grazing behavior maintains low levels of macroalgae (Williams et al. 2001), allowing for increased growth and recruitment of reef-building scleractinian corals, and thus high ecosystem biodiversity (Thacker et al. 2001). As a result of high levels of grazing, shallow back reef communities become dominated by corals, crustose coralline algae, and algal turfs (Lewis 1986).

Herbivores are so important to coral reef ecosystems that if removed, drastic changes to community structure can occur. For example, the long-spined sea urchin, *Diadema antillarum*, suffered a mass mortality in the Caribbean, which was coupled with rapid increases in algal growth. Mortality was first noted in Panama in 1983, but then extended throughout the Caribbean, including the Gulf of Mexico (Williams and Polunin 2001; Carpenter and Edmunds 2006; Alvarez-Filip et al. 2009). A water-borne

species-specific pathogen led to documented mortalities of 97.3% – 100% between 1983 and 1984 (Bak et al. 1984; Lessios et al. 1984; Hunte and Younglao 1988; Debrot and Nagelkerken 2006). Only five days after the mass mortality in St. Croix, U.S. Virgin Islands, algal biomass increased by 20%, indicating the rapid rate of algal growth in the absence of *D. antillarum* (Carpenter 1988). Throughout the Caribbean, overall coral cover decreased while algae cover increased. The outward growth of existing and new coral colonies was thus limited by the percent cover of macroalgae (Williams and Polunin 2001; Idjadi et al. 2010).

The removal of *D. antillarum* from many Caribbean coral reefs in combination with other factors such as overfishing and eutrophication contributed to a shift from coral-dominated to algal-dominated communities (Thacker et al. 2001; Williams et al. 2001; McManus and Polsenberg 2004). Coral-algal phase shifts are becoming more prevalent throughout the world and pose great threat to coral reef ecosystem health and biodiversity because of the unusually low levels of coral cover coupled with high fleshy macroalgal cover (McManus and Polsenberg 2004). Not only does the removal of keystone herbivores such as *D. antillarum* contribute to the phase shift, but eutrophication (Thacker et al. 2001; Williams et al. 2001; McManus and Polsenberg 2004; Mumby 2009), hurricanes, coral bleaching (Mumby 2009), and even outbreaks of a coral-eating species (McManus and Polsenberg 2004) can also lead to such a shift.

It is possible that populations of *D. antillarum* are recovering, which could contribute to a reversal of the phase shift (Carpenter 1988; Carpenter 1997; Idjadi et al. 2010). Population recovery of *D. antillarum* post-2006 is occurring at six locations along a 4100 km arc across the Caribbean (Carpenter and Edmund 2006), and in 2010, increased densities of *D. antillarum* on shallow Jamaican reefs were coupled with improved scleractinian coral growth and survivorship and a decrease in

abundance of macro and turf algae (Idjadi et al. 2010). Through benthic community sampling of scleractinian corals, macroalgae, algal turfs, and crustose coralline algae, it was found that increased scleractinian coral growth was linked to grazing by *D. antillarum* (Carpenter and Edmund 2006). Macroalgal reduction is typically followed by increases in crustose coralline algae cover, which may attract coral larvae and induce juvenile coral metamorphosis (Idjadi et al. 2010).

Several *in situ* experiments have been conducted in order to determine the impact of grazers such as *D. antillarum* and herbivorous fishes on the biomass of algae on coral reefs. Exclusion of both herbivorous fishes and *D. antillarum* from Caribbean reef communities resulted in a rapid accumulation of algae. In areas subjected to only herbivorous fish grazing, algal biomass was 2-4 times higher than that in treatments grazed by fishes and *D. antillarum* (Carpenter 1986). Furthermore, on a Caribbean patch reef, the removal of *D. antillarum* led to a marked shift to algal dominance (Sammarco et al. 1974), suggesting that grazing by the echinoid *D. antillarum* has a major impact on macroalgal biomass.

This study aimed to examine the impact of grazers on the biomass of algae in areas with and without *D. antillarum* in Bonaire, Dutch Caribbean. Because *D. antillarum* populations in the Caribbean may be recovering since the mass mortality of 1983-1984, it is important to compare the grazing of this echinoid to other grazers such as herbivorous fishes and large invertebrates in order to determine the relative impact of individual grazers on algal biomass. I aimed to identify the major grazers in Bonaire, Dutch Caribbean, by excluding certain herbivores from algae access. The following hypotheses were tested:

- H₁: Algal dry mass is greatest when herbivores are excluded, regardless of their proximity to *D. antillarum*.
- H₂: Algal biomass will decrease with increased grazer access.

This study can provide insight into the possible reversal of coral-algal phase shifts through high levels of grazing. Herbivory is oftentimes considered a top-down control of algal biomass in coral reef ecosystems (Ogden 1976; Carpenter 1986; Lewis 1986; Thacker et al. 2001; Williams et al. 2001). It is therefore important to monitor if such herbivores are keeping algal biomass down to a level that enables coral growth and ecosystem biodiversity.

Materials and Methods

Study Site

Bonaire is located in the southern Caribbean Sea, about 80 km north of Venezuela. Bonaire is a volcanic island surrounded by a fringing coral reef. The study took place at Yellow Submarine dive site in Kralendijk, Bonaire, Dutch Caribbean (12° 09'36.6" N, 068° 16'54.9" W), from late February to early April, 2012. The study site is located on the fringing reef of the leeward side of Bonaire (Fig. 1).

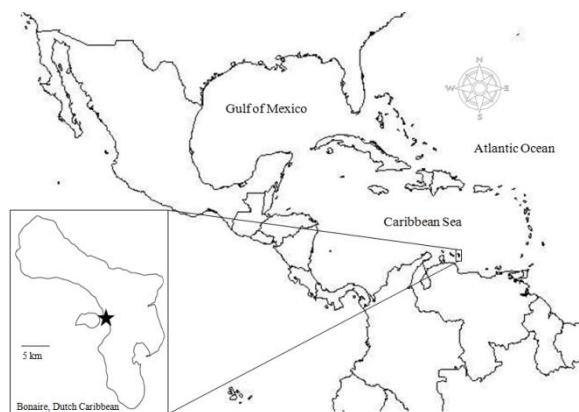


Fig. 1 Map of Bonaire, Dutch Caribbean. Black star indicates Yellow Submarine dive site, Kralendijk (12° 09'36.6" N, 068° 16'54.9" W)

Herbivorous fishes, such as Scarids and Acanthurids, are abundant on the reefs in Bonaire. At the study site, there are distinct patches where *D. antillarum* are present and areas of similar topography where the urchin is not found, making for an ideal site for a comparative field study of the differing

impact of grazing on the reefs in Bonaire. In addition, cages can be utilized to create other grazing treatments along with the patches with and without *D. antillarum*.

Experimental Design

In order to compare the effect of different levels of herbivory on algal biomass, ten sets of three different herbivore exclusion cages were prepared from wire mesh with a 1 cm grid size. The first type of cage was fully closed to exclude all herbivores, the second type had an open-top enabling only fish grazers and the third type was a ceramic tile attached bottom-up to a 20 cm x 20 cm piece of wire mesh with a 1 cm grid size via fishing line (hereafter termed "tile treatment"). Twenty 20 cm x 20 cm x 20 cm cages were made from the wire mesh, with ten having an open-top and ten being fully closed. One 15 cm x 15 cm ceramic tile was attached bottom-up on the bottom inside of every cage treatment using fishing line. One dive weight (~0.45 – 2.7 kg) was attached to the bottom outside of each cage treatment using a zip tie. The tops of the closed cages were secured shut with a zip tie.

Because of the different cage treatments and proximity to *D. antillarum*, different herbivorous grazers were assumed to have access to the ceramic tiles in the cage treatments. Four grazer treatments were created using cages and proximity to *D. antillarum* as follows: (1) *D. antillarum*, large invertebrates, and herbivorous fishes ("D, I, F" treatment) were immediately adjacent to *D. antillarum*, having access to cages with ceramic tiles only and a cage bottom; (2) large invertebrates and herbivorous fishes ("I, F" treatment) were in areas similar in topography to the cages near *D. antillarum* but lacked present urchins and had access to ceramic tiles only with cage bottoms; (3) herbivorous fishes ("F" treatment) from sites with and without *D. antillarum* from open-top cages; and (4) no grazers ("N" treatment) from closed cage from sites with and without *D. antillarum* (Fig. 2).

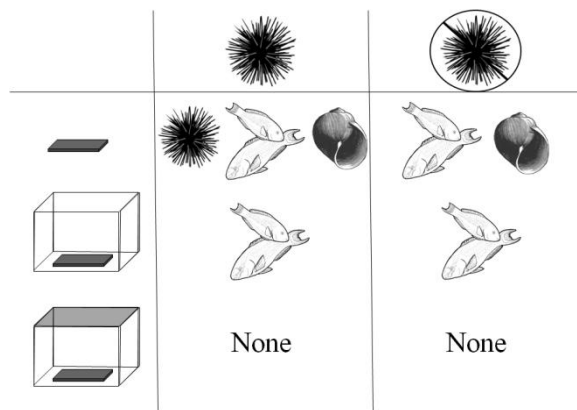


Fig. 2 Grazer categories based on cage treatments (first column) and presence or absence of *D. antillarum* (second and third columns, respectively). The first cage is a single ceramic tile with access to all grazers (*D. antillarum*, herbivorous fishes, and large invertebrates such as gastropods and other urchins, (large invertebrates denoted by *gastropod shell*)), the second is an open-top cage enabling only fish grazers, and the third is a closed-top cage excluding all grazers

Using SCUBA, the reef crest (~7-8 m depth) was scanned for three sites containing one *D. antillarum* individual and for three nearby sites that did not contain the urchin. Via snorkel, two sites with *D. antillarum* and two adjacent sites without *D. antillarum* were located along the shallow coral rock (≤ 1 m depth). At all ten sites, one of each exclusion cage type was placed in the sand, allowing five replicates for each treatment (Fig. 3). At sites containing *D. antillarum*, the cages were placed in the sand within 0.5 m of the sea urchin to ensure grazing. *D. antillarum* were assumed to leave their site of refuge (usually a hole or crevice) at dusk and then return to the same location to shelter in the morning (Bak et al. 1984; Carpenter 1997; Debrot and Nagelkerken 2006). Cages were left to grow algae for 2.5 weeks. In order to determine what herbivores were present at each site, weekly 5-min observations were made between 1730 and 1830 h at a distance of 4 m.

After the 2.5 weeks, using SCUBA, tiles were removed from cages and placed in plastic bags to eliminate algal loss during transfer to laboratory. With a single-edged razor, algae was scraped from tiles and

transferred to aluminum pans. Any water remaining in the plastic bags was vacuum-filtered to collect any remaining algae. The vacuum filter papers containing any remaining algae were then added to the pans with the algae and were placed in a 100°C oven to dry for two days. The algal dry mass in g cm^{-2} was then measured.

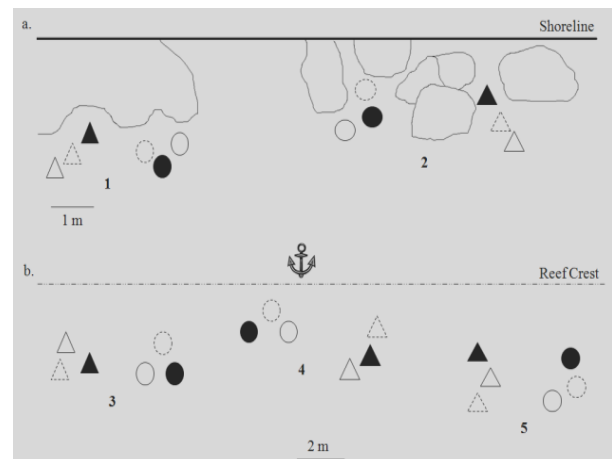


Fig. 3 Diagram of *in situ* herbivore exclusion experiment. Panel a shows two replicate sets of cages that were placed along a shallow (≤ 1 m depth) coral rock outcrop (irregular rounded shapes) at the shoreline (black line). Panel b shows three replicate sets of cages that were placed along the reef crest (dashed line) at ~7 - 8 m depth. Anchor symbolizes underwater anchor used for navigation. Circles denote cages placed in a location inhabited by *D. antillarum* while triangles denote cages placed in areas without *D. antillarum*. Three cage treatments were used: closed-top (fully shaded), open-top (dashed outline), and single ceramic tile (white shape with full outline)

Data Analysis

The mean algal dry mass per unit area across the five replicates for each cage type and presence or absence of *D. antillarum* was calculated in g m^{-2} . Statistical analyses and data manipulation were conducted using Analysis Toolpack in Microsoft Excel 2007. A t-test was conducted in order to test for differences between different cage treatments with the same grazer category. A one-way ANOVA was performed in order to determine significant differences in algal biomass between grazer categories. The mean algal dry masses of the different grazer

categories were compared to isolate the effect of individual grazers on algal biomass.

Results

Experimental cages were in place from 10 March to 29 March, 2012. During the weekly observations, filefish, damselfish, and *D. antillarum* were seen feeding on the algae from single tiles at different locations and times. Small crustaceans, gobies, and juvenile fish were found residing on ceramic tiles of all cage types; however these are not herbivores and should not affect algal growth. Sand was incorporated into the algae collected from all cage types.

At locations containing *D. antillarum*, there was a general increase in the mean algal dry mass (\pm SD) with increased herbivore exclusion (tile: 366.14 ± 184.76 g m⁻², open-top cage: 414.82 ± 220.97 g m⁻², closed cage: 549.12 ± 298.35 g m⁻²). At locations not containing *D. antillarum*, the opposite trend was seen, with a decrease in the mean algal dry mass (\pm SD) with increased exclusion. However the decrease was very small, providing for no general change in algal dry mass (tile: 377.13 ± 253.30 g m⁻², open-top cage: 353.76 ± 56.25 g m⁻², closed cage: 347.96 ± 59.33 g m⁻²; Fig. 4). Between different cage treatments with the same grazer category, no significant difference in mean algal dry mass was found between sites with and without *D. antillarum* (open-top: $t = 0.599$, $p = 0.566$; closed: $t = 1.479$, $p = 0.177$).

The mean algal dry mass (\pm SD) greatly decreased with increased grazer access (N: 448.54 ± 228.83 g m⁻²; F: 384.29 ± 155.38 g m⁻²; I, F: 377.13 ± 253.30 g m⁻²; D, I, F: 366.14 ± 184.76 g m⁻²), however, no statistically significant difference was found (ANOVA; $df = 3$, $F = 0.263$, $p = 0.852$; Fig. 5). Herbivorous fishes removed the highest amount of algae from tiles (14.32%), followed by *D. antillarum* (2.45%), and large invertebrates (1.60%; Table 1).

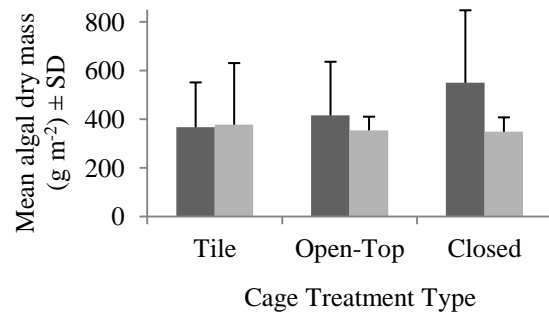


Fig. 4 Comparison of mean algal dry mass on ceramic tiles in bottom only, open-top, and closed cage treatments in areas with and without *D. antillarum*. Dark gray indicates presence of *D. antillarum* and light gray indicates absence of *D. antillarum*

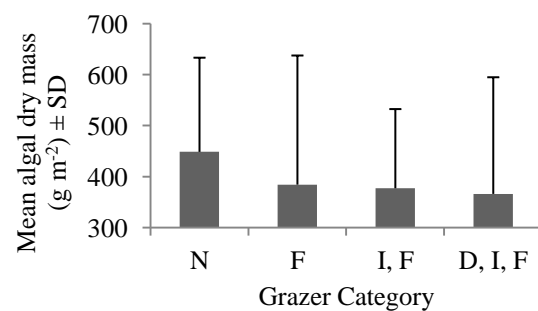


Fig. 5 Comparison of mean algal dry mass in four treatments of grazer access to ceramic tiles using herbivore exclusion cages. Grazer categories denoted by the following: N = no grazing (closed cages); F = herbivorous fish (open-top cages); I, F = large invertebrates, herbivorous fishes (single tiles in areas without *D. antillarum*); D, I, F = *D. antillarum*, large invertebrates, and herbivorous fishes (single tiles in areas with *D. antillarum*)

Table 1 Comparison of the mean dry weight of algae removed in four grazer treatments

Herbivore	Mean dry weight of algae removed (g m ⁻³)	Percent dry weight of algae removed (%)
Herbivorous fishes	64.24	14.32
<i>D. antillarum</i>	10.99	2.45
Large invertebrates	7.15	1.60

Discussion

This study aimed to isolate the impact of different herbivores on algal biomass in Bonaire, Dutch Caribbean, using herbivore exclusion cages in sites with and without the long-spined sea urchin, *D. antillarum*. There was a general increase in the mean algal dry

mass with increased herbivore exclusion at locations containing *D. antillarum*, but there was no increase at locations without *D. antillarum*. The reverse trend was found at locations without *D. antillarum*, with decreases in algal biomass with increased herbivore exclusion (Fig. 4). The decrease in algal biomass with increased herbivore exclusion at sites without *D. antillarum* could be because *D. antillarum* presence may induce algal growth, although this claim has not been thoroughly studied. The hypothesis that algal biomass increases with increased herbivore exclusion was therefore not supported by this study. There was an overall reduction in the mean algal dry mass with increased grazer access; however, no statistical significant difference was found (Fig. 5).

The variability in the mean algal dry mass after grazing could be due limitations brought upon by the short time frame of the study. The algae that grew on experimental tiles during this study may not have had enough time to diversify into the various types most commonly grazed by the herbivorous fish communities of Bonaire, and thus could have led to the discontinuity of the results. This could also provide insight into why no Acanthurid or Scarid species were found feeding on the tiles, and only filefish and damselfish species.

When individual grazing intensity was calculated, herbivorous fishes were found to remove the highest amount of algae from tiles, followed by *D. antillarum*, and large invertebrates (Table 1). This suggests that herbivorous fishes are the key species involved in maintaining low algal biomass, despite the evidence to support that *D. antillarum* once played a major role in limiting algae growth. These results could be due to the mass mortality of *D. antillarum* in 1983-1984 (Bak et al. 1984; Lessios et al. 1984; Hunte and Younglao 1988; Williams and Polunin 2001; Carpenter and Edmunds 2006; Debrot and Nagelkerken 2006; Alvarez-Filip et al. 2009) or because individuals may have moved from the sites where experimental cages were placed, thus

having no effect on algal biomass in those areas. Throughout the Caribbean, populations of *D. antillarum* were decimated and are only now starting to recover (Carpenter and Edmund 2006; Idjadi et al. 2010). Their populations may not be very high around Bonaire, allowing for other herbivores, such as fishes, to increase in abundance and to feed on the high levels of algae left in the absence of the echinoid. For example, around Curacao, the mean density of *D. antillarum* before the mass mortality was $6.4 \text{ ind } (100 \text{ m})^{-2}$, while after it was 0.00 to $0.01 \text{ ind } (100 \text{ m})^{-2}$ (Bak et al. 1984), showing that populations were drastically reduced and it might take some time before they begin to recover.

After the mass mortality of *D. antillarum* from 1983-1984, Caribbean coral reefs began to shift from having coral-dominated to algal-dominated communities (Bak et al. 1984; Lessios et al. 1984; Hunte and Younglao 1988; Williams and Polunin 2001; Carpenter and Edmunds 2006; Debrot and Nagelkerken 2006; Alvarez-Filip et al. 2009). If populations of *D. antillarum* are indeed recovering, as suggested by Carpenter and Edmund (2006) and Idjadi et al. (2010), then a reversal of the phase shift could be possible, if grazing by the echinoid is coupled with grazing by other herbivores.

This study found that the combined grazing effect of all three herbivore types resulted in the lowest mean algal dry mass observed as compared to the other herbivore categories (Fig. 5), indicating the importance of herbivory in maintaining low algal biomass in coral reef ecosystems. In Caribbean reef communities, when grazing by *D. antillarum* was combined with grazing by herbivorous fishes, algal biomass was 2-4 times lower than in treatments solely grazed by fishes (Carpenter 1986). Similarly, in a three-year study on the Great Barrier Reef in Australia, when large herbivorous fishes were excluded from experimental plots, algal cover exceeded 91% and was 9 to 20 times higher than that of open plots exposed to herbivorous grazing (Hughes et al. 2007).

Algae cover is therefore limited by high levels of grazing.

A limitation in algae cover can lead to improved coral growth and recruitment, which is essential in maintaining coral-dominated reefs and preventing algal domination. In the same study on the Great Barrier Reef, when herbivory was limited by exclusion cages, coral recruitment was approximately two-thirds lower compared to open plots, indicating that a lack of herbivory is correlated to a decrease in coral recruitment (Hughes et al. 2007). Grazing by *D. antillarum* has also been shown to promote scleractinian coral growth and recruitment (Carpenter and Edmund 2006; Idjadi et al. 2010). The results of this study did not examine coral recruitment and growth, however, but did show that mean algal dry mass was reduced when exposed more grazers. When levels of macroalgae kept down by herbivory, hard substratum becomes available for recruitment by corals, thus leading to increases in overall ecosystem biodiversity (Ogden 1976; Carpenter 1986; Thacker et al. 2001).

Herbivory is often times considered the top-down control of algal biomass in tropical coral reef ecosystems (Ogden 1976; Carpenter 1986; Thacker et al. 2001; Williams et al. 2001). If herbivores are removed, then drastic changes to community structure occur, as can be seen by increases in algal cover throughout the Caribbean after the mass mortality of *D. antillarum* (Bak et al. 1984; Lessios et al. 1984; Hunte and Younglao 1988; Williams and Polunin 2001; Carpenter and Edmunds 2006; Debrot and Nagelkerken 2006; Alvarez-Filip et al. 2009). This study further supports the importance of herbivores in maintaining low algal biomass in coral reefs, showing that combined grazing by multiple herbivores keeps algal biomass low, which is essential for maintaining high ecosystem biodiversity.

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Density of benthic meiofauna and macrofauna with relationship to depth in sandy coral reef substrate

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Abstract

The relationship that benthic organisms have on fish that live on the reef is well known. Some benthic organisms can be regarded as bio-indicators, acting as indicators of nutrient levels in an ecosystem. Benthic organisms are also an important food source for fish and other invertebrates. In this study, organisms from sediment cores at five different depths were analyzed. Meiofauna and macrofauna cores were collected at each depth and the organisms were identified to family level. This study provides information on where these organisms prefer to live, and if there are any depths that are more favorable or diverse. The abundance of species increased in macrofauna samples from depth 10 m to 20 m. This was after a decrease in density from 5 m, which had the highest density, to 10 m depths, which had the lowest density. This trend was also present in macrofauna species richness. The meiofauna samples also had the highest species richness and density of individuals at 5 m depth for species, but both variables decreased with increasing depths. The data shows an increase at the 5 m depth. With further testing we can better understand the relationship depth has on the diversity of the benthic zone in the southern Caribbean.

Introduction

Benthic organisms play an important role in aquatic food webs by providing nutrition for predators such as fish and other benthic feeding organisms, and are especially important in shallow marine habitats like coral reefs (Snelgrove et al 2000). The predation on benthic organisms living in soft sediments is an important process controlling community structure (Bell 1980). Benthic organisms are grouped into two major categories: 1) macrofauna, which are organisms $> 500 \mu\text{m}$ and 2) meiofauna, which are organisms $62 \mu\text{m} < 500 \mu\text{m}$. Reef fish that live in the habitat use the meiofauna and macrofauna as an important source of food, transferring nutrients from the benthic region up to the water column. Large diversity is very important because it provides more nutrition for larger predators on the reef. When there is more food the fish population increases greatly. The benthic community is also used as a good bio-indicator, because of this the diversity and density is important when trying to display

trends and trace chemicals which are passed from one trophic level to the next.

The diversity of benthic meiofauna is higher in areas where there are more sponges and other various creatures to feed on (Schiel et al. 1986). The diversity is increased in areas where more sponges because the polychaetes feed on the sponges. Sponges also emit and erode the reef, because of this the sand is very fine around sponges, and allows deposit feeders to thrive. Riddle (1988) observed diversity of the benthic organisms and found the most abundant was errant polychaetes along the continental shelf in the central region of the Great Barrier Reef, which outnumbered sedentary polychaetes at all sites except for the inner shelf. The second most abundant macrofauna found was crustaceans. Riddle (1988) found that the diversity was lowest on the outer and middle reefs because of the harder substrate caused by the higher abundance of hard corals. The highest diversity was seen in the inner reef and shallows.

Another factor that plays a role in the diversity of the benthic meiofauna and

macrofauna is the sediment type in which the organisms live (Riddle et al. 1988). Depth is a controlling factor when considering benthic organisms because the sediment type varies with depth, location, and the amount of human impact (Hutchings et al. 2001). In New Zealand the diversity of organic matter and macrofauna and meiofauna is more than two times diverse in firm silt sediment than in a hard/course sediment (Waikato et al. 2004). The type of sediment that the organisms live in is decided by the organism's body type and feeding style (Simon et al. 1974). Hutchings and Frouin (2001) studied the effects of human impact and sediment in a lagoon near the French Polynesian. Five core samples were obtained at different depths from random sites, and preserved; Hutchings and Frouin (2001) separated the organisms into different taxonomic categories, and calculated the times of year the biomass was high and low. The difference in biomass at different times of the year correlated to various seasonal feeding activity of fish on the macrofauna and meiofauna caused by low algal abundance; in all tests polychaetes were dominant by 53% (Hutchings et al. 2001).

In sandy coral areas in Amitori Bay, Iriomote Island, Japan there was a higher biomass in gastropods and polychaetes as opposed to other areas of the reef where the substrate was not so firm, according to a study by Sano et al. (2005). The present study, done in Bonaire, is unique because of the fact that the density and diversity of benthic organisms has not been studied in this part of the Caribbean. The importance of this study is, in part, the addition of benthic organism diversity and density baseline information to the scientific community.

H₁: The density of benthic macrofauna organisms will decrease with increasing depths.

H₂: The number of families in the benthic macrofauna cores will decrease with increasing depths.

H₃: The density of benthic meiofauna organisms will decrease with increasing depths.

H₄: The number of families in the benthic meiofauna cores will decrease with increasing depths.

Most of the studies on the benthic organisms were from the Indo-Pacific. There were a few studies in the northern Caribbean near the American coast of Florida, but these studies have measured density in nursery areas, not on the coral reef.

Materials and Methods

Study Site

The study site, Yellow Sub, is located in the southern Caribbean, on the west coast of Bonaire, to the east of Klein Bonaire (Fig. 1).

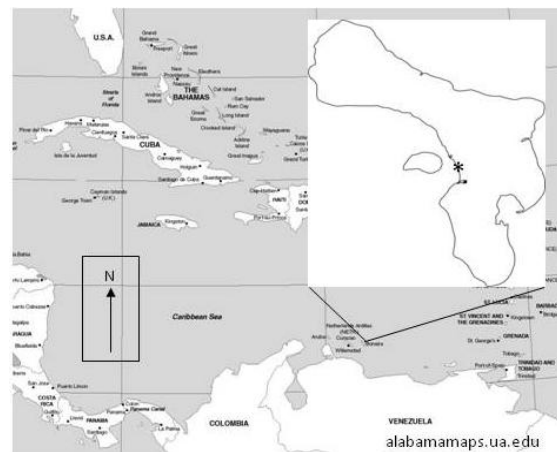


Fig. 1 Map of Caribbean. Bonaire is shown with *black lines*. Asterisk shows dive site where the research was conducted from February – March 2012

Along the leeward side of Bonaire is a fringing, tropical, coral reef system. From the shore to the edge of the reef is sandy bottom and the depth is from 0-5 m. The water temperature varies from approximately 23°C to 27°C. Various types of herbivorous and carnivorous fishes live in and around the reef structures, including several different species of parrotfishes, butterflyfishes, groupers, grunts, damselfishes, snappers, and jacks.

Sample Collection

In order to achieve random sampling, mapping of sandy areas in a 100 m x 100 m

sampling area was completed at five depths: 1 m, 5 m, 10 m, 15 m, and 20 m. From the sandy areas, ten core samples were taken at each depth, five for macrofauna (10.5 cm-dia. x 10 cm) and five for meiofauna (2.2 cm-dia. x 3 cm). The cores were sieved on the shore using a 500-micrometer sieve for the macrofauna cores, and a 62-micrometer sieve for the meiofauna cores. The cores were then taken to the lab and fixed with a 10% formalin solution with the vital stain, Rose Bengal. The samples were then transferred from the 10% formalin solution to a 70% ethanol solution. After 48 h the organisms were sorted from the remaining sand, counted and identified to family level when possible.

Data Analysis

The number of families found at each depth for macrofauna and meiofauna were used to calculate the means \pm SD. The means for species density and richness were compared among depths using a one-way analysis of variance (ANOVA) with depth as the main factor. The samples that showed a significant difference ($p < 0.05$) were then tested using Tukey *post-hoc* tests between the depths.

Results

There were nine families in the macrofauna samples. These families were found at all depths. One family was a sub order of amphipods (Hyperiididae), and the other eight families were from the polychaete class (Chaetopteridae, Poeobiidae, Spintheridae, Pholoididae, Eulepethidae, Polyodontidae, Protodrilidae, Dinophilidae, and Chrysopetalidae). There was a mean \pm SD of about two different families for each core depths (Table 1). There was a significant difference in macrofauna mean species richness between depths 1 m, 5 m, 10 m, 15 m, and 20 m ($F = 2.52$, $df = 4$, 20 , $p = 0.03$), 1 m and 5 m ($p = 0.01$), 5 m and 20 m ($p = 0.01$), 5 m and 15 m ($p = 0.01$), and 5 m and 10 m ($p = 0.01$). There was no significant difference between depths: 1 m

and 10 m ($p = 0.95$), 1 m and 15 m ($p = 0.95$), 1 m and 20 m ($p = 0.99$), 10 m and 15 m ($p = 1.00$), 10 m and 20 m ($p = 0.82$), and 15 m and 20 m ($p = 0.82$; Fig. 2).

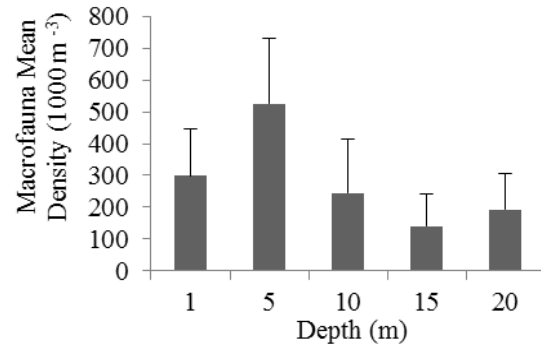


Fig. 2 Mean macrofauna species richness m^{-3} (\pm SD) at each depth ($n = 25$)

There was no significant difference in macrofauna density ($ind\ m^{-3}$) between depths ($F = 2.52$, $df = 4$, 20 , $p = 0.07$; Fig. 3). Although not significant, the highest number of $ind\ m^{-3}$ was at the 5 m depth ($5,545.9 \pm 966.6$).

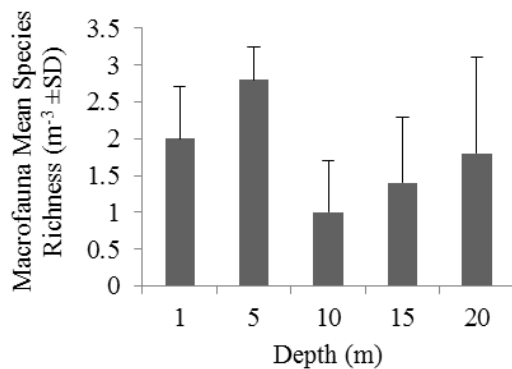
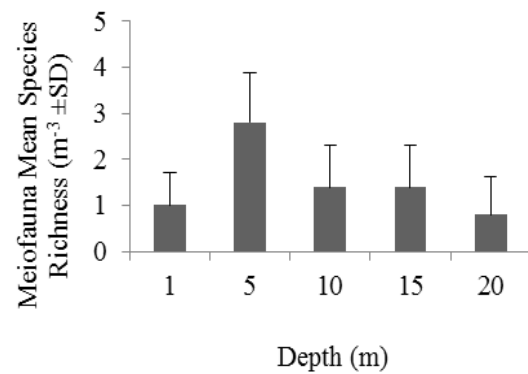
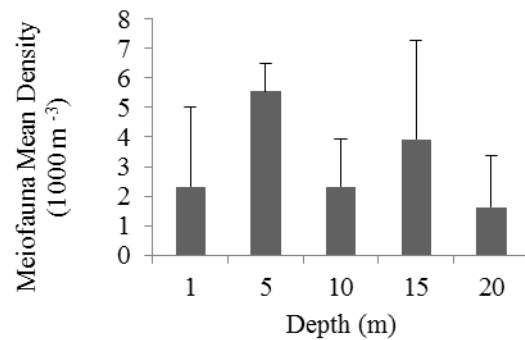
There were a total of 11 different families in the meiofauna samples. These families were found at all depths. One family in the amphipod class (Hyperiididae), one from the Isopod class (Anthuridae), and nine from the polychaete class (Chrysopetalidae, Chaetopteridae, Dinophilidae, Protodrilidae, Eulepethidae, Poeobiidae, Pholoididae, Polynoidae, and Spintheridae). There was a mean \pm SD of about two different families for each core at most of the sampled depths (Table 1). There was a significant difference in meiofauna mean species richness among ($F = 3.11$, $df = 4$, 20 , $p = 0.03$). Tukey's *post-hoc* simultaneous test between all depths showed there were no significant differences between 1 m depth and depths 5 m ($p = 0.59$), 10 m ($p = 0.38$), 15 m ($p = 0.8$), and 20 m ($p = 0.9$). There was a significant difference between 5 m depth and 10 m depth ($p = 0.02$), but not between depths 15 m ($p = 0.1$), and 20 m ($p = 0.38$). There was also no significant difference between depth 10 m and depth 15 m

Table 1. Macrofauna and meiofauna mean density ($\text{m}^{-3} \pm \text{SD}$) and species richness ($\pm \text{SD}$)

Depth	Macrofauna		Meiofauna	
	Mean species richness ($\pm \text{SD}$)	Mean density (1000 $\text{m}^{-3} \pm \text{SD}$)	Mean species richness ($\pm \text{SD}$)	Mean density (1000 $\text{m}^{-3} \pm \text{SD}$)
1	1.0 ± 0.7	2.3 ± 2.7	2.0 ± 0.7	$298.2 \pm 1,267.0$
5	2.8 ± 1.0	5.5 ± 0.9	2.8 ± 0.4	526.3 ± 205.7
10	1.4 ± 0.8	2.3 ± 1.6	1.0 ± 0.7	245.6 ± 168.7
15	1.4 ± 0.8	3.9 ± 3.3	1.4 ± 0.8	140.3 ± 100.0
20	0.8 ± 0.8	1.6 ± 1.7	1.8 ± 1.3	192.9 ± 114.3

($p = 0.11$), 20 m ($p = 0.38$), and between depths 15 m and 20 m ($p = 0.94$; Fig. 4).

The mean \pm SD (m^{-3}) for the meiofauna density at depths shows a large increase at 5 m over all other depths (Table 1). The meiofauna density (ind m^{-3}) indicated a significant difference when ANOVAs test was used ($F = 5.22$, $df = 4, 20$, $p = 0.005$) (Fig. 5). There was a significance between depth 5 m and depths 10 m ($p = 0.0298$), 15 m ($p = 0.0107$), and 20 m ($p = 0.0167$). There was no significance between 1 m depth and depths 5 m ($p = 0.6406$), 10 m ($p = 0.3741$), 15 m ($p = 0.1792$), 20 m ($p = 0.2500$), as well as 10 m depth and depths 15 m ($p = 0.9892$), and 20 m ($p = 0.9987$). 15 m depth and 20 m depth ($p = 0.9996$) were also not significant with a p value of nearly 1.0.

**Fig. 3** Mean macrofauna density in individuals m^{-3} ($\pm \text{SD}$) at each depth ($n = 25$)**Fig. 4** Mean meiofauna species richness m^{-3} ($\pm \text{SD}$) at each depth ($n = 25$)**Fig. 5** Mean meiofauna density in individuals m^{-3} ($\pm \text{SD}$) at each depth ($n=25$)

Discussion

The hypothesis that the density of benthic macrofauna organisms will decrease with increasing depths was rejected because the data does not represent a decrease in density with increasing depth from 5 m to 20 m. The results were not significant, the graph (Fig.3)

shows an increase in density at depth 5 m, and a steady decrease as the depth increases. The hypothesis that the number of families in the benthic macrofauna cores will decrease with increasing depths was not supported by this study. There was an increase in number of families and highest species richness was at 5 m depth. The richness dropped drastically from 5 m – 10 m depth, but then gradually increased with each depth (Fig. 2). There were a total of nine families in the macrofauna samples, which were found at all depths.

The feeding styles for these families vary from deposit feeders which feed in the sediment, predatory which feed on the sponges, and filter/suspension feeders, which feed from the water column (Brusca et al. 2003). The three feeding types were found at all depths. There was not a relationship of the feeding type to depth.

The density of benthic meiofauna organisms will decrease with increasing depths. The study showed there was a significant difference in density among depths ($p = 0.005$). The chart in Fig. 5 shows the relationship of density and the five depths. The depth with the highest density is at 5 m, followed by 15 m. At depths of 10 m and 20 m, nearly half of the density found at 5 m was found. This could have been because of the type of habitat available at 5 m depth. The abundance of sand and substrate not shadowed by corals is much more appealing to the polychaete order. The 5 m depth also has much deeper sandy bottom than any other depth sampled. When the depths were compared to each other using tukey *post-hoc* there was a considerable difference when depth 5 m was compared between depths 10, 15 m, and 20 m. The other were not significantly different, although there was an increase in the chart at 15 m these figures were not significantly different. Density at 5 m depth is statistically significant as compared to all depths, possibly because there may be better living conditions in this part of the reef, or some other factors that should be further studied.

The hypothesis that the number of families in the benthic meiofauna cores will decrease with increasing depths was supported by this study. The data show an increase in species richness from 1 m – 5 m. Then there is a decrease in species richness from 5 m – 10 m with a continued trend between 15 m and 20 m. There were a total of 11 different families in the meiofauna samples. As the macrofauna samples, these families were also interchangeably found at all depths. The feeding styles for these families vary from deposit feeders which feed in the sediment, predatory which feed on the sponges, and filter/suspension feeders, which feed from the water column. The three feeding types were found at all depths. There was not a relationship with these families' feeding styles and depth. This data was used with ANOVA and considered to be significant ($p = 0.005$). There was a significant difference among depth 5 m and all other depths sampled. All other depths did not display significant results between them, but when shown visually (Fig. 5) displayed a general increase among depths 5 m and 15 m.

The significance of the high density and species richness at 5 m for both meiofauna and macrofauna shows that further research should be conducted. The future studies should consider the sea floor and a wider research site. There was a study done in St. Croix during the 1980's where they were checking for holes in the sediment. The study found that more habitat holes were found in the fore reef (Moran et al. 1986). These results are similar to the density and species richness results found at Yellow Sub at 5 m. A large part of the nitrogen derived from particulate sources could be supplied by bacteria. This suggests that such efficient linkage between these reef organisms and the pelagic microbial communities explains the increasing/continued abundance of such benthic organisms on deteriorating Caribbean reefs (Bak et al. 1998). These are just a few reasons for further increasing understanding of the benthic organisms in the southern Caribbean.

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Physical and behavioral differences between the three color morphologies of *Aulostomus maculatus*

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Abstract

Behavioral and physical differences are sometimes the result of a particular color morphology of a species. *Aulostomus maculatus*, the west Atlantic Trumpetfish, has three color morphs, and was studied to determine if behavioral or physical differences exist between the three color morphs. This study was conducted at Yellow Sub dive site, located on the leeward side of Bonaire, Dutch Caribbean. Data were collected using SCUBA transects at four depths, and each transect was repeated 6 times. Size (total length, cm), depth (m), environment type (distinguishing corals or objects), substrate type, distance from substrate, body position, and body movement of *A. maculatus* was recorded for each trumpetfish observed. Significant differences were seen between size and color morph (One-way ANOVA: $df = 2, 184$, $F = 4.30$, $p < 0.05$), depth and color morph (Kruskal-Wallis: $df = 2$, Chi-square = 35.11, $p < 0.0$), and mean density of color morphs at each transect depth (Kruskal-Wallis: $df = 2$, Chi-square = 11.15, $p < 0.01$). These results indicate that there are significant differences between the three color morphs of *A. maculatus*.

Introduction

Color variation occurs throughout several species on earth, and sometimes determines behaviors within the species. A New Mexico lizard, *Urosaurus ornatus*, displayed one specific color morph dominating another color morph more than would happen by chance (Hover 1985). Similarly, side-blotched lizards were observed settling close to other lizards of a similar color morph (Sinervo and Colbert 2003). Accordingly, and individual's size, habitat and behavior may be influenced by its color morphology (color morph).

Aulostomus maculatus, the west Atlantic trumpetfish, has three different color morphs, yellow, red/brown, and blue (Lochmann 1989). They have a long slender body, and reach a size upwards of 80 cm in length. *A. maculatus* has the ability to change the intensity of its coloration. An individual can range from a pale to dark shade, but cannot change their underlying color morph (Lochmann 1989). Predatory behavior in *A. maculatus* can include shadowing other predators and trumpet fish appear to match the color of the species they

shadow to their own color morph. Although as one may expect, there is sufficient variation (Baehr 2007). *A. maculatus* spends almost all day hunting, while occasionally stopping to be cleaned. There are three main stalking strategies employed by *A. maculatus*. The first involves shadowing a fish to remain invisible to smaller prey above or below (Aronson 1983). They also swim diagonally through the water column in open water to take advantage of the large numbers of *Chromis multilineata* that school in the midwater (Auster 2008). The last technique is positioning their slender body next to an object that is similar in appearance to themselves, such as a rope or gorgonian. The present study examined the behavioral and physical differences, and similarities, between the three color morphs of *A. maculatus* in Bonaire, Dutch Caribbean (Fig. 1).

Color morph affects behavior in other animals (Forsman and Appleqvist 1998) and it is therefore predicted that the behavior of *A. maculatus* will also differ according to color morph. Color may affect hunting strategy, as well as predator avoidance. This

objects), substrate type, distance from substrate (m), body position (vertical, horizontal or diagonal), and body movement was recorded for all *A. maculatus* observed along 100 m long transects conducted at depths of 16 m, 12 m, 8 m, and 2.5 m using SCUBA. Six transects were performed at each depth.

Size was estimated against a 28 cm slate. Environment type was recorded as the closest structure (e.g. coral head, gorgonian, mooring block, rope, etc.). However, the individual was recorded as moving if it was drifting without solid position near a distinguishable object.

Data analyses

A G-test was used to compare the frequency of body positions with color morph. One-way ANOVAs were used to assess whether body position and size differed with color morph. Kruskal-Wallis tests were used to assess whether the depths at which individuals were found differed with color morph and to determine whether relative density differed between color morphs (non parametric test was used because data were not normally distributed). A linear regression was used determine the relationship between color and distance from substrate.

Results

Data were recorded for a total of 186 trumpetfish, 66 brown, 101 blue, and 19 yellow. Observation depths ranged from 1.5 m to 18.5 m. Trumpetfish were observed near corals (both live and dead), sponges (primarily rope sponges), man made structures (mooring blocks, ropes, dock piles), and other reef structures.

A significant difference was found between the mean sizes of different color morphs (One-way ANOVA; $df = 2, 184$, $F = 4.30$, $p < 0.05$; Fig. 2). The mean distance from the substrate did not differ significantly with color morph (One-way ANOVA; $df = 2, 184$, $F = 2.61$, $p > 0.05$).

However, a significant difference was found between the mean depths of different color morphs (Kruskal-Wallis; $df = 2$, Chi-square = 35.11, $p < 0.05$; Fig. 3). A weak, but significant difference was found between size and distance from substrate (Linear regression; $df = 1, 186$, $F = 3.91$, $R^2 = .021$, $p = 0.050$; Fig. 4). The mean density of trumpetfish (number of individuals observed transect⁻¹) differed significantly between color morphs (Kruskal-Wallis; $df = 2$, Chi-square = 11.15, $p < 0.01$; Fig. 5). Finally, the frequency of individuals found in a vertical, horizontal, or diagonal body position did not differ significantly between the three color morphs (Chi squared test; $df = 4$, Chi-square = 5.93, $p > 0.05$; Fig. 6).

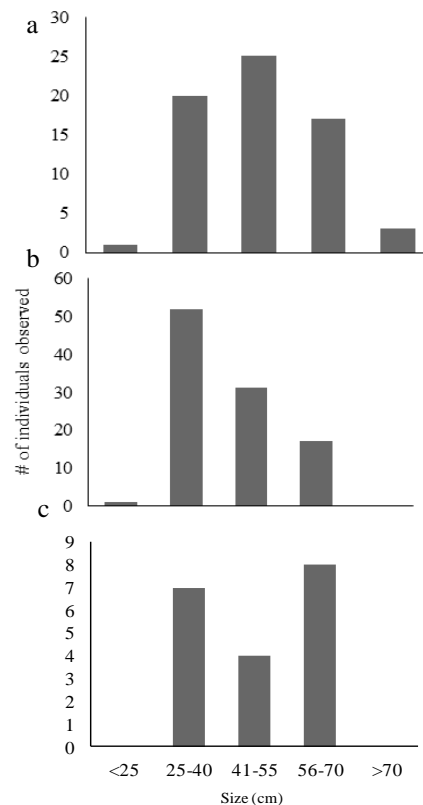


Fig. 2 Number of trumpetfish for each color morph observed for each *size category* ($p < 0.05$) in Bonaire, Dutch Caribbean. Each graph represents one color morph. **a.** represents brown trumpetfish, **b.** blue, and the **c.** yellow

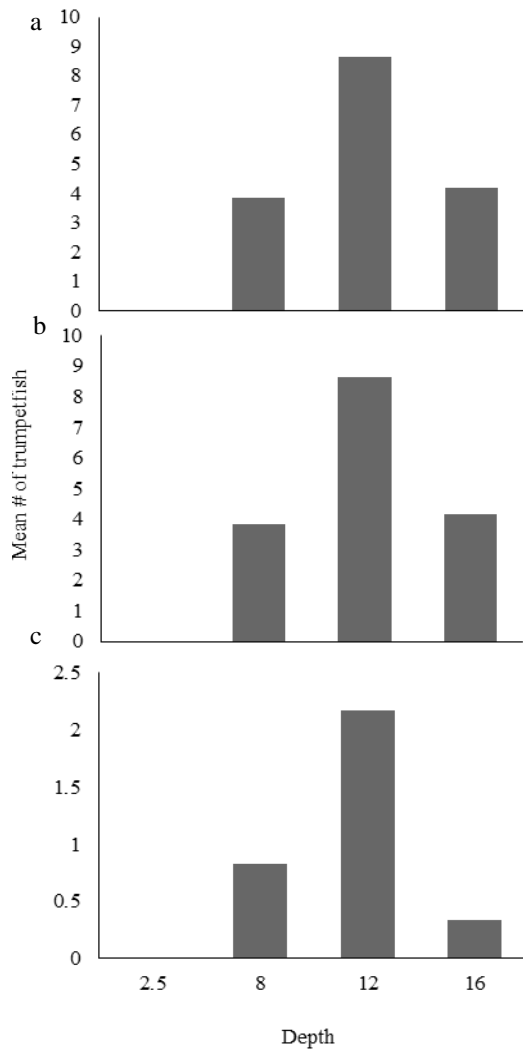


Fig. 3 Mean number of trumpetfish observed at each transect depth ($p < 0.05$) in Bonaire, Dutch Caribbean. **a.** represents brown trumpetfish, **b.** blue, **c.** yellow

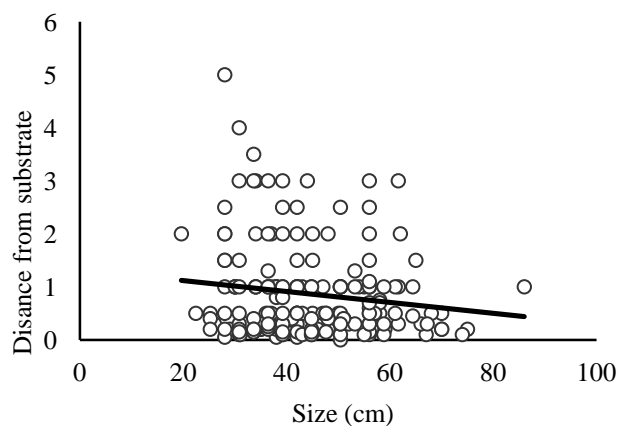


Fig. 4 Relationship between distance from substrate and size ($p = 0.050$) of trumpetfish in Bonaire, Dutch Caribbean. Each dot on the graph represents one trumpetfish. The line of best fit is corresponds to the R^2 value

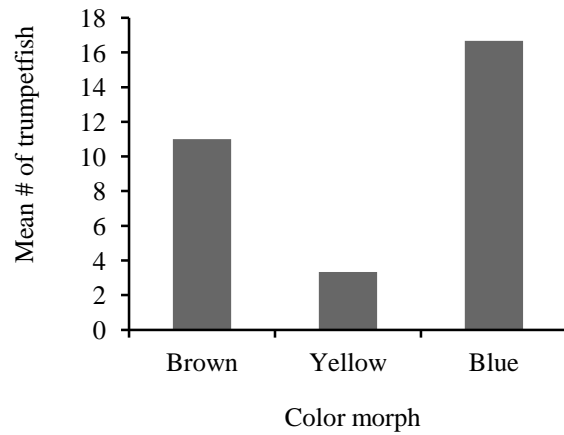


Fig. 5 Mean number of trumpetfish seen per survey for each color morph ($p < 0.05$)

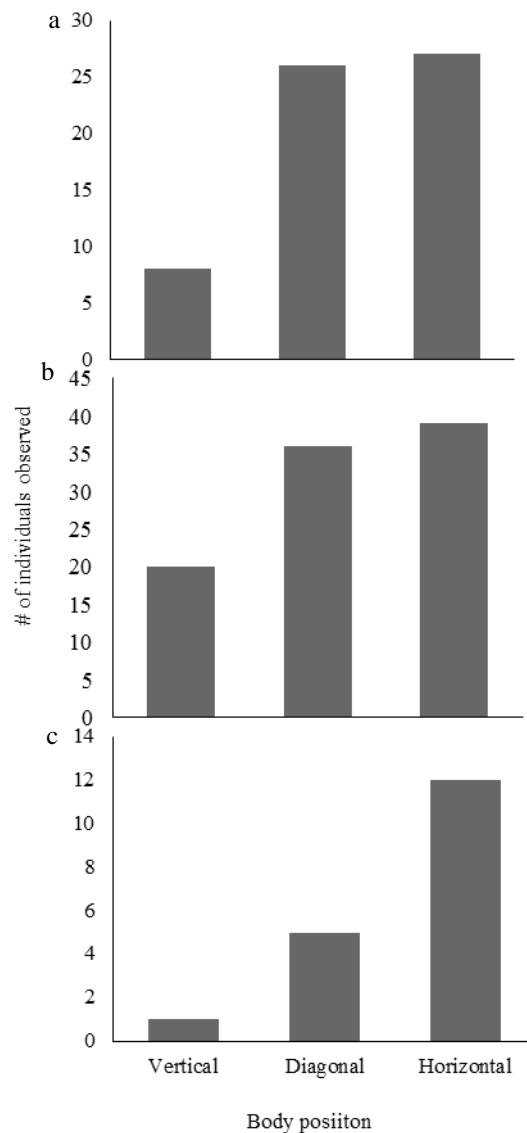


Fig. 6 Number of trumpetfish observed in each body position for each color morph ($p > 0.05$). **a.** represents brown trumpetfish, **b.** blue, and **c.** yellow

Discussion

Results support the prediction that color is related to size, although yellow is not the smallest of the color morphs as initially hypothesized. Yellow individuals were expected to be the smallest because they are more easily spotted by predators, making them more vulnerable (Forsman and Appleqvist 1998) but blue individuals were found to have the smallest mean size. There may be a higher number of small blue trumpetfish because they were the most abundant of the color morphs. The same results also support the hypothesis that brown individuals are the largest of the color morphs. This may suggest that the camouflage abilities of brown trumpetfish among rope sponges and sandy substrate are superior to those of the blue and yellow color morphs.

The hypothesis that yellow individuals are the least abundant color morph was supported by the results. Yellow individuals may also be the product of a double recessive allele, leaving them as a minority in the total population. This may also indicate that the most ecologically fit yellow trumpetfish survive (Forsman et al. 2002).

The depth where trumpetfish were found changed significantly with color morph. Blue individuals were found more often in deeper water compared to brown and yellow. These data do not support the hypothesis that blue individuals will be found higher in the water column. Similarly, brown individuals were expected to be found closer to the substrate, which was not supported by the results. Furthermore, color morph was predicted to be related to body position in the water column, which did not vary between color morphs.

Additional studies could further examine the relationship between color morph and distance from substrate. This information was the most difficult to obtain because the trumpetfish were frequently moving during the observation. Also, future

studies may consider other factors ignored in this study; such as skin tone, strike frequency, and non hunting behaviors in order to further the understanding of *A. maculatus*.

Little is known about the behavioral differences between color morphs of species, both terrestrial and marine. This study provides insight to the notion that both physical and behavioral factors are dependent on color morph. *A. maculatus* is one of the most abundant piscivores on the reefs of the Caribbean, and may be particularly suited for future research aimed at understanding the differences among marine color morphs.

Acknowledgements

This study would not have been possible without the guidance and support of my advisor, J. Claydon. Also, I would like to thank F. Ali and J. Flower, who were available at a moment's notice for advice along the way. I would also like to thank the other interns at CIEE, as they were always available to help when needed. Lastly, thanks to my research partner, S. Penn, for making my research possible.

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Diver impact on coral and fish communities: A comparison of sites with varying intensities of diving at Yellow Submarine, Bonaire, Dutch Caribbean

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Abstract

SCUBA diving on coral reefs is a beneficial economic option for small tropical islands, that can have a lower impact on the environment than alternative options, such as the fishing industry. However, diving can also have a negative impact, when divers physically damage the reefs. The effects of diving on reef fish populations have received little study, though alteration of fish distribution or recruitment in areas with high levels of diving is likely. The purpose of this study was to evaluate the impact of diving on coral and fish communities in Bonaire, Dutch Caribbean. Coral and fish communities at six sites adjacent to a popular dive site were studied. Sites studied included two sites immediately adjacent to the entry where most divers pass, 2 sites (120 m from entry) with intermediate levels of diving and 2 sites (240 m from entry) representing less dived sites. Benthic video transects were conducted at two depths (8-10 m, 15 m), recording coral cover and abundance of Atlantic Gulf Rapid Reef Assessment fish species. Coral cover increased with increasing distance to the north of the site, peaking at 31.2%. Coral cover decreased south of the site, which could be attributed to anthropogenic influences occurring due to southern sites proximity to a main population center. A known relationship between *Agaricia spp.* and *Montastrea annularis* complex was observed, with the first increasing at intermediately disturbed site, and the latter decreasing at the same sites. All other factors varied greatly across sites and could not be associated with changes in diver intensity; however they could be associated with anthropogenic pressures. Overall, this study did not show significant diver impact, though it displayed negative trends in relation to anthropogenic factors.

Introduction

Coral reefs support an immense number of marine species, and though reefs take up only 0.09% of the ocean, nearly 25% of total known fish species inhabit this ecosystem (Wilson et al. 2006). Reefs provide resources for coral reef species as well as people, and are influenced by anthropogenic impacts such as: climate change (Carpenter et al. 2008), coastal development (IUCN 2011), run-off, pollution (Richmond 1993; IUCN 2011), boating, anchor dropping (Stokes et al. 2010), snorkeling (Tratalos and Austin 2001) and SCUBA diving (Davis and Tisdell 1996; Hawkins et al. 1999; Tratalos and Austin 2001; Zakai and Chadwick-Furman 2002; Hawkins et al. 2005). Degradation of reefs can lead to a loss in food and economic security, as well as a loss in biodiversity, impacting

resources for human use and environmental stability (IUCN 2011).

Dive tourism is a lucrative business in areas that have intact coral reefs and is considered one of the world's fastest growing outdoor activities (Davis and Tisdell 1996). Increasing economic activity through dive tourism was originally considered to be a low-impact alternative to the harvest of coral and fish species from the reef, however, reefs can be damaged in a number of different ways as a result of diving (Zakai and Chadwick-Furman 2002). Novice divers (those with fewer than 100 dives), divers who use cameras and male divers contact the reef most frequently and cause the most damage (Barker and Roberts 2004). In particular, fins contact with the reef most often, followed by hands, knees and loose equipment (Barker and Roberts 2004). Divers often harm the reefs through direct

physical contact and the contact can result in broken coral, tissue destruction and general damage (Tratalos and Austin 2001). Coral breakage and abrasion can lead to disruption of growth and reproduction patterns as well as increases in mortality and disease rates (Zakai and Chadwick-Furman 2002). Disruption of reproduction can be very detrimental to scleractinian (stony) corals, which have slow growth rates in comparison to soft corals, that recover quickly (Zakai and Chadwick-Furman 2002; Wilson et al. 2006). In addition to breakage, divers can stir up sediments that then settle and accumulate on the surfaces of coral (Tratalos and Austin 2001). Though sedimentation causes less immediate damage than more direct actions, such as dropping anchors on coral reefs or walking directly on reef flats, it still has a visible negative impact (Tratalos and Austin 2001). Sedimentation can cause a decrease in corals' ability to produce energy and alter the nutrient levels in the water (Tratalos and Austin 2001; IUCN 2011).

Divers disrupt fish behavior, particularly if divers are feeding the fish (Tratalos and Austin 2001). Research in Bonaire has shown that diving pressure has had a limited effect on fish biomass and abundance, with the exception of groupers, when comparing sites with high diver intensity to sites with lower diving intensity (Hawkins et al. 1999). Additionally, feeding has the potential to cause an increase in predatory fish biomass in heavily dived areas, however no significant difference in fish biomass between heavily dived sites and less dived sites has been observed (Hawkins et al. 1999). However, it remains possible that divers scare fish, as well as alter their distributions and recruitment.

The fish preferences of divers was studied on degraded reefs of Jamaica, and it was found that divers prefer to see large, unusual fish and high diversity of fish (Williams and Polunin 2000), indicating that the conservation of biodiversity on reefs bolsters the satisfaction of dive tourists, which in turn could increase dive tourism (Williams and Polunin 2000). An increase in

dive tourism could serve as an alternative source of revenue to fishing reefs such as Jamaica's, potentially leading to a greater recovery and stability of fish populations.

In contrast to Jamaica, the island of Bonaire in the Dutch Caribbean is surrounded by a fringing reef extending out to 300 m from shore, and has become an internationally renowned dive destination, specifically for North American divers (Stokes et al. 2010; IUCN 2011). Bonaire hosts approximately 60,000 tourists per year, of which 34,000 are SCUBA divers (IUCN 2011). This level of diving is leading to increased anthropogenic pressures on the environment, causing general environmental degradation in the area, specifically increasing the algae-coral ratios (Stokes et al. 2010). Bonaire was once considered to have some of the most exquisite and unique reefs in the world, however in the last decade extensive degradation has occurred, and some reefs are now considered to be at the point of becoming functionally extinct (IUCN 2011).

As the number of divers and access to coral reefs increases, dive frequency also continues to escalate (Zakai and Chadwick-Furman 2002). While not every citizen or tourist participates in SCUBA, the pressure on the marine environment is increasing, though limited information is known about the effects the divers are having on the local coral reef and fish populations in Bonaire. Research in Grand Cayman showed that sites experiencing high diving pressure maintained a lower percentage of scleractinian coral cover when compared to sites with lower diving pressure (Tratalos and Austin 2001). In a study conducted on Bonaire, sites on Klein Bonaire that incurred approximately 6,000 dives per year were compared to sites on the main island that were restricted to divers (Hawkins et al. 1999). Diver impact had no significant effect on live hard coral coverage in the afore mentioned areas. The study concluded that dive frequency falling below 6,000 divers a year can be considered sustainable, with limited damage to the environment,

however, this is site specific (Hawkins et al. 1999; Hawkins et al. 2005). In Bonaire high intensities of diving had a negative impact on scleractinian corals, causing an 8.2% increase of soft coral (Barker and Roberts 2004). In addition to studies on coral cover as a whole, the relationship between *Agaricia spp.* and the *Montastrea annularis* complex has been linked to diver impact. *Agaricia spp.* has been found to occur at its highest percentages in intermediately disturbed sites, whereas the *M. annularis* complex consistently decreases in areas that are more disturbed (Tratalos and Austin 2001).

Diving tourism is very important to the economy in Bonaire and the effects of diving on corals and fish communities have received little evaluation in recent years. Yellow Submarine dive site (~0.8 km north of the population center, Kralendijk) is a well known dive site in Bonaire that is recognized as a National Geographic Family Dive Center and supports a large number of divers ranging in expertise, from novice divers (<100 dives) to previously certified divers. Typically, there are three types of divers at the site: 1) discover SCUBA divers, who have never been diving and watch an introductory video before entering the water guided by instructors (max depth 12 m) 2) student divers, who complete a certification course and complete four dives with an instructor (max depth 18 m) and 3) certified divers, who participate in an MPA orientation and perform a buoyancy check and dive without an instructor (max depth varies) (C. Caporusso pers comm). Typically divers dive from Yellow Submarine to the south, spending between 20 and 60 min at depth, with discover SCUBA divers restricted from diving beyond the reef crest (C. Caporusso pers comm). Damage to reefs is typically done in three ways: 1) accidentally, by divers who have little control of their buoyancy, 2) intentionally, by divers ignorant of their effect and 3) intentionally, by photographers who disregard the fragility of live coral (C. Caporusso pers comm).

This study examined the current relationship between diving frequency and two ecological metrics: 1) the percent live coral cover and 2) reef fish density and diversity. These metrics were compared from the access point to 240 m to the north and south of the access point. From these metrics four hypotheses were drawn, and were stated as follows:

- H₁: Reef fish density (ind m⁻²) will be lower in dived areas than adjacent less-dived areas
- H₂: Reef fish diversity (Shannon-Weiner Index) and richness will be lower in dived areas than adjacent less-dived areas
- H₃: Percent live coral cover and coral diversity will be lower in dived areas than adjacent less-dived areas
- H₄: Percent live coral cover of *Agaricia spp.* and *M. annularis* complex will increase and decrease respectively in areas with intermediate dive intensity

As numbers of divers continues to increase (Barker and Roberts 2004), the pressure being exerted is also rising, though evaluation of the effects on coral and fish communities has not occurred. This study provides a unique look at the current status of coral and fish communities at dive sites with varying dive pressures in Bonaire. It may also provide important information about the sustainability of dive tourism as an economically beneficial industry on the island of Bonaire.

Materials and Methods

Study Sites

The study occurred off the western coast of Bonaire, Dutch Caribbean, an island in the southern Caribbean 80 km off tnortheastern coast of Venezuela (Fig. 1). Six sites were selected; two at Yellow Submarine (12°09'35.95" N, 68°16'54.10"W), one at 120 m and one at 250 m to the south of Yellow Submarine, and one 120 m and one

250 m to the north of Yellow Submarine (Fig. 2).

Data Collection

Two 30 m transects were laid at each site; one at 15 m depth and the second just below the reef crest (10 ± 2 m). Once the tapes were laid, two min were waited, allowing the fish to re-aggregate to the site before performing the T-Bar analysis. An initial Atlantic Gulf Rapid Reef Assessment (AGRRA) T-Bar belt transect recording abundance of fish for a defined species list (defined species outlined in Kramer et al. 2005) was performed at each transect. Mean fish density, diversity and richness were calculated from the mean of transects at each site. Following the T-Bar belt transects, benthic video transects were then recorded on the shallow side of the tape using a Sony Handy Cam HDR-SR7 camera and an Ocean Images MP1-SR7 housing and a 50 cm wand to position the camera consistently above the benthos. The video was examined by randomly selecting 30 frames from each transect and each frame was then analyzed using coral point count software (CPC). CPC was used to overlay 25 random points on each frame and the substrate was identified under each point. Corals were identified to species level; all other substrate was classed as macro-algae, sponges, turf, gorgonians, old dead coral, sand or rubble. The coral cover at each site was calculated from the mean of the two transects filmed. All surveys were completed between 9:00 and 13:00 h, and in February and March 2012.

Data Analysis

Graphical interpretations were created with the program Numbers v.2.1 (2009). North and south sites were plotted on one graph for each ecological metric. In order to visualize trends in relation to varying distances, each of the following were evaluated: percent coral cover, coral diversity (Hs), *Agaricia spp.* and *M. annularis* complex (*Montastrea annularis*, *M. favelota*, *M. franksii*) cover,

fish density (ind m^{-2}), fish diversity (Hs) and fish species richness (S)

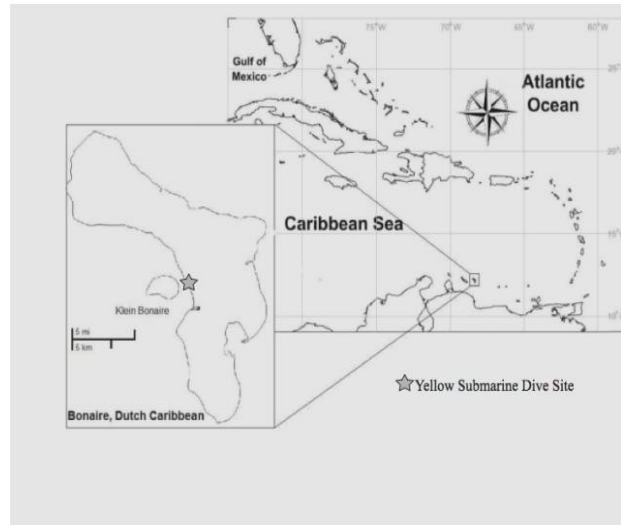


Fig. 1 Map of Bonaire and its position in the Caribbean. Yellow Submarine Dive Site ($12^{\circ}09'35.95''$ N, $68^{\circ}16'54.10''$ W) denoted by gray star on map

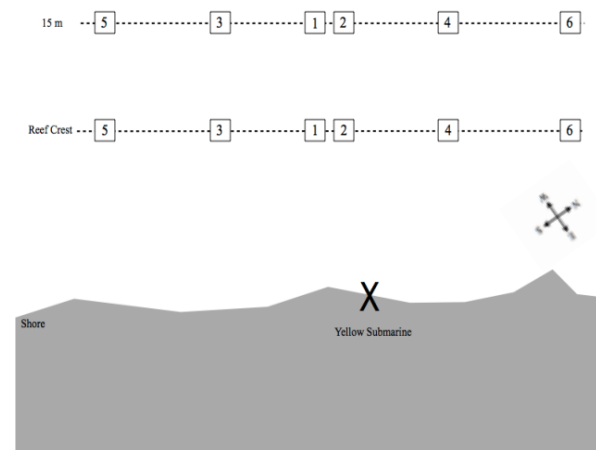


Fig. 2 Map of 30 m transect sites. Sites 1, 3 and 5 are 0 m, 120 m and 240 m, respectively, to the south. Sites 2, 4 and 6 are 0 m, 120 m and 240 m, respectively, to the north. The black X indicates the entry point to Yellow Submarine dive site, dotted lines denote approximate depths (Reef crest is 8-10 m deep)

Results

Percent Coral Cover

The highest mean percent coral cover was found at 240 m north (31.2%) and lowest was at 240 m south (15.4%; Fig. 3a). Generally, mean percent coral cover

decreased to the southernmost research site (240 m south) and increased to the northern most research site (240 m north). Directly in front of Yellow Submarine there was an increase in coral cover, at 0 m south (28.2%), though the cover continued to decline following that site (Fig. 3a).

Agaricia spp. and *Montastrea annularis* Complex Percent Cover

Mean *Agaricia* spp. percent coral cover was highest at 240 m north (12.5%) and lowest at 240 m south (3.1%; Fig. 3b). *Agaricia* spp. cover generally increased from 0 m to 240 m north, and decreased from 0 m to 240 m south, with a slight peak at 120 m south. A difference between mean *Agaricia* spp. cover (7.9%) and mean *M. annularis* complex cover (3.2%) occurred at 120 m north, indicating intermediate disturbance, as well as at 120 m south, with *Agaricia* spp. cover (8.7%) being greater than *M. annularis* cover (6.4%). The mean *M. annularis* complex percent cover maximum was at 0 m south (15.6%) and minimum was seen at 120 m north (3.2%; Fig. 3b).

Coral Diversity

Mean coral diversity peaked at 250 m south ($H_s = 0.56$) and then declined to 120 m south ($H_s = 0.33$; Fig. 3c). At the remaining sites coral diversity was consistently between $H_s = 0.30$ - 0.36 . Initially, coral diversity displays a declining trend with increasing distance from Yellow Submarine, however it then plateaus through remaining sites (Fig. 3c).

Fish Density

Mean fish density was the highest directly in front of Yellow Submarine, at 0 m south (0.790 ind m^{-2} ; Fig. 4a). If 0 m south is excluded, fish density remains fairly constant throughout all other sites, ranging between 0.100 and 0.175 ind m^{-2} (Fig. 4a).

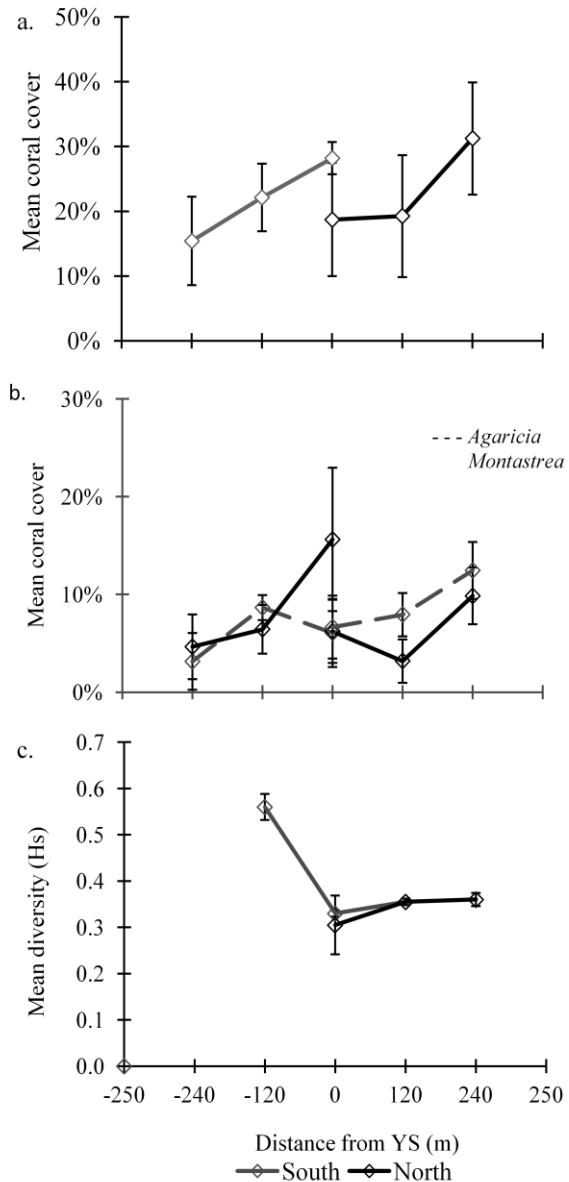


Fig. 3 Comparison of A. complex mean diversity (H_s), B. mean percent coral cover and C. mean percent coral cover of *Agaricia* spp. and *M. annularis* of south sites and north sites at increasing distances from Yellow Submarine dive site. Negative numbers denote distance south from Yellow Submarine. The error bars indicate \pm SD

Fish Richness and Diversity

Mean fish species richness was highest at 0 m south (10) and lowest at 240 m south (3.5; Fig. 4b). Omitting the sites at 0 m south and 240 m south, mean species richness ranged from 5-6. Mean fish diversity peaked at 120 m south ($H_s = 1.67$), and then followed an overall declining pattern from the southernmost site to the northern most site (Fig. 4c).

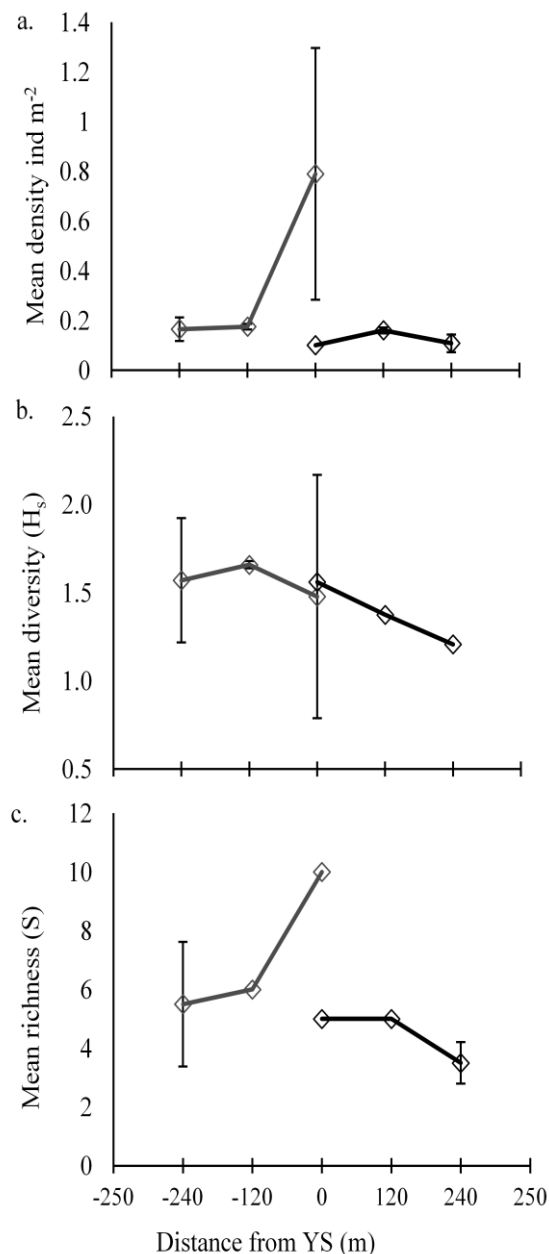


Fig. 4 Comparison of a. mean density of fish (ind m⁻²), b. mean diversity of fish (H_s) and c. mean species richness (S) of south sites and north sites at increasing distances from Yellow Submarine dive site. Negative numbers denote distance south from Yellow Submarine. The error bars indicate ± SD

Discussion

At both intermediately disturbed sites, which were 120 m to the north and south of Yellow Submarine dive site, *Agaricia spp.* cover was higher than *M. annularis* complex cover, supporting the hypothesis about the coral relationship between the two (Tratalos and

Austin 2001). *Agaricia spp.* continued to increase to the north, displaying an increase in coral cover with a decrease in dive intensity, as did *M. annularis* complex, although it showed a slight decrease at 120 m north. However, to the south both *Agaricia spp.* and *M. annularis* complex cover decreased with increasing distance from Yellow Submarine dive site, possibly due to the sites proximity to Kralendijk, the main town on the island. The initial hypotheses that percent coral cover, coral diversity, reef fish density (ind m⁻²), mean reef fish diversity and richness would increase in areas with lower dive intensity were not supported by this study.

Though only one of the hypotheses was directly supported, there were visible trends within the mean percent coral data. The data showed a generally increasing trend to the north as distance from high diver intensity increased, and as distance increased from Kralendijk, again indicating a possible influence of the city on coral cover. There were no clear trends relating to fish communities, as indicated by fairly consistent values of density, diversity and richness throughout all sites. Due to the small study site and few T-Bar belt transects, conclusions about fish density, diversity and richness were limited. Surveying only AGGRA species may have also restricted data collection, as it doesn't include several species that are present on the reefs of Bonaire.

These findings indicate that factors other than diving overshadow diver impact in this region of Bonaire, specifically anthropogenic factors (Richmond 1993). These factors likely include coastal development (IUCN 2011), run-off, pollution (Richmond 1993; IUCN 2011) and climate change (Carpenter et al. 2008). Coastal development is considered to be a large threat to coral reefs in Bonaire, due to the vulnerability of reefs to damage from run-off and sediment which increases due to continued removal of natural vegetation and construction in coastal areas (IUCN 2011). In addition to run-off, the lack of sewage

treatment in Bonaire does not extend beyond the use of septic tanks, which allows for increased nutrient flow onto coral reefs, leading to heightened algal growth, which can have negative impacts on coral growth (Richmond 1993; IUCN 2011). This being said, the most detrimental impact people have on coral reefs is climate change, which compounds and heightens all other anthropogenic influences (Carpenter et al. 2008; IUCN 2011). Change in global temperatures may lead to increased coral degradation, especially in combination with the aforementioned anthropogenic factors. These larger impacts likely shielded the impacts of diving in this study, causing it to be difficult to isolate the direct damage caused by SCUBA divers.

When comparing this study to previous diver impact studies done in Bonaire, it provided limited data for comparison, primarily due its location in relation to Kralendijk. One in-depth study found that diving had little impact on reef fish communities and insubstantial impact on coral cover when comparing known dive sites on Klein Bonaire to sites protected from diving pressure on Bonaire's mainland (Hawkins et al. 1999). The location of their study feasibly shielded the research from many anthropogenic factors as Klein Bonaire lacks any establishments, thus allowing them to focus almost exclusively on the impact of divers. The study of diver impact at Yellow Submarine dive site displayed the effects of Kralendijk more clearly than the direct impact of divers, though it did not refute any of the findings in their research.

When considering future studies looking at diver impact in Bonaire, site location and size should be reviewed carefully. It would be beneficial to use sites further removed from compounding factors, such as cities, as well as bigger study sites in general, in order to focus on diver impact more exclusively. This would plausibly eliminate some of the overarching issues that this study experienced, such as coastal development and sedimentation (IUCN 2011). Another key factor to consider

altering would be the number of replicates; an increased number of replicates and sites could display more complete trends and allow for data to be more aptly statistically analyzed. It would also be interesting to focus primarily on the complex inverse relationship of *Agaricia spp.* and *M. annularis* in relation to diver impact.

Though this study provided little direct evidence supporting the hypothesis that SCUBA divers impact coral reef communities, it is still likely that divers have a clear influence both on corals and associated fauna (Barker and Roberts 2004). Diver impact can be managed in order to limit damage, especially through environmental education and the control of pressure on popular dive sites (Davis and Tisdell 1995; Davis and Tisdell 1996; Wilson et al. 2006). However, it is clear that the impact of SCUBA diving is not the primary negative force afflicting coral reefs. Globally, coral reefs are experiencing visible pressure from anthropogenic factors, and their ability to recover from these factors is being reduced (Wilson et al. 2006; Carpenter et al. 2008). Reef ecosystems are incredible sources of biodiversity (Hawkins et al. 1999; Carpenter et al. 2008), and the continued degradation to them has the potential to lead to extensive global losses (Carpenter et al. 2008).

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The effects of damselfish on coral reef benthic composition

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Abstract

Damselfish have a considerable effect on benthic composition and have been described as a keystone species because of their role in the maintenance of coral and algal assemblages within their communities. By looking at the different densities of damselfish at varying depths and locations, this study aims to see the effects of damselfish densities on coral reef benthic composition and if the presence of damselfish is beneficial to coral reef benthos. The abundance of all damselfish was recorded within 30 x 2 m belt transects. Random photographs within the belts were taken and still images were analyzed using Coral Point Count software to determine total percent coral cover, total percent algal cover, and coral species richness. A total of 702 damselfishes over 4 species were observed. Thirteen species of coral were identified. Contrary to the predictions, a higher density of damselfish was not correlated to increased coral cover and coral species richness. There was significantly more algae than coral cover throughout the study, but such a trend fits with the theory of phase shifts of coral reefs from coral dominated to algae dominated reefs.

Introduction

The world's coral reefs are undergoing a shift from coral dominated reefs to algae dominated reefs, a phenomenon known as phase shifts (Hughes et al. 2010). A variety of anthropogenic and natural processes are accelerating the degradation of coral reefs and a common theme of the effects is a phase shift from coral dominated reefs to algae dominated reefs occurring (Szmant 2002). Most algae are kept under control by grazing and scraping of herbivores, predominantly fish and echinoids in the Caribbean (Klumpp et al. 1987). Herbivorous fish also play a role in promoting coral recovery from degradation and coral resilience by limiting the amount of algal growth (Ledlie et al. 2007). By keeping macro algae growth to a minimum, the herbivores are allowing light to access the photosynthetic zooxanthelle within the coral. However, the extent to which different groups of herbivorous fish promote recovery and resilience depends on their functional niche and the algae they graze (Ledlie et al. 2007).

Certain species of damselfish (Pomacentridae) are rasping or browsing herbivores that maintain territories and

defend them against other herbivores and omnivores (Potts 1977). Similar to some herbivorous fish, damselfish establish and maintain territories, which they will aggressively defend against other fish (Hixon and Brostoff 1983). The territoriality of these fishes is primarily based around defending gardens within their areas, but protection of nesting sites and egg broods also play a role (Potts 1977). Within these territories, damselfishes will establish thick mats of turf algae, which also tend to have lower live coral cover (Ceccarelli 2007). Certain territories have been shown to contain ten times more thick turf algae than surrounding areas (Ceccarelli et al. 2007). Territorial behavior of damselfish can also limit the grazing pressure of other herbivorous fish on macro algae, which can in turn limit the ability of corals to recruit to areas with macro algae (Potts 1977, Hughes et al. 2007).

Despite their reputation, studies also show that damselfish have a considerable positive effect on benthic composition and have been described as a keystone species because of their role in the maintenance of coral and algal assemblages within their communities (Ceccarelli et al. 2005). The

presence of damselfish has been known to increase the diversity of surrounding corals (Gochfeld 2010). Damselfishes have also been shown to cultivate highly diverse and productive algae (Klumpp et al. 1987). Due to their territorial behavior and the management of their individual gardens, damselfishes are capable of altering benthic composition within their territories compared to outside by excluding certain fishes from their territories and promoting the growth of edible algae over others (Hixon and Brostoff 1983).

The degree to which damselfishes defend their territories does not just depend on what they are defending, but also what species they are trying to keep out. Except for very small intruders, certain species of damselfishes distinguish between species when defending their territories, pursuing certain species more than others, some of which might be destructive to the coral cover (Thresher 1976). Threespot damselfish (*Stegastes planifrons*), a species seen in Bonaire, display the majority of their territoriality against Scaridae (parrotfishes), a family known to be harmful to coral in its pursuit to find food (Thresher 1976). Survival of small coral colonies and recruits is increased and branching coral growth is also promoted within damselfish territories (Wellington 1982). Because of the territorial behavior of damselfish, not only do they maintain mats of filamentous turf algae and prevent overgrowth of macroalgae, they also defend the corals against fish that might harm them.

Damselfishes are frequently seen on coral reefs in Bonaire and their populations are increasing (Grimsditch et al. 2009). By looking at the different densities of damselfish at varying depths and locations, this study aims to see the effects of damselfish densities on coral reef benthic composition and if the presence of damselfish is beneficial to coral reef benthos. It is hypothesized that areas that have a higher density of damselfishes will have higher percentage coral cover, higher coral

species richness, and lower percent algal cover.

H₁: Areas with a higher density of damselfish will have higher total percent coral cover, higher species richness of coral and less total percent cover of algae.

Materials and Methods

Study Site

Data collection took place at the Yellow Sub dive site (12°09'36. 6"N, 68°16' 54.9"W) (Fig. 1). Eighteen transects were chosen randomly, 9 at 10-12 m and 9 at 18-20 m. In order to control for changes in fish behavior at different times of the day, all data were collected at 13:30 h on various days over the course of five weeks. In order to randomly select quadrats of coral reef benthos to be photographed, a 4 x 30 grid was made to represent the transect being 30 m long with four 50 cm quadrats across with a total of 240 possible quadrats. Each quadrat was assigned a number and using a random number generator, 30 quadrats of the possible 240 were chosen.

Data Collection

The abundance of all damselfish was recorded within 30 x 2 m belt transects. Only resident fish were recorded. Fish that briefly swam within the 2 m but swam out right away as well as fish swimming in the water column overhead were not counted. Within each transect, benthic composition was assessed by digitally photographing 30 randomly placed 50 x 50 cm quadrats. Still images were analyzed using Coral Point Count (CPC) software, version 3.6 to determine total percent coral cover, total percent algal cover, and coral species richness. A total of 450 points were analyzed per transect, 15 points per image. Fifteen points per image were chosen in order to increase the likelihood of the correct estimation of coral cover whilst decreasing bias (Pante and Dustan 2012). Each transect

was analyzed independently and total percent coral cover, total percent algae cover, and coral species richness were recorded for each transect.

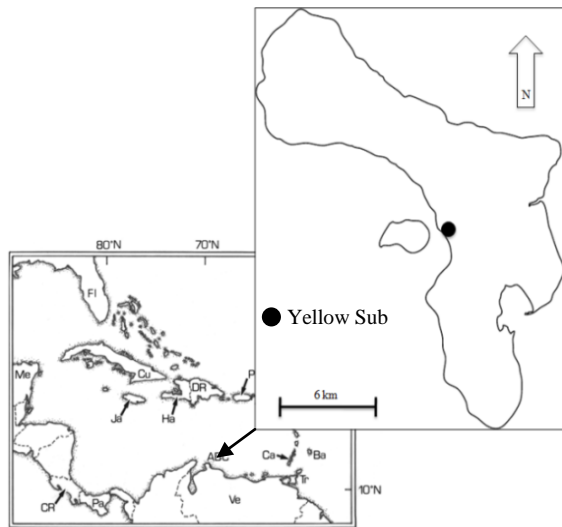


Fig. 1 Map of Bonaire, Dutch Caribbean in context of the Caribbean. Black circle indicates location of Yellow Sub dive site (12°09'36. 6" N, 68°16' 54.9" W)

Data Analysis

Parametric correlation analysis was performed to analyze associations between damselfish density and coral cover, coral species richness, and algal cover. A Student's t-test was performed to determine if there was a significant difference in coral cover and algae cover.

Results

A total of 702 damselfishes from 4 species were observed. The most frequently observed damselfish was the bicolor damselfish (*Stegastes partitus*) followed by the threespot damselfish (*Stegastes planifrons*), longfin damselfish (*Stegastes diencaeus*), and finally the yellowtail damselfish (*Microspathodon crysurus*). A total of 540 digital photos with a total of

8,100 points were analyzed using CPC. Thirteen species of coral were identified. There was a significant difference between the total percent coral cover and total percent algal cover ($t = 13.07$, $df = 34$, $p < 0.001$; Fig. 2). There was a weak and non-significant trend that with increasing damselfish densities per transect, total percent coral cover transect⁻¹ decreases ($t = -1.63$, $p = 0.12$, $r = -0.37$; Fig. 3) and total percent algae cover increases ($t = 1.33$, $p = 0.20$, $r = 0.31$; Fig. 3). As depth increases, total number of damselfish decreases ($t = -0.193$, $p = 0.07$, $r = -0.43$; Fig. 4a) and total percent coral cover increases ($t = 2.81$, $p = 0.01$, $r = 0.57$; Fig. 4b). As the density of damselfish per transect increased, coral species richness decreased ($t = -0.022$, $p = 0.82$, $r = -0.05$).

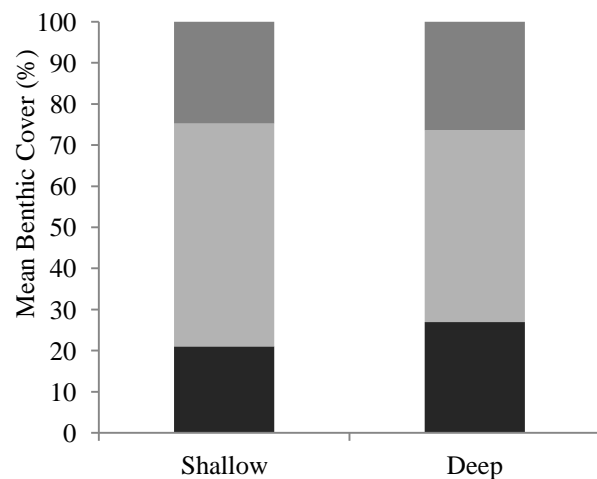


Fig. 2 Mean percent cover of coral, algae, and other at two depth ranges. The shallow depth range is 10-12 m and the deep depth range is 18-20 m. Coral percent cover and algae percent cover were determined by Coral Point Count (CPC). Medium gray bars indicate mean percent 'other' cover, light gray bars indicate mean percent algae cover and black bars indicate mean percent coral cover. There was a significant difference between coral cover and algae cover ($t = 13.07$, $df = 34$, $p > 0.001$)

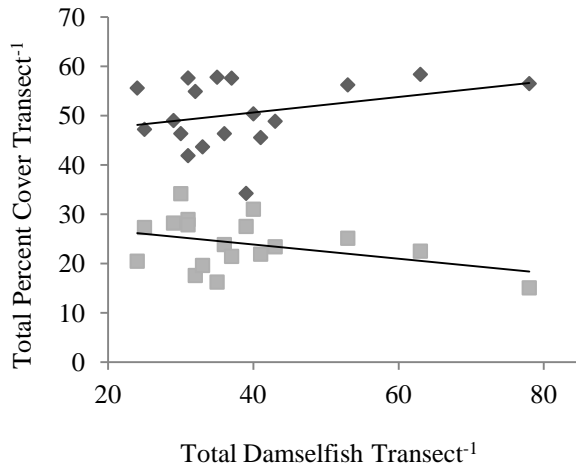


Fig. 3 Association between total number of damselfish and total percent coral cover transect⁻¹. The *dark gray diamonds* represent total percent algae cover transect⁻¹ and the *gray squares* represent total percent coral cover transect⁻¹. The *black lines* represent the trend lines. As total damselfish transect⁻¹ increases, total percent coral cover⁻¹ decreases ($t = -1.63$, $p = 0.12$, $r = -0.37$) and total percent algae cover increases ($t = 1.33$, $p = 0.20$, $r = 0.31$)

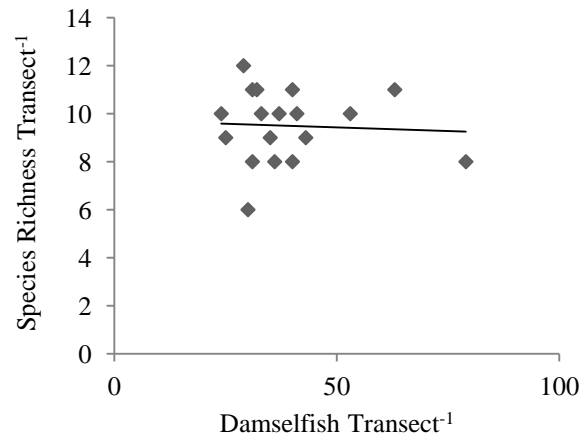


Fig. 5 Comparison of the total damselfish transect⁻¹ and coral species richness transect⁻¹. As the numbers of damselfish per transect increase, coral species richness decreased minimally ($t = -0.022$, $p = 0.82$, $r = -0.05$)

Discussion

Damselfish densities did not have the expected result on coral benthic composition. This study found that algal cover was significantly higher than coral cover. Mean percent algal cover was 50% while the mean coral cover was only 24%. This statistic fits with the theory of phase shifts of coral reefs from coral dominated to algae dominated reefs (Hughes et al. 2010).

As the total numbers of damselfishes per transect increased, the total percent coral decreased, however these data were not statistically significant. The larger populations of damselfish could be associated with their behavior as being both territorial fish and fish with a system of hierarchies within their population (Itzkowitz 1978). This could be due to the established damselfish territories and also clustering of individuals into groups (Itzkowitz 1978). As the total numbers of damselfishes per transect increased, total percent algal cover increased, but not at a significant rate.

Damselfish prefer to establish their territories in better habitats, and with greater densities of damselfish there is greater competition for space (Itzkowitz 1978). With increased competition, many

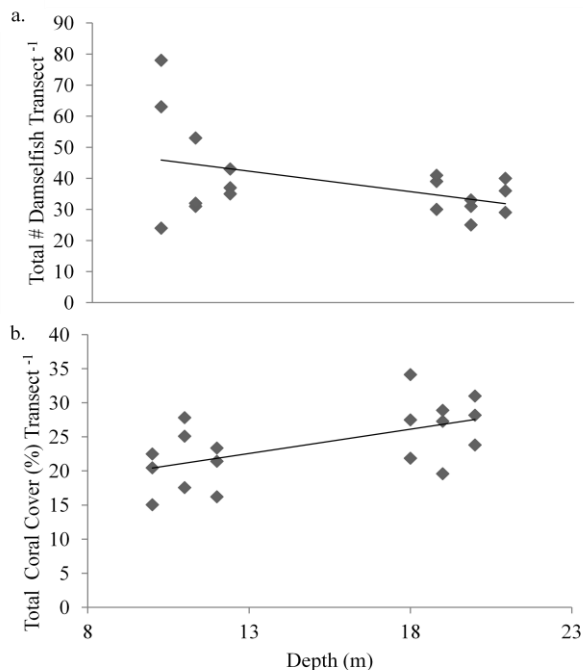


Fig. 4 Comparison of depth to **a.** total number of damselfish transect⁻¹ and **b.** total percent coral cover transect⁻¹. The *black lines* represent trend lines. As depth increases, total number of damselfish decreases ($t = -0.193$, $p = 0.07$, $r = -0.43$) and total percent coral cover increases ($t = 2.81$, $p = 0.01$, $r = 0.57$)

damselfish will use less preferable substratum for their territories (Itzkowitz 1978). Damselfish prefer corals as their habitat and as the amount of coral cover decreases, so does the preferred habitat of the damselfish (Itzkowitz 1978). Also, as depth increased, total number of damselfishes decreased, although not significantly, and total percent coral increased significantly. There was no correlation between damselfish and total percent coral cover. Because there was not a significant positive correlation between damselfish densities and total percent coral cover and coral species richness, areas with higher damselfish densities did not have a greater coral cover or species richness. In fact, the opposite pattern, albeit not significant, was found in the present study.

Damselfish populations in Bonaire are increasing due to overfishing of predatory fish (Grimsditch et al. 2009). The trends seen in this study may be the product of the increasing need for food by damselfish due to the growing population combined with the physical stress of coral reefs in Bonaire. As damselfish populations continue to increase, the demand for territory increases due to the need to make turf algae mats for food (Potts 1977). Damselfishes are an important species that keep macroalgae growth down and promote recruitment of coral within their territories (Wellington 1982). However, it is possible that the combined stresses of declining herbivory, overfishing and climate change are affecting the ability of damselfish to have a positive effect on benthic composition. While their populations may be increasing due to lack of predators, the amount of algae is also increasing at alarming rates due to phase shift trends (Hughes et al. 2010).

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Temporal use of two artificial reef morphologies by coral reef fishes

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Abstract

Coral reefs are important marine ecosystems with high biodiversity that provides food and socioeconomic benefits to people in tropical regions around the world. In the last thirty years coral cover has declined and has been wiped out in some areas. The coral loss affects all benthic organisms and fishes supported by these habitats. Artificial reefs have been used to enhance coral recruitment and to provide a habitat for reef fishes. In Bonaire, Dutch Caribbean, reef fish species richness, diversity, and density were measured at two types of artificial reefs, branching and block-style, at various times of the day. Field observations of reef-fish species richness and density were conducted at midday, dusk, and night. Branching artificial reefs supported higher species richness and diversity. Multiple herbivorous species utilized this habitat for grazing while other species used it for shelter. The block-style reefs supported a higher density (ind m⁻²) of reef fish. Fish density, species richness, and diversity decreased at night at the branching reef. The decrease in fish community complexity could be because the complexity of the block-style reef was not suitable for nighttime sheltering needs. Species richness and diversity also decreased at night at the block-style reefs; but density at night was no different than midday and dusk because the blocks supported a very high number of small individuals during nighttime. Two different artificial reef structures were found to support complex fish assemblages; however the species richness and fish abundance varied between the structures. This suggests that morphology of artificial reefs is a tool that can be utilized to attract specific reef fish communities. During a time of reef degradation and habitat loss, artificial reefs can be used to supply reef fish a habitat to live.

Introduction

Coral reefs are the most productive marine ecosystem on the planet, covering only 0.17% of the earth, reefs hold 4-5% of all known marine species (Nybakken and Bertness 2005). In addition to high biodiversity, coral reefs provide food for more than 500 million people worldwide (Hoegh-Guldberg 2011) and create an economy through tourism (Cesar and van Beukering 2004). However, there has been a global decline in coral reefs worldwide over the past four decades (Bak et al. 2005) that threatens coral reef biodiversity and the capability of reefs to provide food and an economy for people (Jackson et al 2001; Cesar and van Beukering 2004, Alvarez-Filip et al 2009). In 2003, data from 263 sites (63 separate locations) in the Caribbean basin were analyzed to determine the extent of the decline in coral cover over the past 30

years (Gardner et al 2003). Gardner et al. (2003) determined that hard coral cover has been reduced 80% in the Caribbean area since the 1970's. The decline in coral reefs is due to a variety of local and global changes in the environment such as overfishing (Jackson et al 2001), physical damage due to storms (Heron et al 2008), increased nutrient runoff (Fabricius 2005), the rise of ocean temperatures (Kleypas and Hoegh-Guldberg 2008), and changing pH (Hoegh-Guldberg et al 2007).

Development of artificial reefs to increase fishing industries by recruiting algae, shell fish or lobster began in the 1700's. Artificial structures have also been deployed to protect land from rough seas, to decrease erosion and to prevent trawling (Bohnsack and Sutherland 1985). Because of the recent decline of coral reefs, artificial reefs are now increasingly used as habitats for marine organisms. Structures range from

haphazard construction, using a variety of materials including old ships and train cars (Pike and Lindquist 1994), wood (Brown 2005), PVC pipes (Brown 2005), concrete (Gregg 1995), rubber tires (Brown 2005) and tiles (Burt et al 2009). Despite the widespread use of artificial reefs, there are conflicting conclusions about the success of artificial reefs in providing habitats for fishes and other organisms. In a comprehensive review of 413 articles, Bohnsack and Sutherland (1985) found that, when comparing fish communities on natural and artificial reefs, almost all artificial reefs exhibited higher biomass. Fish were using the structures for a variety of purposes such as reproduction, feeding and shelter. However, there were a few artificial reefs that exhibited no difference, and other reefs that had a lower community composition compared to natural reefs. Bohnsack and Sutherland (1985) also found that most articles reporting on fisheries at artificial sites versus natural reefs had higher fish abundances at the artificial sites, but again there were some conflicting results. Bohnsack and Sutherland (1985) suggested that to prevent unsuccessful structures, wasting time and materials, and potentially harming the natural environment, a well-developed plan should be made to attack a specific problem (e.g. overfishing, habitat degradation etc.).

With recent studies finding a reduction in coral cover in Bonaire, Dutch Caribbean (Bak et al 2005), artificial reefs were deployed in October 2011 to help mitigate the loss of coral reefs as an important habitat (Code 2011; Nelson 2011). Surveys were conducted on block-style and branching artificial reefs to measure reef fish species diversity, density and richness over a five-week period immediately after deployment (Code 2011; Nelson 2011). Cement block-style artificial reefs (Code 2011) were designed to have varying hole sizes because artificial reefs with different hole sizes will support fish of different sizes (Hixon and Beets 1989; Gratwicke and Speight 2005). There was a difference among species composition between blocks

of varying hole sizes; smaller fish were more common at blocks with small holes and larger fish were common at blocks with larger holes (Code 2011). The branching style reef was made from tree branches in cement blocks, constructed to mimic *Acropora cervicornis* coral (Nelson 2011). The branching style was chosen because fish density, biomass, and richness all increase with increasing complexity; complexity of a reef is measured by surface area, small chambers and hiding spaces and height of artificial structures (Charbonnel et al 2002; Gratwicke and Speight 2005). There was a higher diversity of reef fish at the branching artificial reefs when compared to naturally branching *A. cervicornis* patches (Nelson 2011). Studies done by Code (2011) and Nelson (2011) concluded that artificial reef structures can provide a habitat for a variety of reef fish for protection, grazing and benthic egg laying. Comparing the results from the branching (Nelson 2011) and block-style reefs (Code 2011) in 2011, species richness was higher on the branching reef and density was higher on the block-style reef.

Although artificial reefs are often monitored following deployment, the temporal variability of fish assemblages is seldom measured (Willis et al. 2005). In reef and kelp bed habitats off the coast of California, the same species of fish were found during the day and night but the abundance was higher during the day (Ebeling and Bray 1976). Diel (daily) movements for marine fishes are common and predictable for certain species (Ogden and Quinn 2002; Willis et al. 2005). Fish move within the water column and to different areas of the reef based on time due to reproduction behaviors, food availability, sharing of space, and transition from foraging to sheltering habitats (Ebeling and Bray 1976; Willis et al. 2005). Most reef fish are active either at night or during the day with dusk and dawn being a transitional period when day and nighttime fish are switching from foraging to sheltering (Hobson 1972; Ebeling and Bray 1976).

The purpose of this study is to compare the species diversity, richness and abundance of reef fish at two types of artificial reefs of different morphology. In addition, the diurnal changes in fish abundance and diversity on the artificial reefs will be investigated. Fish on block-style and branching artificial reefs will be monitored at midday, dusk and night to investigate changes in species composition of reef fish communities over the day. The following hypotheses will be tested:

- H_{1a}: The branching artificial reef will support higher species richness than the block-style.
- H_{1b}: Midday will have the highest species richness, followed by dusk and nighttime will have the lowest species richness.
- H_{2a}: The block style reef will support a higher fish density than branching reef (ind. m⁻²).
- H_{2b}: Midday will have the highest fish density, followed by dusk and nighttime will have the lowest fish density.
- H_{3a}: The branching reef will have higher species diversity than the block-style
- H_{3b}: Midday will have the highest species diversity, followed by dusk and nighttime will have the lowest species diversity.

This study will look at the fish communities supported by two types of artificial reefs five months after being put in place. Results will give insight to the types of fish that branching and block-style reefs support in Bonaire. Since artificial deployment, five months have passed allowing time for additional growth and recruitment to this habitat. Colonization of a new structure by algae, invertebrates and fish communities can take months or even years to reach equilibrium (Bohnsack and Sutherland 1985). Continual monitoring of the artificial structures and fish species compositions would give insight into artificial reef success in Bonaire, DC and the changes of fish abundances and species richness throughout the day.

Materials and Methods

Study Site

This study took place on the island of Bonaire, Dutch Caribbean, located in the southern Caribbean, approximately 80 km off the northern coast of Venezuela (Fig. 1). The artificial reefs utilized in this study are located on the leeward side of the island of Bonaire at Yellow Submarine dive site (12° 09'36.3" N, 68° 16'55.2" W). Bonaire is surrounded by a fringing reef; and at the site used, the reef crest begins approximately 45 m from shore at 9 m depth. Reef fish species richness, density, and abundance were measured on two types of artificial reefs that were deployed in October 2011. Three thickets of branching artificial reefs in 6-7 m of water, 3-4 m shoreward of the reef crest were monitored. Also, three artificial reefs constructed of cement blocks with a combination of hole sizes (two holes 15 cm x 15 cm and four holes 6 cm x 6 cm) in 6 m of water and 8 m from the reef crest were monitored.

Observations

Survey methods were adapted from Code (2011) and Nelson (2011). At each artificial reef unit (branching and block-style) 5 min surveys were conducted from a distance of 4 m, for 2 min, to record fish species and abundance then 2 min observations were made at a distance of 1 m to record fish species and abundance. A final 1 min period was used to identify smaller benthic fish. If possible, a distinction between juvenile and adult fishes was made. All species within 50 cm of the branching reefs and 20 cm of the blocks style reefs were included. Observations were repeated for 3 branching and 3 blocks-style reefs for each sampling period at three times: midday (between 1100-1400 hrs), dusk (1800-1930 hrs) and night (2000-2130 hrs). Survey methods were slightly altered for nighttime observation to accommodate decreased visibility; the first 4

min were spent within 2 m of each artificial reef unit to accurately identify species.

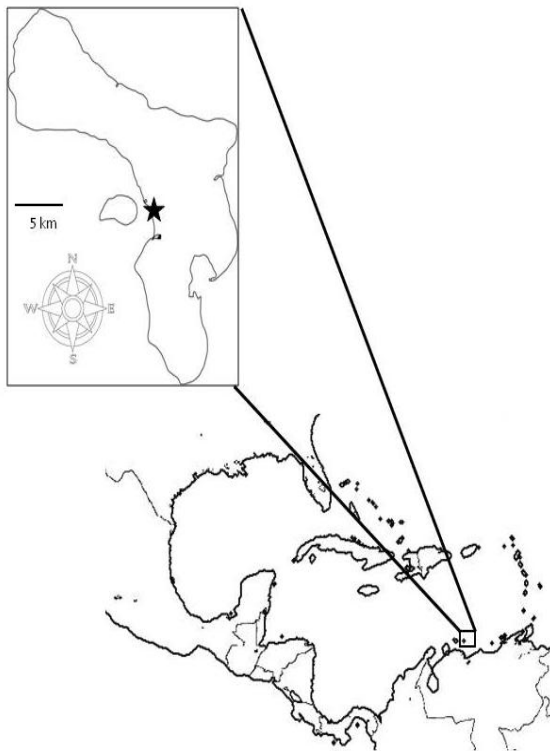


Fig. 1 A map of Bonaire, Dutch Caribbean. Black star indicates study site, Yellow Sub (12° 09'36.3" N, 68° 16'55.2" W)

Data Analysis

Species richness was calculated by summing all fish species seen either in one time period or at one type of artificial reef. Fish density (individuals m⁻²) was calculated by summing all fish seen in one survey, at a study site or during a study time, and then dividing it by the total area of the habitat surveyed (branching 7.59 m², blocks style 0.7 m²). Species diversity was calculated using the Shannon-weaver Index, $H = -\sum_{i=1}^S (p_i \ln p_i)$, where p_i was the frequency of a species and an increasing H-value coincided with the increasing reef-fish species diversity. A t-test was used to

compare the mean species richness, fish density, and species between the times of day (midday, dusk, night).

Results

During 21 surveys between 29 February 2012 and 28 March 2012, a total of 1,554 individual reef fish were observed at the branching artificial reefs and 967 individuals were observed at the block-style artificial reefs. A total 44 species were seen between the two artificial reef habitats.

The mean reef fish species richness \pm SD was significantly higher on branching artificial reefs (7.89 ± 2.63) than on block-style artificial reefs (3.44 ± 1.27) ($t = 6.84$, $df = 40$, $p < 0.001$; Fig 2). Multiple herbivorous fish species, such as parrotfish (*Scaridae*) and surgeonfish (*Acanthuridae*), were seen at the branching reefs, but only one major herbivore, the ocean surgeonfish (*Acanthurus bahianus*) was seen at the block-style reef. Trumpetfish (*Aulostomus maculatus*) were never observed at the block-style reef but were commonly seen at the branching reefs during midday, dusk and night. Sergeant majors (*Abudefduf saxatilis*) were the only species consistently seen on the block-style reef but never observed utilizing the branching habitat.

Fish species richness between the times of day was significantly different at the branching reef (ANOVA; $F = 15.327$, $df = 2.18$, $p < 0.001$; Fig. 3a). The mean species richness \pm SD at midday (9.43 ± 0.90) and dusk (8.79 ± 2.34) were significantly greater than at night (4.61 ± 1.29 ; Table 1). Similar to the branching reef, the mean fish species richness at the block-style reef was also significantly higher at midday (4.23 ± 0.76) and dusk (3.79 ± 1.11) than nighttime (2.00 ± 0.60) (ANOVA; $F = 11.973$, $df = 2, 18$, $p < 0.001$; Fig 3b).

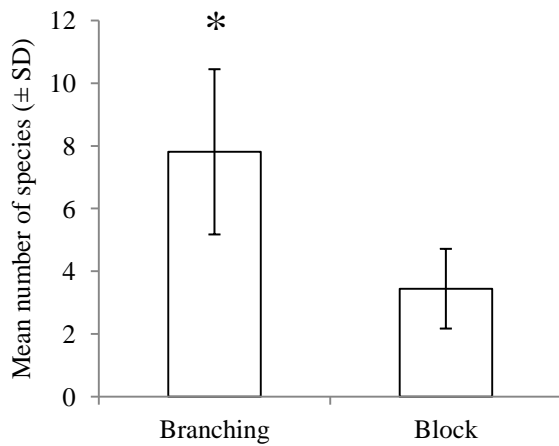


Fig. 2 Comparison of reef fish species richness on branching ($n = 21$) and block-style ($n = 21$) artificial reefs. Asterisk indicates a significant difference ($t = 6.84$, $df = 40$, $p < 0.001$)

Table 1 Tukey *post-hoc* results of reef fish species richness between branching and block-style artificial reefs. Asterisk indicates a significant difference

	Mean difference	Standard error	Significance
Branching			
Midday-Dusk	0.637	0.874	0.750
Midday-Night	4.817	0.940	0.001 *
Dusk-Night	44.18	0.912	0.001 *
Block			
Midday-Dusk	0.494	0.454	0.533
Midday-Night	2.286	0.488	0.001 *
Dusk-Night	1.792	0.474	0.004 *

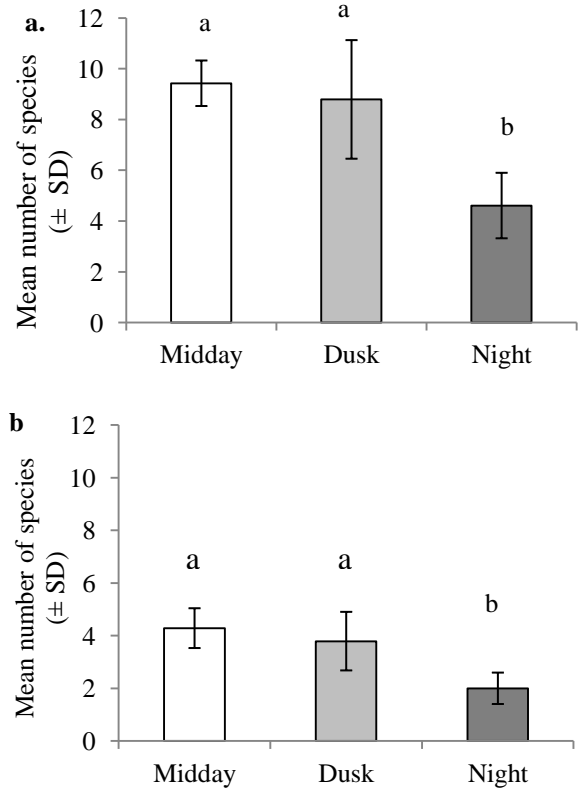


Fig. 3 Comparison of reef fish species richness at branching (**a.**; $n = 21$) and block-style (**b.**; $n = 21$) artificial reefs between three times of the day. *a* and *b* indicate a significant difference between the means (branching $F = 15.327$, $df = 2, 18$, $p < 0.001$) (block type $F = 11.973$, $df = 2, 18$, $p < 0.001$)

Reef fish density \pm SD was significantly higher on the block-style reef (21.93 ± 6.26) compared to the branching reefs (5.37 ± 2.68) ($t = -11.14$, $df = 40$, $p < 0.001$; Fig. 4). The block-style reef supported large densities of small fish species such as masked gobies (*Coryphopterus personatus*) and peppermint gobies (*Coryphopterus lipernes*), which were seen in groups of 40-60 individuals during night surveys. The large abundance of small fish at night resulted in no significant difference of mean density \pm SD when comparing midday (26.87 ± 3.72) and night (20.40 ± 5.40). There was only a significant difference in density at the block-style reef between midday (26.87 ± 3.72) and dusk (18.75 ± 6.43) (ANOVA; $F = 4.615$, $df = 2, 18$, $p = 0.024$; Fig 5b and Table 2). Similar to other fish complexes, reef fish density was significantly greater at midday (7.00 ± 1.00) and dusk (6.44 ± 1.89) than

nighttime (1.77 ± 0.78 ; Table 2) on the branching reefs (ANOVA; $F = 28.943$, $df = 2, 18$ $p < 0.001$; Fig 5a and Table 2).

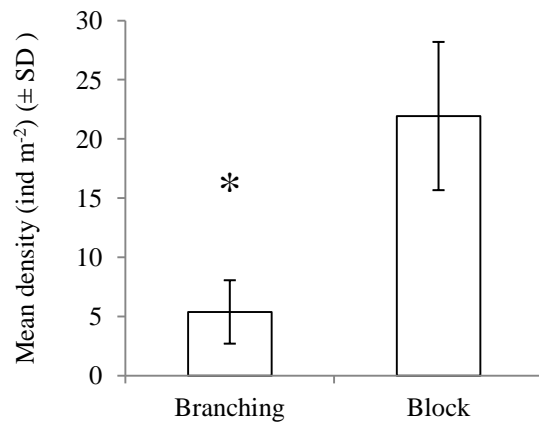


Fig. 4 Comparison of reef fish density on branching ($n = 20$) and block-style ($n = 20$) artificial reefs on. Asterisk indicates a significant difference ($t = -11.14$, $df = 40$, $p < 0.001$)

Table 2 Tukey *post-hoc* results of reef fish density between branching and block-style artificial reefs. Asterisk indicates a significant difference

	Mean difference	Standard error	Significance
Branching			
Midday-Dusk	0.358	0.712	0.871
Midday-Night	5.236	0.765	0.000 *
Dusk-Night	4.878	0.743	0.000*
Block			
Midday-Dusk	8.121	2.778	0.023*
Midday-Night	6.474	2.986	0.104
Dusk-Night	-1.647	2.898	0.839

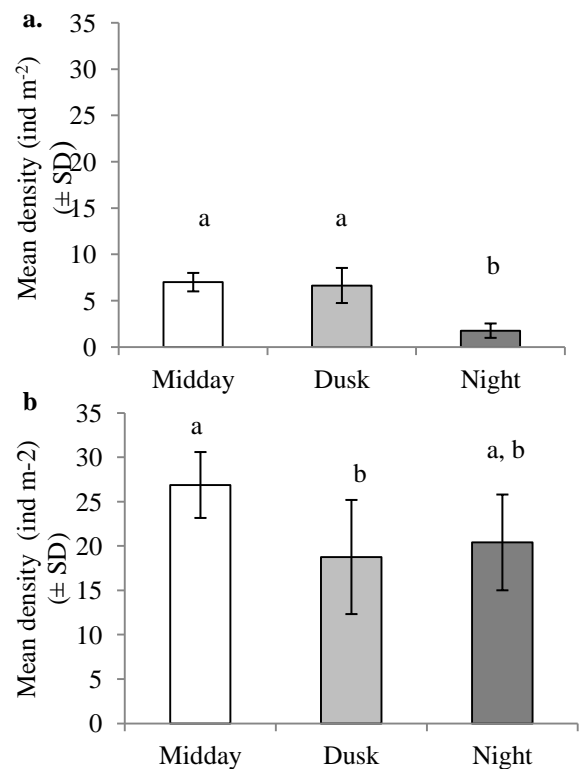


Fig. 5 Comparison of reef fish density (ind m⁻²) on branching (**a**; $n = 21$) and block-style (**b**; $n = 21$) artificial reef at three times of the day. *a* and *b* indicate a significant difference between the means (branching $F = 28.943$, $df = 2, 18$ $p < 0.001$) (block-style $F = 4.615$, $df = 2, 18$ $p = 0.024$)

The mean reef fish species diversity \pm SD at the branching artificial reefs (1.66 ± 0.33) was significantly higher than block-style reefs (0.83 ± 0.36 ; Fig 6). Multiple large fish species and individual fish were observed utilizing the branching style artificial reef at one time. For example many herbivorous fishes (both different species and different individuals) were grazing on the algae while other fish were utilizing the branches for a sheltering habitat. However, at the block-style reef only small fish such as gobies and bicolor damselfish (*Stegates partitus*) were able to utilize a block at one time. Reef fish diversity \pm SD on branching artificial reefs at midday (1.78 ± 0.17) and dusk (1.79 ± 0.27) was significantly higher than at night (1.34 ± 0.26) (ANOVA; $F = 7.385$, $df = 2, 18$, $p = 0.005$; Fig 7a and Table 3). Similarly, reef fish diversity at the block-style reefs was significantly higher at

midday (0.98 ± 0.17) and dusk (1.01 ± 0.29) compared to night (0.42 ± 0.29) (ANOVA; $F = 11.039$, $df = 2, 18$ $p = 0.001$; Fig 7b and Table 3).

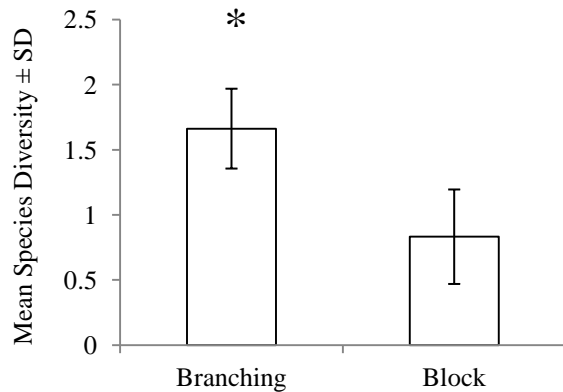


Fig. 6 Comparison of reef fish species diversity of branching ($n = 20$) and block-style ($n = 20$) artificial reefs. Asterisk indicates a significant difference ($t = 8.01$, $df = 40$, $p < 0.001$)

Table 3 Tukey *post-hoc* results of reef fish species diversity between branching and block-style artificial reefs. Asterisk indicates a significant difference

	Mean difference	Standard error	Significance
Branching			
Midday-Dusk	-0.008	0.124	0.997
Midday-Night	0.440	0.133	0.011 *
Dusk-Night	0.448	0.129	0.007 *
Block style			
Midday-Dusk	-0.030	0.133	0.972
Midday-Night	0.565	0.143	0.002 *
Dusk-Night	0.595	0.138	0.001*

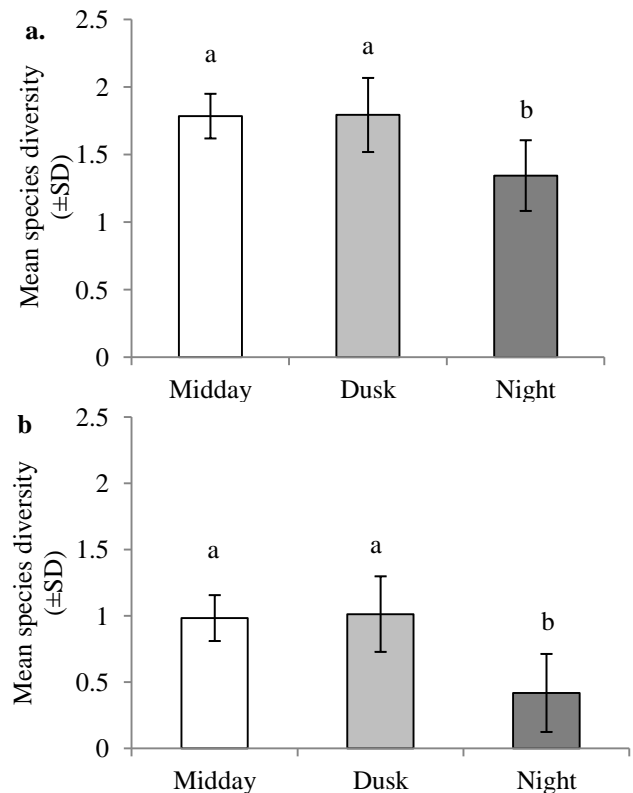


Fig. 7 Comparison of reef fish species diversity (using the Shannon-Winer Index) at branching (**a**; $n = 21$) and block-style (**b**; $n = 21$) artificial reefs between three times of day. *a* and *b* indicate a significant difference between the means using (branching $F = 7.385$, $df = 2, 18$, $p = 0.005$) (block-style $F = 11.039$, $df = 2, 18$ $p = 0.001$)

Discussion

The hypotheses predicting higher fish species richness, lower density and higher diversity at the branching artificial reefs than the block-style reefs were supported in this study. The increased number of fish species on the branching reefs may be explained by the difference in habitat utilization of the two artificial reefs. The branching reef had a higher complexity which increased the species richness. Algae growing on the branches attracted more herbivorous fishes to the branching reef and the proximity of the branching artificial reef to the natural reef crest may have increased the species richness as well. The higher complexity of the branching style reef acted as a suitable sheltering habitat for species such as the trumpetfish (*Aulostomus maculatus*) and

french grunt (*Haemulon flavolineatum*). Trumpetfish were seen at the branching reefs at all times of the day using the branches as camouflage, which made the fish more difficult to see. Trumpetfish were not observed at the block-style reef, which may be because the block-style reef did not offer any protection for this species. French grunts were also often observed on the branching reef at all times of the day and only observed swimming around the block-style reef but not using it for shelter or protection. The grunts and trumpetfish were observed sheltering at the branching reef during the day and night which may have been preferable to crowded natural reefs providing less complexity or the block reefs which are not suitable for the size of these species. Previous studies found decreased predation on reef fish with increasing complexity (Almany 2004). So the higher complexity on the branching reef compared to the block-style could account for the higher species richness.

The high density of algae growing on the tree branches of the branching reef attracted numerous herbivorous fish, adding to the higher species richness on the branching reefs. Several species of parrotfish and three species of surgeonfish were observed to be feeding on the branching reef however only one herbivore was observed on the block-style reef. Although herbivorous fishes scrape hard, old dead coral and ingest some of the calcium carbonate substrate, concrete may not be edible, whereas tree branches could be more suitable. The fish using the branching reef as a feeding ground added to the higher species richness on the branching reef compared to the block-style artificial reef.

The proximity of the branching structure to the reef crest may have also played a role in the presence of some species. Initial phase bluehead wrasses (*Thalassoma bifasciatum*) were often observed moving from large coral heads on the reef crest into the branching artificial reef area. Although the branching reef may not have anything to offer the bluehead wrasse

species, the location of the branching reef could have increased the species richness when compared to the block style reef.

Sergeant majors were the only fish species never observed on the branching reef but often seen on the block-style reefs. Sergeant majors laid their eggs on all sides of the cement blocks and even within the large holes. Sergeant majors would chase away other fish while protecting the egg broods, which may have lead to a decrease in block utilization by other species. Competition of the same resource between two species may impact the distribution of species in an area (Connell 1961). Since sergeant majors are aggressive and protective of their egg broods, when they claim the cement blocks this could decrease the presence of other fish species.

Results also support the original hypothesis that fish density would be higher on block-style reefs than on branching. The block-style reef was able to support a high number of small fish which influenced the total fish density when compared to the branching reefs. On some surveys a higher number of bridled gobies (*Coryphopterus glaucofraenum*) were observed around the branching reefs, but high densities of this species were also consistently seen at the block-style reefs. During the night surveys 40-60 masked/glass gobies and peppermint gobies could be seen which influenced overall density of block-style reef. Another small fish species, the slippery dick (*Halichoeres bivittatus*) was present at both the branching and block-style artificial reef locations. However, upwards of 20 individuals were often seen at one specific block. The species that were consistently seen in larger numbers at the block-style reefs contributed to its overall greater density of fish when compared to the branching reefs.

The species diversity of reef fish was also compared between branching and block-style reefs using the Shannon-Weaver index. This index accounts for species prevalence and frequency. The original hypothesis that branching reefs will support higher species

diversity than block style reefs has been supported. These results may be because the branching reef provided a habitat for a wider range of uses when compared to the block-style reefs. The branching structure was utilized for grazing for multiple herbivorous fish species, which was hardly seen at the block-style reefs. The bigger overall area of the branching reefs supported a lower mean density but gave more area for grazing and shelter so multiple fish could use the reef at one time. This was often observed; during surveys parrotfish and surgeonfish were feeding at the same time trumpetfish were utilizing the branches for shelter. Smaller species such as the bicolor damselfish and sharpnose puffers (*Canthigaster rostrata*) could also utilize the branching structure as protection without being too close to the larger fish. The blocks were smaller, which did not support many large individual fish utilizing this space at all and when large fish were there it was not favorable for other fish nearby. Only small fish like bicolor damselfish and slippery dicks were observed utilizing the blocks at the same time. The wide ranges of habitat utilization at the branching style reef lead to a greater diversity than the block-style reef.

The hypotheses that reef fish species richness, density and diversity would decrease throughout the day were rejected; there was typically no difference these measurements at midday and dusk. The decrease in species richness, diversity and density at night may be attributed to the species' activities at this time. The differences in fish assemblages between day and night show that the fish seen during the daylight hours are only active during this time and are sheltering at night which is common for reef fish species (Hobson 1972; Ebeling and Bray 1976). At night, french grunts and trumpetfish were often seen utilizing the branching reefs as sheltering habitats and the soapfish (*Rypticus saponaceus*) did the same at the block-style reefs, but these habitats may not be as suitable for sheltering activities of other reef fish species. Previous studies conclude that

habitat complexity has a greater effect on fish communities at night than during the day (Walsh 1985) which may account for the presence of fish here. On the branching reef, parrotfish were observed in abundance during midday and dusk observations but only one individual was ever seen at night. The branching structure was an obvious feeding area during the day but as discussed in other studies after dark fish are no longer attracted to rich feeding grounds but are more focused on seeking a suitable shelter (Ebeling and Bray 1976). Nelson (2011) concluded that the artificial branching reef was not as complex as natural branching coral habitats which could contribute to the limited number of fish observed to be sheltering on these reefs at night.

Previous studies have seen colonization of a new structure by reef fish taking several months or years to reach equilibrium (Bohnsack and Sutherland 1985; Golani and Diamant 1999). Measures of species richness, diversity and fish density, directly after the installation of the branching and block-style reefs in October, 2011 (Code 2011; Nelson 2011), may not have been the most accurate evaluation of the fish these structures support. Golani and Diamant (1999) found that colonization of an artificial reef was rapid at first but over seven months species diversity declined and then leveled off. Comparing results from previous studies this may have been the case on the branching and block-style artificial reefs in Bonaire. The current study shows a decrease in species richness on the branching artificial reef from 23 species recorded by Nelson (2011) to 9 species in 2012. The fish density also decreased from 23 ind m⁻² to 7 ind m⁻². However, species diversity increased from 0.71 to 1.78. Measurements of fish density and species richness on the block style reefs from 2011 to 2012 show similar trends (Code 2011). Species richness decreased from 11 species to 4 species and density decreased from 136 ind m⁻² to 27 ind m⁻². Results are consistent with previous findings where species compositions were high immediately after artificial reefs were

deployed (Bohnsack and Sutherland 1985; Golani and Diamant 1999).

The current study shows the importance of continual monitoring of artificial reefs for an extended amount of time. The results from this study are different from the results obtained immediately after the branching and block-style reef were placed in 2011. With such a difference between species richness, diversity, and density at both types of reefs it is hard to tell whether the fish communities have reached a stable equilibrium, which should be further investigated. Future studies could compare the fish communities using non-parametric multidimensional scaling to see if different species utilize the artificial reefs compared to the natural reefs because previous studies on artificial reefs have shown fish communities to be dissimilar between natural and artificial structures (Clark and Edwards 1994). In addition to supporting fish assemblages, the hard cement of both artificial reefs morphologies could provide a suitable habitat for coral recruits in the near future (Clark and Edwards 1994) which would add to the biodiversity that these structures support.

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Herbivory and predatory pressures on artificial reefs in Bonaire, Dutch Caribbean

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Abstract

Artificial reefs are commonly used to increase habitat space for reef-dwelling organisms. Coral reefs in Bonaire, Dutch Caribbean, are degrading due to factors such as disease, bleaching events, and heavy storms, reducing habitat space for reef fish. Two different artificial reefs were deployed on the leeward side of Bonaire in 2011: one block and one branching structure. Studies found that both reefs supported fish communities but utilization of the reefs by fish was not studied. The current study examines utilization of branching and block-style artificial reefs for foraging and feeding activities by herbivores and predators to assess which reef structure provides more resources for fish. Herbivore grazing and predator stalking rates were calculated as well as herbivory and predation pressure at increasing distances away from the artificial reef. The branching artificial reef supported more herbivore and predator activity compared to the block reef, suggesting structural complexity increases important sheltering and feeding areas for reef fish. Predation and herbivory pressures showed no trend with increasing distance from the artificial reef while predation pressure decreased with increasing distances from the natural reef. This suggests that the artificial reef may act as a shelter between the reef crest and the surrounding sand and rubble area, thereby increasing foraging distances of fish coming from the reef crest. Not only resident, but transient individuals, were found to use the artificial reefs for feeding and sheltering, suggesting that artificial reefs do not need to create permanent habitats in order to be important habitat for reef fish.

Introduction

Coral reefs world wide have been severely degraded over the past few decades (Clark and Edwards 1994; Hughes 1994; Rilov and Benayahu 2002; Pandolfi et al. 2003; Worm et al. 2006). Overfishing (Jackson et al. 2001), pollution (Fabricius 2005), raising ocean temperatures (Bouchon et al. 2005), increases in the ocean's acidity (Kleypas and Hoegh-Guldberg 2005), coral mining (Shepherd et al. 1992), developing of coastlines (Rogers 1990), and destructive tourist activities (Cesar et al. 2003) have all been attributed to declining coral reef health. It has been predicted that 70% of reefs will seriously decline in the next 3 decades (Clark and Edwards 1999) and that large, formerly rare bleaching events will become biannual occurrences for the next 20 to 30 years (Donner 2005). Coral reef degradation has led to decreases in habitat for many marine

organisms, causing a loss in biodiversity and species richness (Rilov and Benayahu 2002), which is predicted to cause a decrease in the stability of the ecosystems (Worm et al. 2006).

The construction and deployment of artificial reefs has been occurring for over 200 years (Clark and Edwards 1994). Recently, artificial reefs have been used as management tools to increase the diminishing coral habitats reef fish depend upon (Grossman et al. 1997). A variety of materials including concrete blocks, car tires, pipes, shells, rocks, steel, rubber, vehicles, and even vascular plants have been used in artificial reef construction (Bohnsack and Sutherland 1985; Jesse et al. 1985). Artificial reefs are generally built in order to attract commercially valuable fish, to create new settlement and recruitment areas for fish and benthic organisms, to act as recreational diving and fishing sites, and to increase the

productivity of an ecosystem by increasing fish biomass and growth (Golani and Diamant 1999). Artificial reefs increase fish growth and population biomass by increasing available food (Hueckel and Stayton 1982; Bohnsack and Sutherland 1985), shelter and resting habitats (Pickering and Whitmarsh 1997), foraging efficiency (Pickering and Whitmarsh 1997; Rilov and Benayahu 2002), and recruitment habitats (Rilov and Benayahu 2002). Whether artificial reefs create a permanent habitat for reef fish (Stone et al. 1979; Bohnsack and Sutherland 1985), or just serve to aggregate transient fish to an area that can be used to exploit populations through fishing (Polovina 1989; Grossman et al. 1997) is still up for debate and is an important issue when discussing the effectiveness of artificial reefs increasing population sizes.

Foraging and feeding have been observed as frequent behaviors near or on artificial reefs (Hueckel and Stayton 1982; Bohnsack and Sutherland 1985; Bohnsack 1989; Johnson et al. 1994; Pickering and Whitmarsh 1997; Charbonnel et al. 2002) and gut content analyses have suggested that most fish on artificial reefs obtain their diets primarily from the reefs (Johnson et al. 1994). Because fish spend the majority of their time foraging or feeding in order to maximize reproductive capacity (Deloach and Humann 1999), the availability of food at artificial reefs has been determined to be a key component in attracting fish to the area (Hueckel and Stayton 1982; Bohnsack 1989; Charbonnel et al. 2002).

Fish have also been shown to abide by the optimal foraging theory, which states that individuals will maximize foraging efficiency by spending more time or energy to find food which returns the most gain for the energy output (Hueckel and Stayton 1982; Bohnsack 1989; Kurz 1995). The optimal foraging theory has been proposed as a possible method to predict fish distribution and abundance patterns (Kurz, 1995) suggesting that higher fish abundance should be found in areas with more food based upon optimizing foraging strategies.

Artificial reefs with increased food abundance should, therefore, have larger fish densities. The increased surface area created by artificial reefs allows for growth of benthic flora, thereby increasing food availability for herbivores (Pickering and Whitmarsh 1997). In addition, the aggregation of fish and benthic organisms near the reefs can be important for predators and an increased predator presence has been observed on artificial reefs supporting larger fish populations (Bohnsack 1989).

Concurrent with the optimal foraging theory, fish seek to minimize risks while foraging and foraging behaviors have been found to be higher near artificial reefs in several cases (Bortone et al. 1988; Kurz 1995; Einbinder et al. 2006). Infaunal communities adjacent to artificial reef structures were less diverse than communities farther away, which was attributed to increased predation by fish living on the artificial reefs foraging more frequently near the reef (Bortone et al. 1988 and Kurz 1995). Herbivory has also been found to decrease with increasing distance from artificial reefs, due to increased risk of predation as herbivores move farther away from the shelter of the artificial reefs (Einbinder et al. 2006).

The coral reefs surrounding the island of Bonaire, Dutch Caribbean are tourism hot spots because the reefs are so well preserved. However, even the fringing reefs in Bonaire have been degraded due to disease, bleaching and mass mortalities. The *Acropora cervicornis* and *Acropora palmata* that used to be two of the most plentiful corals in the area underwent mass mortalities due to white band disease in the 1980's. In 1999, Hurricane Lenny decimated most of the remaining *Acropora* colonies. The loss of *Acropora* has turned shallow areas, once occupied by *Acropora* thickets, into sand and rubble, causing a loss in structural complexity and habitat space for reef fish. The reefs in Bonaire are also undergoing changes in coral cover due to the loss of the important grazer, *Diadema antillarum*, bleaching events, and eutrophication caused

by increasing development along the coastline (Bries et al. 2004). With coral degradation in Bonaire, reef-dwelling fish are losing important habitat.

In October of 2011, two types of artificial reef were deployed on the leeward side of Bonaire. One was designed to study the effects of various hole sizes on reef fish diversity and abundance (Code 2011); the other artificial reef was designed to mimic the structure of *A. cervicornis* and study the differences in diversity and abundance of reef fish between the natural *A. cervicornis* and artificial patch reefs. Studies were conducted immediately after reef deployment and observation of reef fish communities were made for several weeks (Code 2011; Nelson 2011).

The theory of optimal foraging strategies, suggests that fish found in a particular area should aggregate for the use of an important resource. Feeding efforts on an artificial reef, therefore, should indicate the availability of important resources such as food and shelter. The present study assesses the effectiveness of the two different artificial reef types in Bonaire as reef fish habitat by analyzing the feeding behaviors of herbivores and predators at both reefs.

The surfaces of both artificial reefs have gathered turf algae, which herbivores feed on and have attracted organisms for predators to feed upon. The study compares the feeding and foraging rate of herbivores and predators between the block and branching artificial reefs to assess which structure is utilized more for herbivore feeding and predator foraging. The branching artificial reef has a larger surface area than the cement block artificial reef, giving more space for turf algae to grow and thereby increasing potential food for herbivores.

The branching artificial reef is also more structurally complex than the cement block artificial reef with the overlapping branches creating more coverage, allowing for more areas for fish to find shelter. This should increase the fish abundance and

thereby the predator feeding behavior at this reef. However, it has been found that predation decreases with increased habitat complexity, as found with mud crab predation on oysters and toadfish predation on mud crabs (Grabowski 2004). Predation pressure on adult fish was also found to be lower in more complex live reef habitats, which could be attributed to the increased shelter from predation found at more complex reef sites (Almany 2004). Herbivory and predation on the two artificial reefs were studied by testing the following hypotheses:

H₁: Herbivore feeding rate will be greater at the branching artificial reef than at the cement block artificial reef.

H₂: Predator feeding rate will be greater at the cement block artificial reef than at the branching artificial reef.

H₃: Herbivory pressure will decrease with increasing distance away from the artificial reef.

H₄: Predation pressure will decrease with increasing distance away from the artificial reef.

The previous studies conducted on the artificial reefs in Bonaire have not addressed the habitat utilization of the structures by reef fish. By assessing the amount of herbivory and predation at the artificial reefs, the present study will help to determine which reef structure is more effective at increasing food resources for reef fish. Because foraging and feeding comprise such a large portion of fish activity, the herbivory and predation activity observed on the reefs should help give a larger picture of the effectiveness of the two reefs as contributing important resources for reef-fish.

Other studies have also compared herbivory and predation pressure away from artificial structures to monitor if artificial reefs alter the feeding patterns of reef fish. Past studies assume that predation and herbivory pressures are caused by individuals living directly on the reef without having any data about what individuals are accounting for the results. The present study aims to give a more accurate reflection of

what organisms are causing predation and herbivory pressures and increase the understanding of foraging behaviors by fish in Bonaire in regards to the artificial reefs.

Materials and Methods

Study Site

The study was conducted at a site on the fringing reefs of Bonaire, Dutch Caribbean. Bonaire is an island situated in the Southern Caribbean around 80 km to the north of Venezuela (Fig. 1).

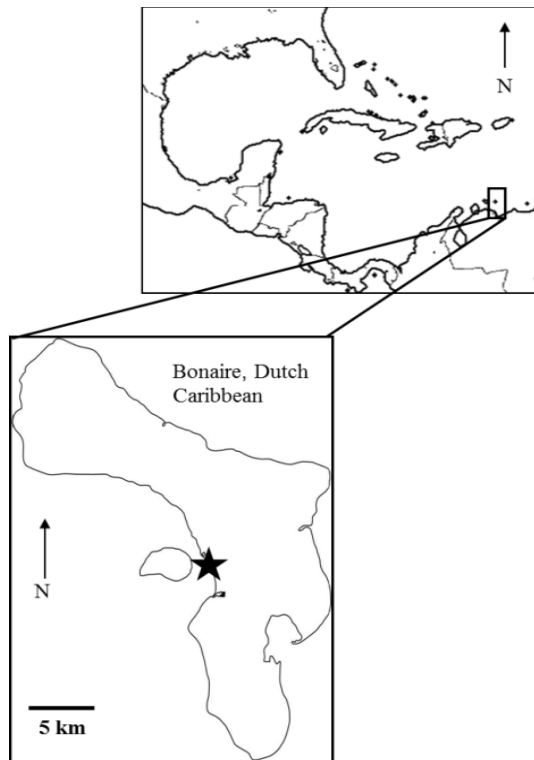


Fig. 1 Map of Bonaire, Dutch Caribbean. *Black star* indicates Yellow Sub dive site ($12^{\circ} 09'36.3''$ N, $60^{\circ} 16'55.2''$ W)

Studies were conducted at Yellow Sub ($12^{\circ} 09'36.3''$ N, $60^{\circ} 16' 55.2''$ W) on the leeward side of Bonaire where two artificial reefs were deployed in October of 2011 (Fig. 2). At Yellow Sub, there is a large sand flat before the reef crest. The artificial reefs are placed in this sand flat near the edge of the reef crest. The two

artificial reefs vary in size and structure.

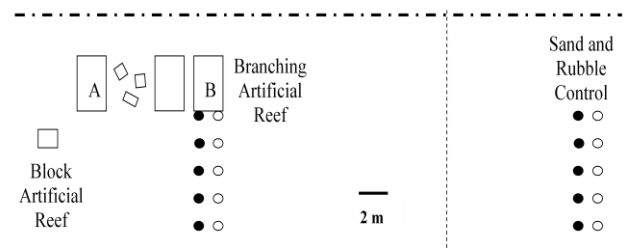


Fig. 2 Site map of study sites at Yellow Sub. Blocks are not to scale. *Square* represents the cement block artificial reef used for herbivory/predation behavior studies. *Rectangles* represent the branching artificial reef. *A* is the branching patch used for the herbivory/predation activity studies and *B* is the patch used for herbivory/predation pressure at increasing distance study. *Sand and Rubble Control* is the control site for the herbivory/predation pressure at increasing distance study. *Circles* represent rebar for the herbivory/predation behavior study. *Black circles* represent rebar used to hang algae while *white circles* represent rebar used to hang fish. *Vertical grey line* represents a 10 m gap and *horizontal bold dashed line* represents the reef crest

Block Artificial Reef

There are 16 concrete blocks (40 cm x 60 cm x 30 cm) with varying sizes of holes. The blocks are 8 m away from the reef crest at a depth of approximately 6 m and are 10 m away from one another. The northernmost block was the block used in the present study. There are six (6 cm x 6 cm) holes in one side of the block (Fig. 3).

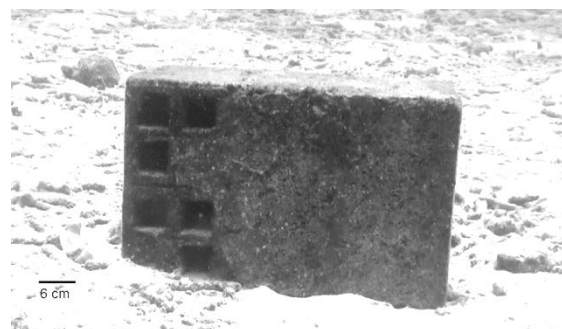


Fig. 3 Photograph of the block artificial reef at Yellow Sub dive site in Bonaire, Dutch Caribbean. The block-style artificial reef was deployed in October of 2011 and consists of 16 blocks of equal size, with varying hole sizes within each block

Branching Artificial Reef

The reef is constructed of concrete blocks (40 cm x 20 cm) with tree branches sticking out of the cement, simulating a patch reef of *A. cervicornis*. There are three 3 block x 3 block patches (1.7 m x 1.4 m) and a group of 3 solitary blocks (1.7 m x 1.5 m). The patches are placed 3 m away from the reef crest at a depth of approximately 6 m. The southernmost 3 x 3 patch (patch A) was the study site for the herbivory/predation rate study while the northernmost 3 x 3 patch (patch B) was the study site for foraging distance study. The branching reef patches are approximately 2 m away from the concrete block artificial reef (Fig. 4).

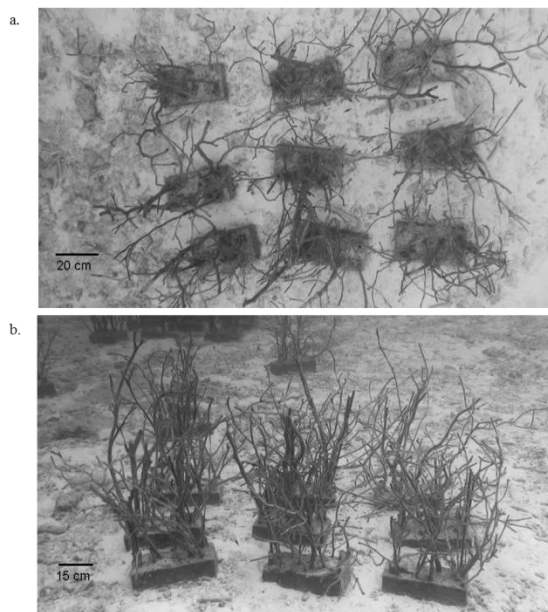


Fig. 4 a. Photograph of the branching artificial reef an aerial view. **b.** Photograph of the branching artificial reef from a side view. The branching artificial reef was deployed at Yellow Sub dive site in Bonaire, Dutch Caribbean in October 2011 and consists of three patches of nine blocks and three solitary blocks.

Study sites included a 20 cm parameter around the block artificial reef and the branching reef patches.

A third study site was used as a control site for the foraging distance study. This site consisted of sand and rubble substrate approximately 3 m from the reef crest and 6 m deep and is approximately 35 m from the other two study sites. The site

is removed from other large structures such as mooring blocks that may act as fish aggregates that could influence predation and herbivory at the control site.

The study took place between February and April of 2012. All surveys were conducted between 1100 and 1700 hrs using SCUBA.

Herbivore and Predation Activity

Distinct herbivores and predators (Table 1) were observed at the block and branching artificial reefs for 20 min surveys. A total of five surveys were conducted at the block artificial reef and patch A of the branching artificial reef (Fig. 2). While in the survey area, herbivore and predator feeding and foraging activity were monitored. Herbivore activity was measured using number of bites individuals took off of the artificial reef. Predator activity was measured as the amount of time predators spent at the artificial reefs stalking prey.

Table 1 Herbivores and predators monitored at the branching and block artificial reefs to determine herbivory and predation activity at two artificial reef structures in Bonaire, Dutch Caribbean

Common name	Herbivore/ predator	Family	Scientific name
Blue Tang	Herbivore	Acanthuridae	<i>Acanthurus coeruleus</i>
Ocean Surgeonfish	Herbivore	Acanthuridae	<i>Acanthurus bahianus</i>
Stoplight Parrotfish	Herbivore	Scaridae	<i>Sparisoma viride</i>
Princess Parrotfish	Herbivore	Scaridae	<i>Scarus taeniopterus</i>
Striped Parrotfish	Herbivore	Scaridae	<i>Scarus iserti</i>
Redband Parrotfish	Herbivore	Scaridae	<i>Sparisoma aurofrenatum</i>
Redtail Parrotfish	Herbivore	Scaridae	<i>Sparisoma chrysopteron</i>
Yellowtail Parrotfish	Herbivore	Scaridae	<i>Sparisoma rubripinne</i>
Trumpetfish	Predator	Aulostomidae	<i>Aulostomus maculatus</i>
Bar Jack	Predator	Carangidae	<i>Caranx ruber</i>
French Grunt	Predator	Haemulidae	<i>Haemulon flavolineatum</i>
Bluestriped Grunt	Predator	Haemulidae	<i>Haemulon sciurus</i>
Spanish Hogfish	Predator	Labridae	<i>Bodianus rufus</i>
Butter Hamlet	Predator	Serranidae	<i>Hypoplectrus unicolor</i>
Coney	Predator	Serranidae	<i>Cephalopholis fulva</i>

The total number of bites taken at the study site by herbivores and the total amount

of time predators spent stalking prey at each study site was recorded during each 20 min survey period. Because of the complexity of the branching artificial reef, video monitoring was used and videos were later analyzed to accurately assess bite rates and stalking time on the larger structure.

Surface areas for both reefs were calculated. The surface area for the cement block was calculated without accounting for the six holes because they are too small for the herbivores to access. The surface area of the branching artificial reef was calculated by measuring the lengths and circumferences of the individual branches in a single block of the patch. The surface area of the branches as well as the surface area of the concrete block supporting the branches was calculated. The total surface area of the single block was multiplied by nine to give an estimate of the surface area for the entire patch reef.

The number of bites on each site was used to calculate the bite rate m^{-2} for herbivores. Predator stalking times were used to calculate stalking times m^{-2} . Data were analyzed using t-tests ($\alpha = 0.05$) to test for significant differences between the bite rate m^{-2} and stalking time m^{-2} between block and branching artificial reef sites.

Herbivory/Predation Pressure at Increasing Distance

Rebar was hammered into the substrate at 2 m increments up to 8 m away from patch B (Fig. 2) of the branching artificial reef and the sand and rubble study sites (at 0 m, 2 m, 4 m, 6 m, and 8 m). Two rows of rebar were placed 1 m away from one another shoreward of the reef crest. Pre-weighed pieces of fish (*Pterois volitans*) were attached to rebar using fishing line in one row and in the other row pre-weighed pieces of algae (*Enteromorpha linolata*) were attached to the rebar in the same manner.

Video surveys were used to record herbivory and predation pressure at the two sites for 20 min after pieces of fish and algae

were hung. Videos were later used to compare species responsible for herbivory and predation on the artificial reef and control site by recording which species were observed taking bites from the fish or algae snares and at which distance species were feeding. After the 20 min survey was completed, the remaining algae and fish were removed and later weighed to determine the percent of fish or algae remaining after 20 min from each meter mark.

Percentages of algae and fish remaining after surveys from each area were analyzed using a one-way ANOVA to test for significance ($\alpha = 0.05$) between remaining amounts of algae and amounts of fish at 0 m, 2 m, 4 m, 6 m, and 8 m. Pearson's correlations ($\alpha = 0.05$) were completed for percentages of remaining algae/fish against increasing distances.

Results

Herbivore and Predation Rates

Of the observed herbivores and predators (Table 1), seven different herbivorous species and four different predatory species were observed at the block-style reef. At the branching artificial reef there were eight species of herbivores and three species of predators. Initial phase princess parrotfish (*Scarus taeniopterus*) were the most common species around both artificial reef sites.

Higher mean (\pm SD) herbivory bite rate was observed at the branching artificial reef ($131.54 \pm 72.84 \text{ bites min}^{-1} \text{ m}^{-2}$) than at the concrete block artificial reef ($8.97 \pm 8.67 \text{ bites min}^{-1} \text{ m}^{-2}$), with a significantly greater bite rates ($t = -3.9$, $df = 8.0$, $p = 0.004$; Fig. 5).

There was a higher mean (\pm SD) predator stalking time at the branching artificial reef ($171.80 \pm 169.85 \text{ s m}^{-2}$) than at the block artificial reef ($32.22 \pm 72.05 \text{ s m}^{-2}$), however no statistical differences were found ($t = -1.69$, $df = 8$, $p = 0.13$; Fig. 6).

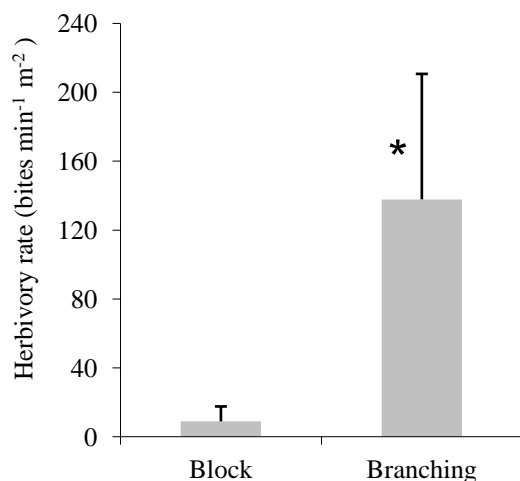


Fig. 5 Comparison of herbivory between block and branching artificial reefs. Herbivory determined by number of bites min⁻¹ m⁻². Data were collected in 20 min observational surveys (n = 5). Error bars show + SD. Asterisk denotes a statistically significant difference (t-test, $\alpha = 0.05$)

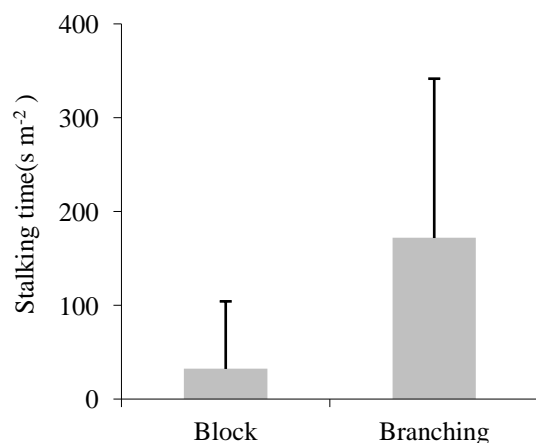


Fig. 6 Comparison of predation between block and branching artificial reefs. Predation determined by time spent stalking at the artificial reef (s m⁻²). Data were collected in 20 min observational surveys (n = 5). Error bars show + SD

Herbivory/ Predation Pressure at Increasing Distance

A total of 20 different species were recorded feeding at the artificial reef site from 9 families. At the control site, 14 different species from 8 different families fed from the fish and algae snares. Most species observed to be feeding on the fish at both the artificial reef site and the control site were species

known to be herbivorous (individuals from Scaridae and Acanthuridae families). Most species of Scaridae were observed to feed primarily on the fish but rarely on the algae.

Omnivores (Pomacanthidae, Chaetodontidae, Labridae, and Pomacentridae), carnivores (Labridae and Bothidae) and piscivores (Haemulidae and Serranidae) were also observed taking bites from the fish. Algae at the artificial reef site was eaten primarily by herbivores with a few instances by omnivores (Pomacanthidae and Labridae). At the control site, most algae was eaten by herbivores. Several omnivores were also observed feeding on the algae (Chaetodontidae and Pomacentridae).

Of the fish feeding on the fish and algae snares, only one french grunt and several initial phase princess parrotfish were observed coming directly from the artificial reef. Only the french grunt (*Haemulon flavolineatum*) was observed to consistently come from and return to the artificial reef. All other individuals came from other surrounding areas. At the control site, several princess parrotfish, yellowhead wrasses (*Halichoeres garnoti*), redband parrotfish (*Sparisoma aurofrenatum*), and a coney (*Cephalopholis fulva*) were observed to come to feed from the fish or algae snares from the reef crest. Two coneys feeding from the fish snares were observed coming from a nearby mooring block several times. Many ocean surgeonfish (*Acanthurus bahianus*), french angelfish (*Pomacanthus paru*), princess parrotfish, and redband parrotfish were observed coming to feed at the control site from the surrounding sandy areas.

At the artificial reef site, the largest mean percentages of algae remaining (\pm SD) was found at 0 m ($52.00 \pm 38.34\%$) and 4 m ($52.00 \pm 47.64\%$) with the least percent remaining found at 2 m ($44.00 \pm 43.93\%$), however no statistical difference was found between percentage of algae remaining between distances (ANOVA; $F = 0.04$, $df = 20, 4$, $p = 0.9$) and there was no significant pattern in the percentage of remaining algae

as distance increased ($r = 0.00$, $p = 1.0$; Fig 7).

Differences between percentages of algae remaining at distances at the control site were found, with most algae remaining at 2 m (58.00 ± 23.63 %) and smallest percentages of algae remaining at 0 m (28.00 ± 0.33 %), although no statistical significance was found (ANOVA; $F = 0.61$, $df = 20, 4$, $p = 0.6$). Like the artificial reef site, there was no pattern among percentages of algae remaining and increasing distances ($r = 0.00$, $p = 1.0$; Fig 7).

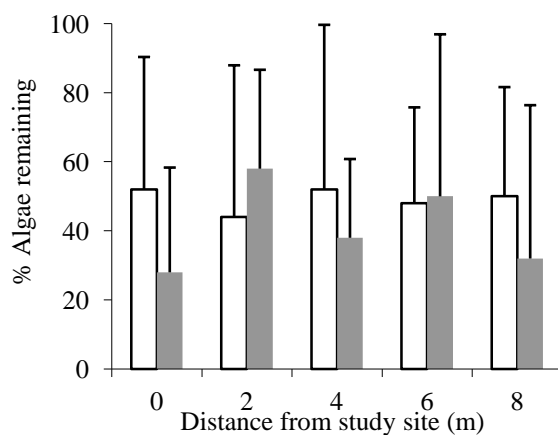


Fig. 7 Comparison of mean herbivory pressure between an artificial reef site and a control site. Herbivory was determined by the percent of algae remaining after being left out for 20 min at increasing distances from study site. Error bars show + SD, white bars denote artificial reef site, and gray bars denote control site

Greatest mean percentages of fish remaining (\pm SD) at the artificial reef were at 6 m (9.60 ± 16.47 %) with the lowest percentage of remaining fish at 2 m (0.00 ± 0.00 %). Differences between percentages of fish remaining at distances away from the artificial reef showed no statistical differences (ANOVA; $F = 0.96$, $df = 20, 4$, $p = 0.4$; Fig. 8) and there was no statistical trend between distances and remaining fish percentages at the artificial reef site ($r = 0.275$, $p = 0.6$; Fig 8).

There was a significant positive correlation at the control site between the percent of fish remaining with increasing distances ($r = 0.924$, $p = 0.025$) with the largest remaining percentage of fish found at

8 m (17.60 ± 25.54 %) and the smallest percent remaining found at 0 m (0.00 ± 0.00 %). However no statistical significance was found between percentage of remaining fish and different distances (ANOVA; $F = 1.16$, $df = 20, 4$, $p = 0.3$; Fig. 8).

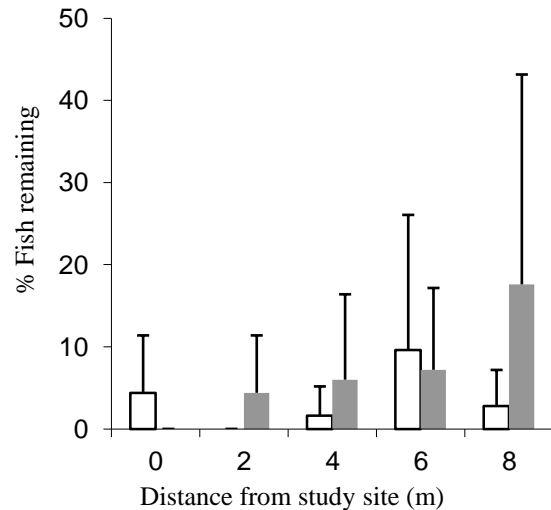


Fig. 8 Comparison of predation pressure between an artificial reef site and a control site. Predation was determined by the percent of fish remaining after being left out for 20 min at increasing distances. Error bars show + SD, white bars denote artificial reef site, and gray bars denote control site.

Discussion

It was hypothesized that there would be increased herbivory at the branching artificial reef compared to the block-style reef. Significantly more bites were taken at the branching artificial reef surface than at the block surface, supporting the hypothesis. In former studies, increased habitat complexity of artificial reefs has increased fish biomass and species richness. Since food was not a limiting resource in the study, the increase was attributed to the increase in shelter areas at the reef (Charbonnel et al. 2002). These results apply to the current study because amount and type of algae growing on both structures appeared to be the same and both sites were comparable in location away from the reef crest and other structures. The surface area of each reef was also taken into consideration when calculating herbivore activity, thereby removing the possibility that the increased

area at the branching reef accumulated more algae to graze upon. The added complexity of the branching artificial reef, which offers more cover for grazing herbivores to shelter from predators, could be the reason for the increased herbivory at the branching reef.

This study focused only on larger herbivores which could not seek shelter within the holes of the block. An analysis of smaller grazers at both reefs may show different outcomes. Because there was no shelter at the block for the larger herbivores, the presence of observers during surveys may have affected feeding during the survey time. However, even after observers had moved much farther away after surveys were completed, no large change in the amounts of herbivores feeding at the block was observed.

Predator presence was higher at the branching artificial reef, refuting the hypothesis that predators would spend more time stalking at the block artificial reef. The higher predator stalking presence at the more structurally complex reef does not agree with previous findings that predator abundance was lower on more complex structures (Almany 2004). This may be due to the fact that large predators were not found at either artificial reef and the smaller predators such as small grunts and trumpetfish (*Aulostomus maculatus*) that were observed during this study were small enough to fit into spaces created by the branching structure and may have been using the artificial reef as a shelter from larger predators. The predators were never observed preying upon the herbivores at the artificial reefs, suggesting that the predators attracted to the reefs are not stalking the herbivores studied but rather smaller organisms that may be sheltering in the reefs. Gobies (*Gobiidae* spp.) and Sharpnose puffers (*Canthigaster rostrata*) are commonly found at both artificial reefs and there may be more small organisms in the branching structure because of the increased sheltering areas provided compared to the block reef. Small predators able to swim into the branching reef structure may be able to prey upon the small

organisms sheltering in the reef and therefore increase their foraging efforts at the branching reef while simultaneously utilizing the habitat for shelter themselves.

Because of the increasing sheltering areas at the artificial reefs compared to the surrounding sand flats, it was expected that fish would not travel increasing distances away from the reef site to forage, causing more predation and herbivory pressure near the reef than at increasing distances away. There was no significant difference between the feeding pressures at increasing distances away from the artificial reef site as has been shown in other studies (Bortone et al. 1988; Kurz, 1995; Einbinder et al. 2006). The hypothesis that herbivory and predation pressures would be lower at increasing distances away from the artificial reef can neither be supported nor refuted.

At the control site there were significantly higher percentages of fish remaining at increasing distances from the reef crest. The lack of a relationship at the artificial reef between predation and herbivory pressure at increasing distances could be due to the fact that many of the fish coming to take bites from the fish and algae snares were transient fish coming from other fish aggregates (such as mooring blocks and other patches of the branching reef) or were coming from surrounding sand flats. The artificial reef is also very close to the reef crest, giving a further origin of foraging fish. The control site, however, is separated from other aggregates. Fish feeding on the algae and fish at this site were coming either from the reef crest (approximately 6 m away from the study site), mooring blocks (approximately 5 m away from the study site), or were transient species coming from the surrounding sand flats. Two coney were observed coming from the nearest mooring block to feed on fish several times, however they swam back to the mooring block very quickly after taking bites from the fish, suggesting that they were using the mooring blocks for shelter. Similarly, several princess parrotfish and a coney were observed coming from the reef crest to take bites of

fish and then returning to the reef crest after feeding.

The shelter area created by the natural reef and other structures at the control site was separated from the fish and algae snares by several meters of sand and rubble, while the snares were directly adjacent to the branching artificial reef structure and surrounded by several other structures offering shelter to fish. The correlation between increased feeding pressure at distances closer to the reef crest at the control site could have been caused by the larger distance between shelter areas and the fish and algae snares at the control site.

The fact that there was no trend in predation or herbivory pressure at the artificial reef does not necessarily indicate that the reef is not being utilized by fish. In fact, several fish, including princess parrotfish and a french grunt, were observed to come from the artificial reef to feed on the fish and algae snares. The french grunt was only ever observed to go as far as the 4 m mark to take bites from the fish snares. After bites were taken, the french grunt would return to the artificial reef. The princess parrotfish did not seem to have as restricted of a foraging range and were observed taking bites as far away as 8 m. This behavior indicates the branching artificial reef is likely being utilized as a shelter habitat. The reef structure may be acting as a shelter breaking up the sand area and the reef crest, allowing fish to forage farther distances away from the reef crest than normal. If distances had been increased away from the artificial reef, it is possible that there would have been a trend in the data, as seen at the control site.

Future studies should continue to study predation and herbivory pressure away from the artificial reef. Increasing distances should be investigated to determine if there is a trend in predation and herbivory pressure away from the artificial reef and the other nearby fish aggregates or if most of the pressure comes from transient individuals coming from the surrounding sand flats. The method used to observe predation and herbivory pressure may have affected the

fish coming to feed at the sites as well. Methods that do not risk scaring away fish such as snorkeling or observing from farther away may change the species feeding away from the reef. Studies should be continued to monitor if the artificial reefs in Bonaire become used increasingly for resources by reef fish.

The results from this study suggest that increasing surface areas of artificial reef, thereby adding structural complexity and availability of food and shelter, may increase fish attraction to the reef. In addition, artificial reefs may serve as effective tools in increasing foraging ranges of reef fish. While it has been suggested that artificial reefs are placed distances far away from natural reefs (Bohnsack and Sutherland 1985; Herrera 2002), placing artificial reefs near the reef crests in areas where natural reefs were previously found, such as the formerly existing *Acropora* thickets in Bonaire, may increase food and shelter habitats for reef-dwelling fish.

This study also shows the importance of artificial reefs for transient species. Previous studies have tried to measure the efficiency of artificial reefs based on the number of resident individuals (Bohnsack and Sutherland 1985; Bohnsack 1989; Szedlemayer and Shipp 1994; Grossman et al. 1997; Charbonnel et al. 2002). During this study, many transient individuals were observed to come to the artificial reefs to feed and forage. Some of these transient individuals likely use the artificial reefs as shelter while feeding and these structures may be important for reef-dwellers which do not utilize the artificial reef as a permanent habitat. Effectiveness of artificial reefs should therefore not be judged only on the number of residents utilizing the reef, but also the effective utilization by transient fish as well.

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Brood location preference and paternal care behavior by sergeant majors (*Abudefduf saxatilis*)

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Abstract

Sergeant majors (*Abudefduf saxatilis*) engage in male-dominated reproduction: males establish territories, females are courted, mate with the male and then depart – leaving protection of the eggs to the male. *A. saxatilis* prefer smooth, artificial substrate for egg laying; in Bonaire, Dutch Caribbean, they tend to mate on large, concrete mooring blocks which have surfaces of varying degrees of exposure to the water column. Preferred nesting locations were expected to be those offering the least exposure, as broods would be shielded from predators and guarding males would expend minimal energy. The number of threats, chases and feeds in a 5 min interval were recorded as an indicator of energy expenditure and consumption. The length of the fish and size of corresponding egg brood were measured as indicators of mating potential. The number of threats, chases and feeds did not differ significantly between degrees of exposure, but threats and chases differed between sites, being greatest at the site closest to the reef, possibly indicating that exposure does not play a role in energy expenditure and therefore may not affect preferred mating locations. However, larger fish and larger broods were observed on the most protected sides of the mooring blocks offering some support to the notion that *A. saxatilis* prefer to nest in more protected locations. An increase in the number of artificial structures could lead to an *A. saxatilis* population rise.

Introduction

Paternal care is rare in the animal kingdom. Typically, it is thought that males giving care to eggs or young decrease their opportunities to mate (Kvarnemo 2005). However, paternal care of eggs has been shown to be common in some groups of animals such as birds, insects and fish (Kvarnemo 2005). In some species, paternal care actually increases the males' mating opportunity and overall fitness level (Gross and Sargent 1985; Kvarnemo 2005). The damselfish, *Abudefduf* spp., are some of the fish that utilize paternal care as a mating strategy (Fishelson 1970; Foster 1987; Manica 2010). *Abudefduf* spp. are found around the world, however mating cycles vary with location (Fishelson 1970; Foster 1987; Allen 1991;). For example, while *A. vaigiensis* in the Red Sea (originally misreported as *A. saxatilis*) have a restricted mating season, *A. troschelii* in the Pacific Ocean (originally misreported as *A. saxatilis*) follow a lunar mating pattern and *A. saxatilis* in the Caribbean have

random, year-round mating that follows no set pattern (Fishelson 1970; Foster 1987; Allen 1991). Regardless of differences in mating periodicity, throughout the genus, males establish territories and protect egg broods without males (Fishelson 1970; Foster 1987; Allen 1991; Manica 2010 Harper 2011).

Reproduction in *Abudefduf* spp. is male dominated (Fishelson 1970). A reproductive school is led by a few males (which turn dark purple) in search of an attractive nesting area (Fishelson 1970). Males break away from the school and establish individual territories with a preference for uncovered surfaces along vertical walls and smooth, artificial surfaces (Fishelson 1970; Itzkowitz et. al 2001; Harper 2011). Once the males establish territories, females are courted. High value males mate with multiple females over a 5-6 h spawning period (Fishelson 1970). Females mate with a single male and leave the site while the male stays to protect the

eggs until they hatch 4 days later (Fishelson 1970).

Protection of an egg brood requires energy expenditure. Males must threaten, chase, or even bite other fish in order to prevent them from preying upon their eggs (Fishelson 1970). Male *A. sexfasciatus* sometimes engage in filial cannibalism due to the high energetic cost of protection (Manica 2004). An egg brood that is too large and too energetically taxing to defend is reduced in size by consumption of some of the eggs (Manica 2004). Males that have more access to food engage in less filial cannibalism, indicating that energy expenditure is the driving force behind the consumption, not brood size (Manica 2004). This would imply that sheltered surfaces offering the most protection from predators and areas offering more feeding opportunities would offer the best nesting sites for *Abudefduf* spp.

In Bonaire, Dutch Caribbean, sergeant majors (*Abudefduf saxatilis*) are abundant. In order to prevent boats from anchoring on the coral reef surrounding the island, Bonaire has installed mooring blocks. These concrete blocks, at depth between 5-6 m, offer a homogeneous nesting place for *A. saxatilis*. Within each set of blocks there are different degrees of exposure to the water column. Using aggressive interactions and feeding capability as an indicator of energy expenditure, locations on the mooring blocks were examined with the aim of establishing the preferred nesting sites for *A. saxatilis*.

H₁: *A. saxatilis* expend less energy on brood protection on more protected, less exposed surfaces of the mooring block.

The order of protection from least to most is: (1) unexposed vertical wall, (2) partially exposed vertical wall, (3) exposed vertical wall, (4) exposed horizontal wall.

Male fish size could also play a role in sexual selection. Larger male fish have been shown in a variety of species to be stronger competitors, provide better care to offspring and be more fertile (Darwin 1871; Côte and Haute 1989; Kolm 2002). Other

studies have shown that females preferentially choose larger males to mate with (Kolm 2001; Itzkowitz et al. 2001; Young et al. 2010). A study on *A. sexfasciatus* showed that certain, large females would lay “test” eggs for a male to defend (Manica 2010). If the males demonstrated that they were capable protectors, the female would then mate with that male and lay a full brood (Manica 2010). Brood size can also be an indicator of female reproductive capability and mate choice (Kolm 2001; Manica 2010). Using male fish size as an indicator of female sexual selection and brood size as an indicator of fertility, a relationship sexual selection and fertility was explored.

H₂: Larger males protect larger egg broods

If females display a preference for larger males by laying more eggs (larger brood) then a relationship could be established between larger males, brood size, and preferential nesting site.

H₃: Larger males guard broods on the most protected nesting locations on the mooring blocks

The mooring blocks are just one of many artificial surfaces on Bonaire’s reefs for *A. saxatilis* to lay eggs on. They have been seen protecting broods on cement blocks, pipes, oil drums, and even wooden planks (M Mossler personal observation). The effect of artificial structures on the reproductive capabilities of these fish is relatively unknown. This study could show whether an artificial surface that offers more protection could lead to an increase in mating potential. An increase in the number of artificial structures, whether purposeful (such as the mooring blocks) or not (e.g. dumping) could shift the mating potential in *A. saxatilis*.

Materials and Methods

Study Site

Data were recorded at the Yellow Submarine dive location on Bonaire, Dutch Caribbean, (Fig. 1) (12°09’36.39 N 68°16’54.84 W).

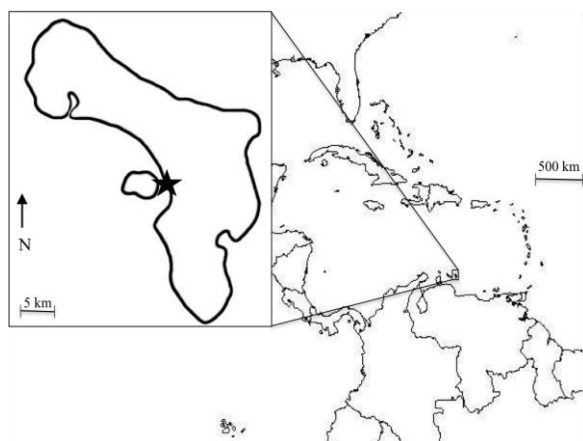


Fig. 1 Study site: Yellow Submarine, Bonaire, Dutch Caribbean. *Black star* indicates study site (12°09'36.39"N, 68°16'54.84"W)

The location offered eleven sites of large (3-part) mooring blocks to study. Three sites with similar degrees of exposure were chosen to study (Table 1). All sites were composed of three 1 x 1 x 1 m cement blocks connected by steel cable and rope at a depth of 5-6 m. Site 1 was ~1 m from the reef crest; Site 2 was ~8 m from the reef crest; Site 3 was ~5 m from the reef crest.

Table 1 Study Sites. Surfaces offered by varying degrees of exposure

Degree of exposure:	1	2	3	4
Site 1	3	6	3	3
Site 2	1	2	6	3
Site 3	2	4	5	3

Data Collection

Over a period of 4 weeks in March 2012, male *A. saxatilis* and their corresponding egg broods were studied. Males were watched until an individual rubbed his underside on an egg brood (cleaning) – it could then be established that those eggs belonged to that particular male. Once a male had been established as the protector of a brood, the numbers of aggressive interactions by the fish were recorded in a 5 min time span. Digital photographs were taken against a scale and the size of the egg brood and

length of the male fish were estimated using Image J (Collins 2007).

Using SCUBA, divers visited each site three times per week to record data. The spawning periods of each site varied; close attention was paid to each site so as not to record data on the same fish during the same spawning period.

Aggressive interactions were categorized as threatening, chasing, and biting (as per Fishelson 1970). Threatening was defined as a male swimming the fish swimming quickly towards another fish and turning sharply before exiting his egg brood boundary. Chasing involves the fish chasing an intruder past the egg brood border. Biting is a more extreme form of energy expenditure and was not seen (Fishelson 1970). The number of feeds (defined as the fish entering the water column and quickly thrusting his mouth outward and then inward) by each male in the 5 min time span were counted as well starting in week 2. Data were collected between 1330 hrs and 1530 hrs to standardize relative fish activity.

Data Analysis

Two way ANOVAs were performed with site and degree of exposure as factors to test for differences in the number of threats, chases, and feeds, and in the size of fish and broods. Tukey's HSD post hoc tests were used to further explore differences in means. A relationship between fish size and the natural log of brood size was explored using a linear regression.

Results

A total of 71 male fish were studied; there were 34, 26, and 11 at Site 1, 2 and 3; and 8, 26, 23 and 14 at degrees of exposure 1, 2, 3 and 4, respectively. However, none were recorded at Site 3 with degree of exposure 4. Total number of feed recordings was 54. A total of 60 fish lengths were measured and 63 brood sizes were recorded.

There were significant differences in the mean number of threats and chases

Table 2 ANOVA results. Threats, chases, feeds, fish size and brood size as variables; site and exposure as levels. Asterisk indicates significance

Variable	Site			Exposure		
	df	F	p	df	F	p
Threats	2,60	14.02	*	2,60	0.38	n.s.
Chases	2,60	3.62	*	2,60	1.54	n.s.
Feeds	2,44	0.69	n.s.	2,44	0.20	n.s.
Fish Size	2,50	4.64	*	2,50	5.60	*
Brood Size	2,53	2.80	n.s.	2,53	4.87	*

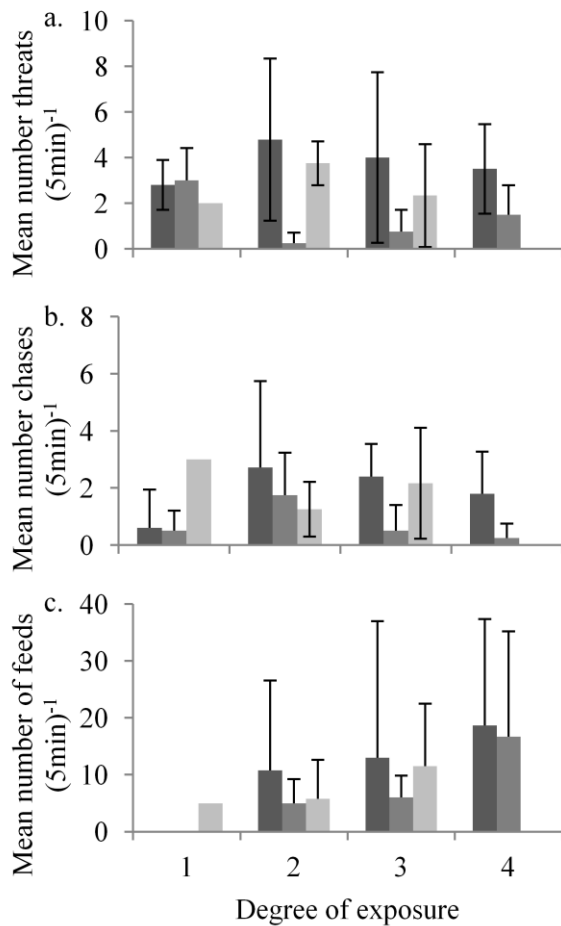


Fig. 2 Mean number of threats (a), chases (b) and feeds (c) 5 min⁻¹. Dark gray indicates Site 1, gray indicates Site 2, light gray indicates Site 3. Error bars indicate ± SD

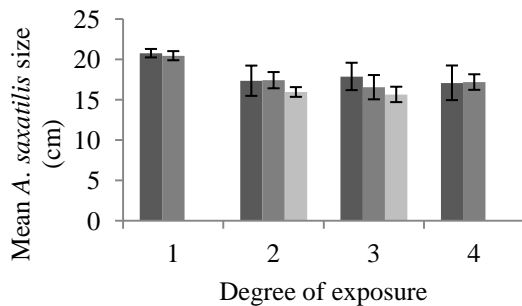


Fig. 3 Mean *A. saxatilis* size (TL cm). Dark gray indicates Site 1, gray indicates Site 2, and light gray indicates Site 3. Error bars indicate ± SD

between sites, but not between degree of exposure (Table 2 and Fig. 2).

Post hoc tests showed that there were significantly more threats and chases at Site 1 than Site 2, but no other differences between sites. There was no significant difference in mean feeds between sites or degree of exposure (Fig. 2). *A. saxatilis* size differed between both site and degree of exposure (Fig. 3)

Post hoc tests showed that *A. saxatilis* size was significantly bigger at Site 1 than Site 3, but no other differences between sites. *A. saxatilis* size was significantly bigger at degree of exposure 1 than all other degrees of exposure, but no other differences were found between *A. saxatilis* size and other degrees of exposure. Brood size was not significantly different between sites, but was between degree of exposure (Fig. 4). Post hoc tests showed that brood size was significantly larger on degree of exposure 1 than all other degrees of exposure, but no other differences were found between brood size and degree of exposure.

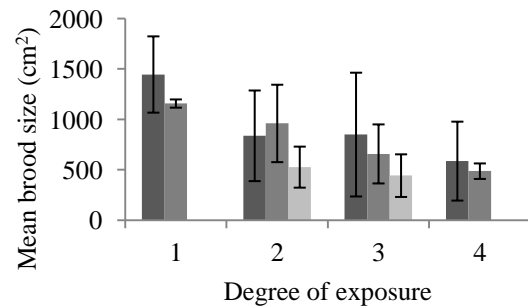


Fig. 4 Mean brood size (cm²). Dark gray indicates Site 1, gray indicates Site 2, light gray indicates Site 3. Error bars indicate ± SD

There was a significant positive linear relationship between *A. saxatilis* size and brood size ($R^2 = 0.603$, $df = 1,58$, $F = 88.13$, $p < 0.05$; Fig. 5).

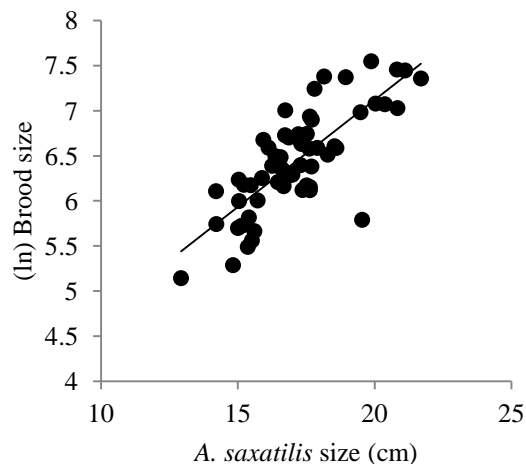


Fig. 5 Natural log of brood size and size of *A. saxatilis*. Gray line shows linear regression ($R^2 = 0.603$, $df = 1,58$, $F = 88.13$, $p < 0.05$)

Discussion

The hypothesis that *A. saxatilis* would expend less energy on mooring block surfaces with varying degrees of exposure was not supported by this study. However, for threats and chases, there was a significant difference between sites. This could be due to the distance between site and reef crest. Aggressive interactions were significantly higher at Site 1, which was the closest study site to the reef crest. The proximity to the reef crest possibly increases the amount of activity around Site 1, thus increasing opportunities for fish to prey on *A. saxatilis* eggs. There could be a multitude of factors as to why the counted number of feeds did not show any kind of relationship. The most likely one is that, because feeds were only counted starting in week 2, there were not enough data to make a conclusion. Though the times of data collection were standardized, the feeding schedules of *A. saxatilis* are unknown and could have varied during the 3-h data collection period. *A. saxatilis* feed on plankton, hence plankton density could have affected the mean number of feeds.

The relationship between fish size and egg brood size showed a significant, positive trend, supporting the hypothesis that larger fish protect larger broods. These data indicate that larger males have a higher

mating potential as they are able to acquire more mates and fertilize more eggs.

Although paternal defense of egg broods is thought to limit reproductive success of males, in other animals this has been shown to be offset by defending overlapping broods using an evolutionary model. The model demonstrates that males protecting a brood of eggs can, over a single spawning period, simply collect more eggs from different females and maintain their territory (Manica and Johnstone 2004). Researchers observed this behavior during five spawning periods witnessed at the different sites. Once males were finished mating with a female, they would chase the female off and begin courting another female. The new female would then lay her eggs next to, around, or on top of eggs that the male was already protecting. In a population of *A. sexfasciatus*, females were found to prefer mating with males guarding a preexisting egg brood (Manica 2010). During the spawning period, *A. saxatilis* eggs are bright purple, but over time the eggs fade to a deeper color, making it possible to tell the difference between female eggs during the spawning period as some are brighter than others, dependent on the time they were laid. By the next day, however, all the eggs appear the same color and it appears that the male is guarding a single brood. The observations of overlapping broods support the notion that *A. saxatilis* evolved this mating strategy to mitigate the energetic cost of brood protection (Manica and Johnstone 2004).

Additionally, it was shown that egg brood size and *A. saxatilis* size differed significantly between degrees of exposure. The most protected surface (degree 1) had larger egg broods and larger males than relatively exposed surfaces. The positive relationship between fish size and brood size, coupled with the relationship between brood size and degree of exposure, and *A. saxatilis* size and degree of exposure, lend support to the final hypothesis that larger males establish larger broods on the more protected surfaces.

However, it is evident that at Yellow Submarine dive site, the majority of *A. saxatilis* mating occurs on the mooring blocks; there are no other places in the water column or on the reef where such a large concentration of *A. saxatilis* guarding egg broods can be seen (M Mossler personal observation). Many other studies have also recognized artificial surfaces as the preferred sites for damselfish and *Abudefduf* spp. mating (Fishelson 1970; Itzkowitz et al. 2001; Harper 2011). An increase in artificial structures along Bonaire's coasts would give *A. saxatilis* more preferred nesting locations and could contribute to a population rise. Whether a population rise would be beneficial or detrimental to overall ecological health remains to be seen. Competing species that do not benefit from the artificial structures could be disadvantaged and replaced by larger numbers of *A. saxatilis*. On the other hand, an increase in numbers could provide more food for predators or improve water clarity because they are planktivores. Regardless, the mooring blocks do a great deal to prevent damage to the reef; if more need to be installed and a detrimental *A. saxatilis* increase is imminent, perhaps underground moorings would be the better option.

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Incidence of disease in *Acanthurus bahianus* population, Bonaire, Dutch Caribbean

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Abstract

Disease in the ocean is difficult to study because of the logistics involved in conducting marine research. This in turn has resulted in a lack of recognition when outbreaks do occur. Most diseases do not manifest themselves in an organism unless the individual is subject to stress that weakens its ability to fight disease. In recent years, anthropogenic stressors have increased in the world's oceans; something thought to be increasing the incidence of disease. Recently, ocean surgeonfish, *Acanthurus bahianus*, in Bonaire, Curacao, and Turks and Caicos, have been observed with black spots on their bodies. There has not been any research on the subject and the causative agent has not been identified. The purpose of this study was to develop a basic understanding of the ocean surgeonfish with black spots. Using timed swims and observations, data on frequency of disease at depths, flash rate, bite rate, and percentage of time spent feeding were collected. There was a significant difference in the frequency of individuals across depth. In addition, there was a positive correlation between number of spots and percent of time spent feeding. However, there was no relationship found between number of spots and feeding rate and flash rate. This disease is affecting 89% of ocean surgeonfish. The implications of this disease are important to understand because ocean surgeonfish play a strong ecological role as herbivores in coral reef ecosystem.

Introduction

Over the past 30 years, diseases have increased throughout the world's oceans (Harvell et al. 1999). However, disease in the oceans is difficult to study because of the logistics involved in conducting marine research (Vethaak and Rheinallt 1992; Harvell et al. 2004). Most study sites cannot be reached without equipment such as a vessel (McVicar 1986), and outbreaks of disease are not usually recognized readily as little is known about their origin (Harvell et al. 2004). The information is difficult to compare historically because there have been a lack of baseline data, making it difficult to quantify the increases in disease that are reported (Harvell et al. 2004).

Pathogens spread more quickly in marine habitats than on land (Harvell et al. 2004). Diseases can survive for long periods of time in the ocean without host organisms and unlike on land, there are few barriers to dispersal (Harvell et al. 2004). In addition, pathogens such as spore-forming bacteria and fungi can remain dormant in

sediments for thousands of years and once disturbed, resuspend into the water column (Heidelberg et al. 2002). It is not uncommon for a single pathogen to infect organisms from multiple species and even genera, such as black band disease and white plague disease in corals, broadening potential impact of marine diseases (Green and Bruckner 2000). These characteristics allow marine diseases to travel great distances and infect multiple species over wide geographic areas (Harvell et al. 2004).

Disease can have drastic effects on the community structure of an ecosystem. In the Caribbean, the *Diadema antillarum* die-off (Lessios et al. 1984), *Acropora palmata* disease (Aronson and Precht 2001) and coral bleaching (Bruno et al. 2009) have all lead to a decrease in coral cover. Causes of these diseases include eutrophication (Shotts et al. 1972; Vethaak and Rheinallt 1992), chemical pollutants either as heavy metals such as copper and zinc or organic micropollutants in the form of PCBs and PAHs (Pippy and Hare 1969; Vethaak and Rheinallt 1992), aquaculture effluent and ship ballast water

discharge by directly introducing new pathogens (Harvell et al. 2004), thermal pollution (Meyer 1970; Roth 1972; Snieszko 1974; Walters and Plumb 1980; Vethaak and Rheinallt 1992), turbidity due to runoff from storm waters (Harvell et al. 1999), sewage discharges in the form of nutrients, sediments, and toxic substances that cause harmful algal blooms (Pastorok and Bilyard 1985), algal toxins (Vethaak and Rheinallt 1992), low dissolved oxygen (Snieszko 1974; Walters and Plumb 1980; Vethaak and Rheinallt 1992), habitat destruction and the pressure of fisheries (Vethaak and Rheinallt 1992).

So far, the interest in fish disease has been centered on fish farms (Harvell et al. 2004). As many of the wild fisheries are being or have already been depleted, there has been an increase in aquaculture production (Muir 2005). The fish in aquaculture are generally raised in high densities with poor water quality, factors that increase susceptibility to disease (Rottmann et al. 1992). Combating disease is an economic necessity for aquaculture and thus well studied in this context (Muir 2005). There are many detailed books that list and describe the known pathogens that affect both freshwater and saltwater aquaculture fish species (Austin and Austin 2007; Noga 2010; Plumb and Hanson 2010), but there has been limited research in the wild.

In most cases, pathogens colonize an already weakened host, causing the outbreak of disease (Austin and Austin 2007). Factors such as increasing water temperatures and the ability for a pathogen to survive long periods without a host increases the susceptibility of species to infection (Harvell et al. 2004). Additionally, fish in aquaculture conditions usually live in water with low dissolved oxygen, increased carbon dioxide, nitrate, ammonia, and organic matter (Rottmann et al. 1992). These factors all contribute to weaken or stress the host organism, giving an opportunistic pathogen the chance to colonize (Austin and Austin 2007).

Stress can reduce the ability of the species to perform specific activities (Beitinger and McCauley 1990), and require an increase in energy to maintain body functions (Rottmann et al. 1992). An activity exhibited by stressed fish is flashing (Li et al. 2002). Flashing is when a fish rubs their side against a solid object to itch (Klinger and Floyd 2009), and can be an indicator of poor water quality (Li et al. 2002). Another behavior related to disease and stress is visits to cleaning stations, where individuals with skin infestations visit cleaning stations more frequently (Reinthal and Lewis 1986).

On coral reefs, around 15-25% of fish biomass is represented by herbivores in the Scaridae, Acanthuridae, and Pomacentridae families (Bakus 1964). These fish are ecologically important species on tropical reefs (Lewis 1986), and have a large impact in structuring benthic communities of coral reefs by limiting the distribution, abundance and production of algae (Paddock et al. 2006).

In Bonaire, Curacao (Carmabi 2012) and Turks and Caicos (pers. comms. J. Claydon), ocean surgeonfish (*Acanthurus bahianus*) have been seen with black or dark grey spots on their bodies. It has been suggested that these spots are the result of a disease, hereafter referred to as black spot disease (Carmabi 2012), but the pathogen responsible has not been identified and nothing is currently known of the disease's effect on the host.

The purpose of this research was to develop an understanding of the prevalence of black spot disease in *A. bahianus* within the total population and at different depths. Ocean surgeonfish are important herbivores and mortalities because of disease could lead to increased algal cover and decreased coral cover. There is no literature that describes these spots or provides any information about possible causes or sources. Therefore, the results of this research will begin to provide an understanding of the prevalence and effect of this disease in ocean surgeonfish.

It has been shown that disease spreads more quickly in warmer water (Boyett et al. 2007). In Bonaire, the temperature between 3 and 18.3 m differed from between 0.1 to 1 °C over a period of 42 days (Jones et al. 2008). Therefore it was hypothesized that:

H₁: Prevalence of black spot disease decreases with increasing depth.

In Bonaire, ocean surgeonfish with black spot disease have been observed flashing. Because fish flash in response to disease (Klinger and Floyd 2009), it was hypothesized that:

H₂: Individuals with black spot disease will flash more frequently than healthy individuals.

If the number of spots on an ocean surgeonfish is an indicator of health, where an individual with more spots is more stressed, then individuals with a greater number of spots will have decreased energy. Therefore they will need to increase energy intake to maintain body functions (Rottmann et al. 1992), and it was hypothesized that:

H₃: Individuals with black spot disease will feed more frequently than healthy individuals.

Materials and methods

Study site

All research was conducted at Yellow Submarine dive site, Bonaire, Dutch Caribbean (Fig. 1). The western coastline is characterized by fringing reefs. The study was conducted between February and April, 2012.

Study Species

Acanthurus spp. are frequently found in shallow reef habitats in the tropical North West Atlantic and are one of the most common herbivorous benthic-feeding species (Robertson et al. 2005). *A. bahianus* often swim in mixed-species schools with *A. coeruleus* and *A. chirurgus*. However, 31% of *A. bahianus* individuals have been shown

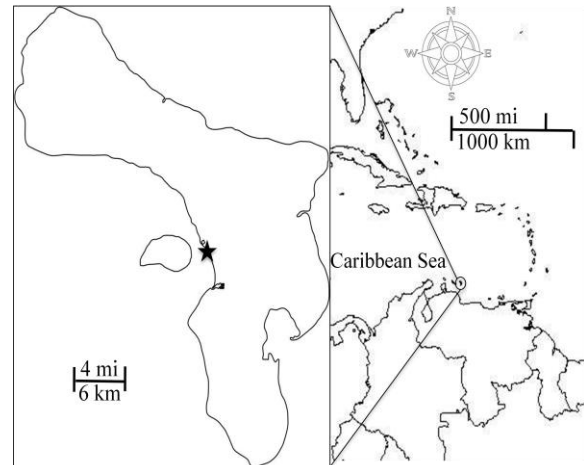


Fig. 1 Map of Bonaire, Dutch Caribbean. *Black star* indicates location of study site, Yellow Submarine (N 12° 09' 36.5" W 068° 16' 54.9")

to exhibit solitary behavior (Reinthal and Lewis 1986). *A. bahianus* also shows a significantly greater density of individuals on the back reef in comparison to the reef crest or spur and groove zones (Lawson et al. 1998), and Reinthal and Lewis (1986) found that 89% of solitary bites of surgeonfish were in the sand and rubble grooves while 72% of bites of schooling fish were on the sides and tops of coral spurs.

Prevalence of disease and depth

Research was conducted using SCUBA. The proportion of individuals with black spot disease was estimated by counting all diseased and healthy *A. bahianus* during 15 min timed swims at depths of 2, 5, 11, and 18 m. Nutrient levels remained unchanged between depths where nitrate was < 0.25 ppm, and nitrite < 0.05 ppm (S. Penn, unpublished results). Percent of diseased individuals was calculated for each depth and frequency of diseased individuals at each depth was analyzed using a χ^2 test.

Behavior

Individual *A. bahianus* were observed *in situ* and recorded on video for 10 min, documenting the number of bites, flashes, and visits to cleaning stations, along with the

number of spots on each individual. Time spent swimming, defined as time that an individual does not bite the substratum for more than 5 s, and time spent feeding, calculated by subtracting feeding time, and was measured for each individual observed from the video footage. Over ten minutes, an individual with black spot disease may take the same number of bites as an individual without black spot disease, but spend more time engaged in feeding behavior. A Pearson's correlation was used to analyze the relation between number of spots and flash rate, number of spots and bite rate, and number of spots and proportion of time spent feeding.

Results

A total of 152 individuals were observed during 630 min of observation. Diseased *A. bahianus* were observed at all depths studied. The number of spots on diseased fish ranged from 0 to over 20.

Prevalence of disease and depth

The proportion of individuals displaying disease ranged from 93.14% at 2 m to 72.41% at 18 m (Fig. 2). There was a significant difference in the frequency of diseased and non-diseased fish found across depths ($\chi^2 = 13.44$, $df = 3$, $p < 0.05$). The largest number of fish was observed at 2 m and the least at 18 m. Results suggest that there are less diseased individuals at depth.

Behavior

A total of 51 fish were observed at 5 m over a total 510 min of observation. There was no significant association between number of dark spots and bite rate ($R = -0.077$, $n = 51$, $p > 0.05$), or flash rate ($R = -0.212$, $n = 51$, $p > 0.05$). Bite rate ranged from 22.9 to 76.8 bites min^{-1} (Fig. 3a), where 22.9 bites min^{-1} was observed by an individual with greater than 20 dark spots. Flash rate ranged from 0 flashes min^{-1} to 0.9 flashes min^{-1} (Fig. 3b). A significant correlation was found between

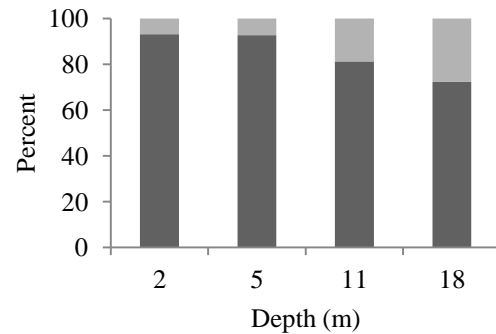


Fig. 2 Proportion of diseased and healthy *A. bahianus* at Yellow Submarine dive site in Bonaire, Dutch Caribbean. Dark gray indicates diseased individuals and light gray indicates healthy individuals. Data were collected during 15 min timed swims at 2, 5, 11, and 18 m depth ($n = 4$ at each depth)

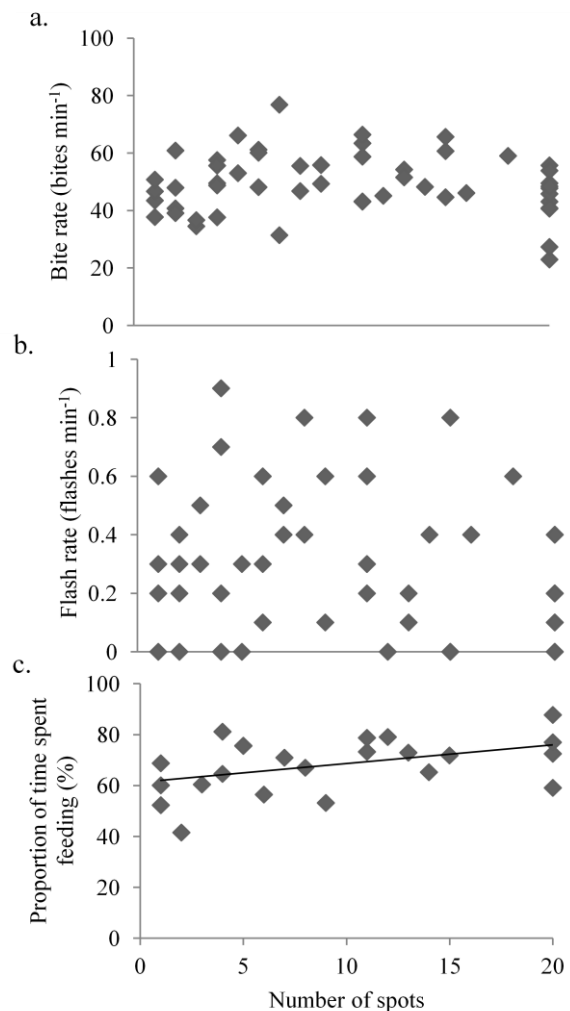


Fig. 3 Number of spots on *A. bahianus* and **a.** Bite rate (bites min^{-1} ; $n = 51$), **b.** flash rate (flashes min^{-1} ; $n = 51$), and **c.** frequency of time spent feeding (%; $n = 23$). Gray line shows linear regression. All data were collected during 10 min observations at Yellow Submarine dive site in Bonaire, Dutch Caribbean

feeding time and number of spots ($R = 0.442$, $n = 23$, $p < 0.05$; Fig. 3c).

Discussion

The results of this study support the hypothesis that black spot disease prevalence in *A. bahianus* decreases with increasing depth. Diseased ocean surgeonfish may be more abundant on the back reef because of a decreased number of damselfish attacks (Reinthal and Lewis 1986), causing less stress for a diseased individual. The disease is more prevalent in higher densities of *A. bahianus* at 2 m than at 18 m. This could be due to the increased rate of disease in warmer, shallower water (Boyett et al. 2007).

Contrary to expectations, *A. bahianus* with black spot disease did not flash more often than healthy individuals. It is possible this behavior is not elicited because the spots are not felt as an irritation like ectoparasites or dead tissue and damaged scales. Instead, there may be various physiological responses in diseased individuals, but future experiments would be needed to test for these changes (Levine 1983; Beitinger and McCauley 1990).

Although it was predicted that individuals with more spots would need to feed more to cope with the added physiological stress of the disease, bite rate did not increase with increasing numbers of spots. However, analysis of feeding time showed that individuals with disease spent more time feeding. It is possible ocean surgeonfish with a greater number of spots are lethargic (Selye 1950, 1973), and must spend more time engaged in the process of feeding.

Ocean surgeonfish are important herbivores on coral reefs (Lewis 1986). It is unclear what the possible future implications of black spot disease are, but if the disease persists and leads to mortality of *A. bahianus*, this species would no longer be able to contribute to herbivory at the same scale. This is especially concerning paired with the trend of increasing algal growth throughout the world's oceans (Lewis 1986).

In addition to ocean surgeonfish, black spot disease has been observed in barracudas, parrotfish, trumpetfish, and grunts in Bonaire (S. Penn, pers. obser.). Black spot disease is affecting 89% of ocean surgeonfish and is a potentially very serious problem. Further research should be conducted to identify the pathogen causing black spot disease and to determine if any internal changes are occurring as a result of this disease. Future studies should also examine the prevalence of black spot disease in other species and locations, including on Bonaire and in the Caribbean, to gain a better understanding of the prevalence of black spot disease in the marine environment.

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Diel vertical migration and luminescent activity of bioluminescent dinoflagellates in Bonaire, Dutch Caribbean

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Abstract

Bioluminescence is a cold-light produced by chemical reactions and has been observed in over 90% of marine organisms. The largely speculated adaptive significance behind bioluminescent activity includes defense against predators, prey distraction, and communication. Bioluminescent photosynthetic dinoflagellates play a major role in the ocean's primary production. Daily vertical migrations (DVMs) through the water column are phototactic movements, where photosynthetic dinoflagellates return to the surface during daylight to photosynthesize. It was hypothesized that dinoflagellates migrate to the surface in daylight and to depth (4 m) in darkness. It was also hypothesized that chemically provoked luminescent activity would be greater after prolonged exposure to daylight rather than darkness. Samples were collected at am and pm intervals using a 30 cm diameter plankton tow with 20 μ m netting. Density of dinoflagellates were estimated under a compound microscope using a Neubauer-improved haemocytometer. Luminescent assays were performed by adding 5% acetic acid to the samples and timing the duration of luminescence in s. A two-way ANOVA with depth and time as factors revealed a significant interaction: at night 4 m density was significantly higher than 0 m density, and in the morning 0 m density was significantly higher than 4 m density. Luminescent activity in the morning was significantly higher than in the evening. A DVM of bioluminescent dinoflagellates was exhibited as a result of a phototactic movement. This study aimed to understand the relatively unknown bioluminescent dinoflagellate activity of one shallow coastal area in Bonaire, Dutch Caribbean.

Introduction

Bioluminescence is the ability for an organism to use chemical energy to produce light through a luciferase-catalyzed reaction with molecular oxygen and luciferin (Wilson and Hastings 1998). Species within 12 phyla of coastal marine organisms ranging from bacteria to fishes emit light (Morin 1983). The functions of luminescence in light emitting organisms vary greatly but are mostly speculated to play important roles in avoiding predation, finding prey, and communication (Young 1983). Dinoflagellates have been speculated to use bioluminescence in startling predators as well as attracting larger predators towards initial predatorial threats also known as the burglar alarm (Young 1983).

Photosynthetic bioluminescent dinoflagellates are unicellular phytoplankton

that luminesce when shear force is experienced on the cell membrane (Karleskint 2009). The necessary bioluminescent chemicals found in dinoflagellates are housed in scintillons, which are special cytoplasmic organelles that react with molecular oxygen creating photons of visible light (Wilson 1998). Although species concerned differ, dinoflagellates are found in all oceans and collectively are the most important contributors to primary production after diatoms (Karleskint 2009).

In addition to playing major roles in the marine food chain, many of the upwards of 10 toxic dinoflagellates exhibit positive phototactic diel vertical migrations that impact the surrounding ecosystem (Schofield et al 2006). The toxins produced during a bloom accumulate in higher trophic levels poisoning shellfish and commercially

important fish, causing a range of problems in humans, marine mammals and birds including paralysis, kidney problems, and death (Karleskint 2009).

Diel vertical migration (DVM) refers to the daily movement through the water column typical of marine dinoflagellates (Blasco 1978) as a result of a phototactic movement, which is a movement in response to light. While photosynthesizing at the surface in daylight, dinoflagellates are also speculated to be sequestering chemicals needed to luminesce, which are then used in the evening when luminescent activity is much higher. Bioluminescent dinoflagellates around Jamaica demonstrate luminescent activity regulated by phototactic movement as well as a DVM to the surface in daylight when luminescence was absent and dinoflagellates were photosynthesizing (Soli 1966). Similarly, dinoflagellate migration through the water column in the North Atlantic Ocean demonstrated maximum luminescence during darkness and minimum luminescence during daylight (Yentsch 1964).

With the exception of a few species and in certain locations, bioluminescent dinoflagellates have been the subject of relatively little research, none of which has been conducted on Bonaire. This relationship between a DVM regulated by phototaxis and the corresponding luminescent activity in tropical marine dinoflagellates is addressed in this study with the following hypotheses:

- H₁: During darkness dinoflagellate density is higher at depth than at the surface, but during daylight dinoflagellate density is higher at the surface than at depth.
- H₂: The ability of dinoflagellates to luminesce is greater in individuals who have experienced prolonged daylight exposure than those who have experienced prolonged darkness exposure.

This research aims to increase the understanding of the daily depth distributions

and luminescent activity of the photosynthetic dinoflagellates at one site in Bonaire, Dutch Caribbean.

Materials and Methods

All dinoflagellate samples were collected at the Yellow Submarine dive site in Kralendijk, Bonaire Dutch Caribbean (Fig. 1).

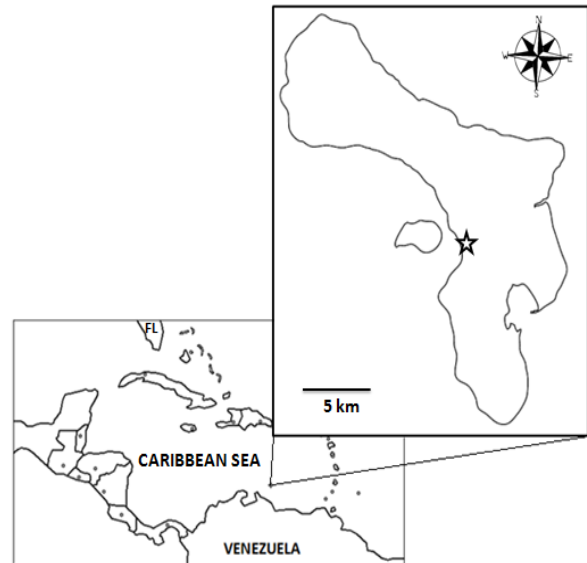


Fig. 1 Map of Bonaire, Dutch Caribbean. Star represents capital city Kralendijk where Yellow Submarine site is located

Over the course of the study, samples were collected at the same randomly selected area 3 m in length using a 30 cm diameter student plankton tow with 20 μ m netting. Samples were collected at two times of the day and at two depths: in the morning between 0630-0800 hrs and in the evening between 1830-2000 hrs, and at the surface (0 m) and close to the substratum (4 m). Date and time were recorded for each sample collected. Sample contamination was avoided by using Ziploc bags, filtered sea water, and capped labeled vials during collection. Samples referred to as being collected at “depth” indicate 4 m deep. A total of 80 samples were collected during a three week period in March of 2012.

Dinoflagellate Density and DVM

Variations in current during the density collection tows were negligible, allowing for a standardized volume of water to be sampled regularly. Samples were sieved with 250 and 20 μm mesh as directed by the Manual on Harmful Marine Microalgae (Hallegraeff 1995) and were preserved and dyed in 0.5 mL of 4% formalin and rose bengal solution (Steidinger 1979). After 12 h of preservation the samples were switched to 0.5 mL of 10% ethanol and pipetted onto a Neubauer-improved haemocytometer to be counted and identified to genus under a compound microscope (Fisher Scientific Micromaster) at 10x and 40x magnification. Density of dinoflagellates in each sample was extrapolated using the volume of water sampled during each collection tow in combination with the volume of the sieved sample used to fill the Neubauer-improved haemocytometer chamber.

Luminescence assay and DVM

Luminescence samples were collected at the surface and at depth in the am and pm using the student plankton tow previously described for a standard distance of 3 m. It was assumed that the length of time over which a sample would luminesce is independent of dinoflagellate density in a sample. Bioluminescent activity was provoked immediately after collection in a dark room by adding 1 mL of 5% acetic acid to the sample. The duration of luminescence was then timed in s.

Data Analyses

Two-way ANOVAs were performed using time of day and sample collection depth as factors, to investigate differences in (1) density and (2) luminescent activity.

Results

Eight genera of bioluminescent dinoflagellates were identified in density samples: *Gonyaulax*, *Ceratium*, *Ornithocerus*, *Procentrum*, *Peridinium*, *Noctiluca*, *Ceratocorys*, and *Alexandrium* (Fig. 2).

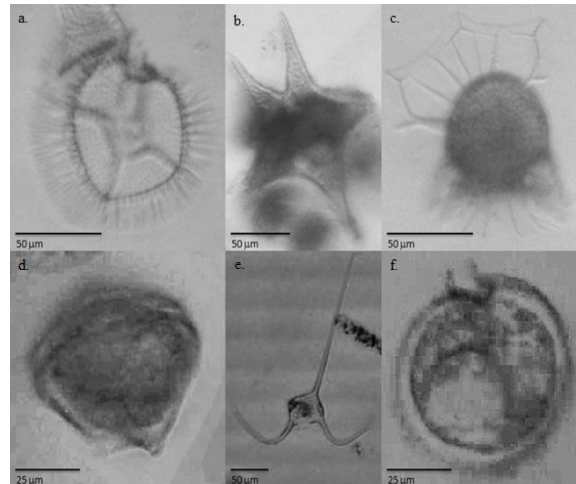


Fig. 2 Compound microscope images of 6 observed genera of bioluminescent dinoflagellates under 40x magnification. **a.** *Ceratocorys* spp. **b.** *Peridinium* spp. **c.** *Ornithocerus* spp. **d.** *Gonyaulax* spp. **e.** *Ceratium* spp. **f.** *Procentrum* spp.

Dinoflagellate Density

Although there were no differences in dinoflagellate density with depth or time of day, there was a significant interaction between these factors: in the morning surface density was significantly higher than at depth and conversely, in the evening density at depth was significantly higher than at the surface ($df = 1, 32, F = 49.20, p < 0.05$; Fig. 3).

Luminescence

Luminescent activity did not differ significantly between samples collected at the surface and those collected at depth (two-way ANOVA; $df = 1, 35, F = 0.73, p > 0.05$). However, luminescent activity was significantly higher in the am when compared to the pm (two-way ANOVA; $df = 1, 35, F = 140.00, p < 0.05$; Fig.4)

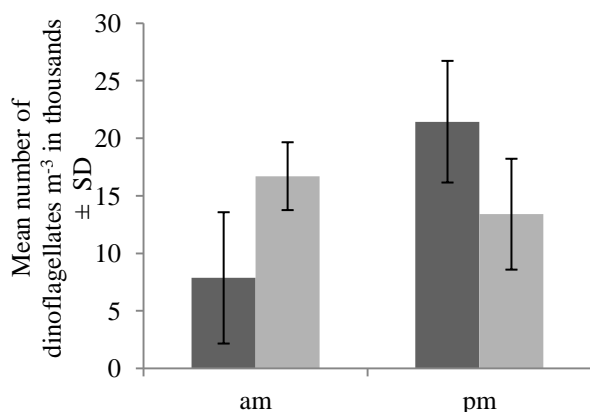


Fig. 3 Density of bioluminescent dinoflagellates at the surface and at depth (4 m) in the am and pm. Error bars indicate \pm SD. Dark gray bars show data from depth and light gray bars show data from the surface. *am* represents samples collected between 06:30-08:00 hrs and *pm* represents samples collected between 18:30-20:00 hrs ($df = 1, 32$, $F = 49.20$, $p < 0.05$)

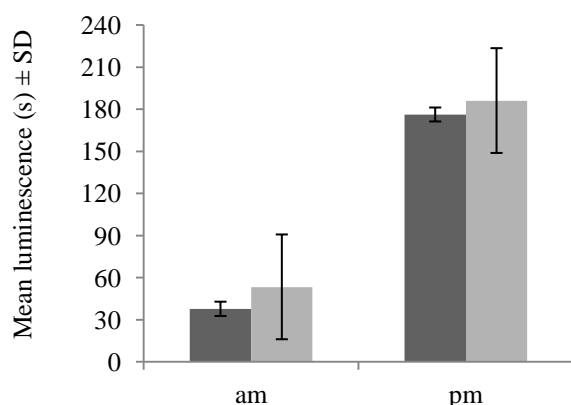


Fig. 4 Mean luminescence of photosynthetic dinoflagellates in seconds at the surface and at depth (4 m) after daylight exposure (*pm*) and darkness exposure (*am*). Error bars indicate \pm SD. Dark gray bars show data from 4 m and light gray bars show data from the surface. *am* represents samples collected between 06:30-08:00 hrs and *pm* represents samples collected between 18:30-20:00 h ($df = 1, 35$, $F = 0.4$, $p > 0.05$)

Discussion

As hypothesized, dinoflagellates of the shallow coastal area in Bonaire appear to migrate to the surface during the day and towards the substratum at night, a behavior also documented in Jamaica (Soli 1966). Higher dinoflagellate density at the surface during the day is most likely a phototactic

movement performed in order to acquire sunlight needed for photosynthesis (Blasco 1978). Environmental factors including temperature, pH, storm systems, nutrients influx from recent construction, and upwelling could have an effect on the DVM of dinoflagellates because dinoflagellates are most successful when the water column is sufficiently stable allowing motility for access to light and nutrients (Fogg 1991). Future studies could be conducted to include some of these factors in order to see how they contribute to dinoflagellate DVMs.

Greater luminescent activity was observed in dinoflagellates after prolonged exposure to daylight compared to darkness. This was also expected as scintillons and luminescent proteins in dinoflagellates have been found to break down completely during the night and then synthesize again during daylight exposure (Wilson and Hasting 1998) and could indicate that chemicals necessary to luminesce are depleted after prolonged luminescent activity in darkness. The degradation and reformation of scintillons and necessary bioluminescent chemicals could be the most energy efficient for photosynthetic dinoflagellates because luminescent behavior has been observed to be nonexistent in these organisms during the day (Soli 1966). Future experimentation on the exact cellular processes involved in specific marine organism's bioluminescent pathways are needed in order to fully understand the mechanisms behind the patterns of exhibited luminescent activity (Young 1983).

Bioluminescence is relatively unexplored considering that over 90% of marine organisms possess this ability (Wilson and Hasting 1998). In addition to the specific cellular processes that take place inside of bioluminescent organisms, there is little known about the definite adaptive significances of bioluminescence. Future research on bioluminescent dinoflagellates could involve sampling different depths and areas influenced by environmental factors including pH, temperature, and nitrogen levels. This could provide further insight into

the dynamics involved between these organisms' bioluminescent activity and their surrounding variable environments.

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Circadian rhythms and food entrainment of lionfish (*Pterois volitans*)

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Abstract

Circadian rhythms are common in many organisms and provide an organism synchrony with its environment, which is vital for survival. Entraining factors, called Zeitgebers, can modulate and synchronize the internal clock. Prey availability can change circadian rhythms and has been modeled in the laboratory with the presentation of food. The anticipation of the presentation of food, called food-anticipatory activity (FAA), has been studied extensively in mammals but is poorly understood in fish. The aim of the present study was to examine circadian rhythms and FAA of the Indo-Pacific lionfish, *Pterois volitans*. *P. volitans* is an invasive species of fish that may alter the composition of coral reefs. Lionfish have become established from the southeastern coast of the United States through the Caribbean Islands and parts of South America. In the wild, lionfish are most active around the crepuscular periods, dawn and dusk, and least active at midday. Lionfish maintained under constant conditions (CC) for two weeks did not demonstrate the same patterns of activity observed in the wild. The pattern that emerged was similar overall but much less distinct, suggesting that the pattern observed in the wild requires a specific Zeitgeber to persist. When fed consistently at 1300 hrs for two weeks under CC, a significant change in activity levels occurred. Lionfish demonstrated clear evidence of FAA through a significant increase in time spent active during the hour preceding prey availability. This study adds to the literature on fish chronobiology and provides insight into the adaptive nature of lionfish.

Introduction

Circadian rhythms consist of periods of approximately 24 hours and control the cyclic activity in many organismal processes such as biochemistry, physiology, electrochemistry, and behavior (Palmer 1973). Present in the animal (Meisel et al. 2003), plant (McClung 2006), and bacterial (Clodong et al. 2007) kingdoms, circadian rhythms are controlled by an endogenous, or self-sustaining, biological clock (Piggins and Guilding 2011). At its molecular core, the clock is comprised of a host of genes that are well-conserved in many vertebrates and have also been identified in a handful of teleost fish species (Feliciano et al. 2011).

The internal clock can be synchronized to an external environmental factor called a Zeitgeber in a process called entrainment (Rusak and Zucker 1975). Entrainment allows an organism to accurately anticipate temporal events like dawn or dusk and adjust its biology

accordingly. An organism is given synchrony with its environment, which is critical for its survival, through entraining environmental cues. Although the major entraining stimulus is the natural light/dark cycle, circadian rhythms can also be modulated by social interactions, temperature, or salinity (Vitaterna et al. 2001).

In order to observe an organism's natural circadian rhythm free of environmental cues, the organism must be placed under constant conditions (CC) such as constant light, constant temperature, or constant pH. The rhythm that emerges under CC is a true representation of an organism's natural cycle (Rusak and Zucker 1975). If a rhythm does not persist under CC, it is not considered circadian but is ecological and requires a specific Zeitgeber to persist (Palmer 1973).

The number of cycles in circadian rhythms, or the number of times the pattern repeats over the course of a solar day, varies

by environment. Most terrestrial organisms experience one cycle whereas marine cycles are usually bimodal as most of the world's coasts experience two tides a day (Palmer 1973). The bimodal rhythmicity has been observed in a number of intertidal organisms. For example, the fiddler crab (*Uca pugnax*) is active during both daily low tides and, under CC, the same bimodal rhythm persists (Palmer 1973). Although the circadian rhythms of intertidal biota are well studied (Palmer 1973; Palmer 1990; Saigusa and Kawagoye 1997), the rhythms of marine organisms outside of the intertidal zone are not.

Lionfish, native to the Indo-Pacific, are an invasive species that have spread quickly across the Caribbean. Over a period of more than a decade, two lionfish species, *Pterois volitans* and *P. miles*, became established along the southeastern coast of the United States. *P. volitans* spread through the Caribbean Islands and parts of South America and reached the island of Bonaire in 2009 (Schofield 2009; Szmant 2010). In each of its non-native ranges, *P. volitans* has proven to be a successful reef predator with densities higher than the densities seen in its native range (Green and Côté 2009).

Lionfish pose a threat to the ecological balance of coral reef ecosystems. Albins and Hixon (2008) did a cage experiment over a period of five weeks and found that lionfish caused decreases in the recruitment of native fish by an average of 79%. Commercial fisheries may be impacted by lionfish predation on the juveniles of economically important fish. Lionfish may also outcompete native midsized predators, resulting in a reef where most of the fish biomass is lionfish biomass (Albins and Hixon 2011).

Although there are many studies on lionfish feeding ecology and the effects that lionfish have on reefs (Albins and Hixon 2008; Morris and Akins 2009; Morris et al. 2009; Albins and Hixon 2011), there are few on lionfish activity patterns. Green et al. (2011) observed activity patterns of lionfish on Bahamian coral reefs and found that

lionfish exhibited spikes in activity during the crepuscular periods, dawn and dusk, and a depression in activity during midday. In the study, the activity pattern was not found to be prey-limited as prey fish biomass was significantly lower at dusk. The study did not make clear, however, whether the pattern of activity is circadian or environmental because the lionfish were only observed in the wild and not under CC. Furthermore, the study only considered lionfish behavior from one hour before sunrise to one hour after sunset, leaving a large portion of the night unexamined. To determine if the activity patterns observed in the wild by Green et al. (2011) are circadian, lionfish were observed under constant light (LL) in the laboratory in order to test the following hypothesis:

H₁: The pattern of high activity lionfish exhibit around the crepuscular periods in the natural environment is not circadian and will not persist when lionfish are observed under constant conditions.

Similar studies of marine organisms outside of the intertidal zone (Meisel et al. 2003) have demonstrated that patterns of activity observed in the wild do not persist once removed from the environmental Zeitgeber. Because marine organisms like lionfish undergo such extreme environmental changes each day, it is unlikely that activity patterns observed in the wild are completely independent of environmental cues.

Lionfish are able to adapt to a number of different environments as evidenced by their wide geographic distribution (Schofield 2009). Anticipation and adaptation to stimuli such as prey availability is critical for survival. Anticipation of food is known as food-anticipatory activity (FAA) and has been studied extensively in vertebrates (Bolles and Stokes 1965; Mendoza 2006; Storch and Weitz 2009). FAA manifests itself as an increase in locomotor activity shortly before the daily presentation of food (Feliciano et al. 2011).

To determine if lionfish can exhibit FAA, lionfish were held under LL and fed at

a consistent time. It is hypothesized that:

H₂: Lionfish will exhibit FAA with an increase in activity levels during the hour prior to the presentation of prey.

Lionfish have been proven to be highly adaptive in the wild. Furthermore, studies of goldfish (*Carassius auratus*) and zebrafish revealed that, when fed consistently, both species of fish exhibited FAA (Sanchez and Sánchez-Vazquez 2009; López-Olmeda et al. 2010; Feliciano et al. 2011). Lionfish, therefore, may also possess the capability to anticipate the availability of prey.

This study seeks to augment the existing literature on circadian rhythms and FAA in fish by studying lionfish behavior under CC. Studying the behavior of lionfish may also provide insights into how lionfish have spread so successfully.

Materials and Methods

Lionfish reached Bonaire, Dutch Caribbean in October 2009 and are now common on the fringing reef surrounding the island. In order to study circadian rhythms and FAA, five lionfish (5-10 cm total body length) were collected by local divers from 3-17 m at Boy Scout, 1000 Steps, Sabadeco Dock, and Pink Beach in Bonaire, Dutch Caribbean (Fig. 1) and brought to the laboratory.

Lionfish were held in 19 l glass aquaria with black plastic separating each tank to reduce outside stimuli to the lionfish, which may potentially influence the fishes' behavior. Gridded paper was placed at the back of each aquarium to help observe movement in all directions and a 19 cm by 7 cm plastic half-circle was placed in each tank for shelter. Two bubblers were placed in each aquarium and lionfish were kept in LL. A 25% water change was done daily and lionfish were fed one to three live fish daily. Lionfish were fed small, live fish caught locally.

A Lorex LH328501C4T22B Edge Security Camera System was used to document lionfish activity levels 24 h a day for the duration of the experiment using a

camera for each aquarium. Video clips of five s every 15 min were continuously migrated from the security system to a computer for analysis (*In sensu* Meisel et al. 2003).

During weeks one and two, times of tank cleaning and feeding were randomly generated to avoid entrainment. During weeks three and four, lionfish were fed and tanks were cleaned at 1300 hrs each day to observe whether the food entrainment would significantly raise activity levels during the hour preceding the 1300 hrs feeding where there was a depression in activity observed from weeks one and two. A significant increase in activity levels would provide evidence for FAA capability in *P. volitans*.

During week five, data were analyzed visually for the proportion of time spent active in each five s clip. Active lionfish were defined as displaying movement in any dimension. Resting lionfish were defined as not exhibiting any movement. Lionfish activity levels from weeks one and two were plotted to visually identify peak levels and depressions in activity. The lowest activity level observed from the first two weeks was used to set the time to test FAA. Activity levels of lionfish during weeks three and four were plotted to detect FAA. The lowest activity level selected for the FAA experiment was compared to the same time during the second two weeks of the experiment using a t-test (significance at $p < 0.05$) to determine if any significant change had occurred as a result of the entrainment.

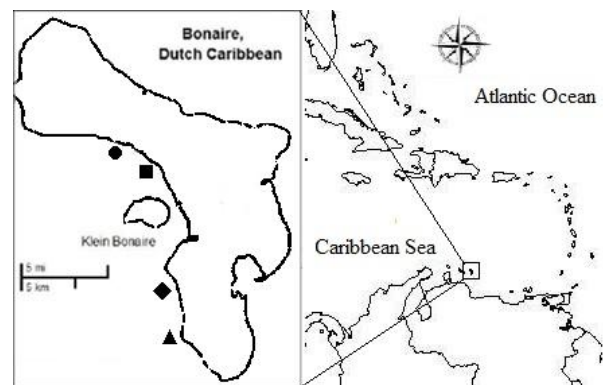


Fig. 1 Map of lionfish collection sites in Bonaire, Dutch Caribbean. Circle represents 1000 Steps. Square represents Sabadeco Dock. Diamond represents Boy Scout. Triangle represents Pink Beach.

Results

During the 21,215 s of lionfish behavior that was analyzed, the mean proportion of time active was 0.25 during the first two weeks and 0.15 during the second two weeks with no significant difference in overall activity levels (t-test; $df = 8$, $p = 0.18$).

During weeks one and two, the activity patterns of Fish A ($n = 550$) and Fish C ($n = 330$) were similar with a peak before dawn and following dusk and a depression of activity during midday. Although the trend of Fish B ($n = 348$) was similar to Fish A and Fish C, the peaks and depression were much less distinct. Fish D ($n = 308$) and Fish E ($n = 310$) showed no discernible patterns (Fig. 2).

The overall trend in activity levels \pm SD ($n = 1846$) followed a similar curve to A and C (Fig. 3). At 0600 hrs, the proportion of activity was 0.34 ± 0.13 , which was the highest proportion of activity observed overall. The second highest proportion, 0.33 ± 0.21 , was observed at 0000 hrs. The lowest proportion observed overall, at 1200 hrs, was 0.12 ± 0.04 . The proportion of activity at 2000 hrs dusk peak was 0.31 ± 0.15 .

During weeks three and four, Fish A ($n = 473$), Fish B ($n = 484$), Fish C ($n = 480$), Fish D ($n = 480$), and Fish E ($n = 480$) all showed similar trends in activity levels. For all five fish, there was a depression in activity levels before dawn and after dusk and a peak in activity levels during midday (Fig. 4).

The overall trend in proportion active \pm SD observed during weeks three and four ($n = 5$) was the inverse of the trend observed during weeks one and two (Fig. 5 and Fig. 6). There was a depression in activity at 0600 hrs with the proportion of time spent active equal to 0.18 ± 0.19 . The proportion during the 1200 hrs was 0.29 ± 0.22 . Finally, at 2000 hrs, the proportion equaled 0.08 ± 0.12 .

A t-test of the six means at the three time ranges (0600, 1200, and 2000 hrs) revealed that a significant change in activity

levels had occurred at all three times ($p = 0.003$; $p = 0.003$; $p < 0.001$; Fig. 7).

Discussion

There was a peak in activity before dawn, a peak after dusk, and a depression at midday in the free-running rhythm that emerged from weeks one and two. Although the overall trend followed a similar curve to the trend observed by Green et al. (2011) in the wild, the differences in activity levels were not as distinct. Green et al. (2011) found a decrease of approximately 0.90 between the proportion of time active before dawn and the proportion of time active at midday. The same difference was found between the proportions active at midday and dusk. In this study, the greatest difference in proportions active observed from weeks one and two was 0.22.

Despite the differences between the data from weeks one and two and the data from observations in the wild, lionfish in this study still exhibited circadian rhythmicity. After two weeks in constant light, there were still peaks and depressions in lionfish activity levels during the times of day the peaks and depression were observed in the wild. Future studies should focus on observing lionfish in the laboratory with a simulated light:dark cycle to see if the added Zeitgeber is sufficient to produce more distinct peaks.

Another point to come out of the data from weeks one and two is the high level of activity observed at 0000 hrs. It was the second highest proportion of time active observed overall by a difference of only 0.003. Because Green et al. (2011) did not examine lionfish behavior during this time period, further study would be worthwhile to determine whether activity levels in the wild are similarly high during the 0000 hrs.

The food entrainment during weeks three and four had a significant effect on the overall activity levels of the lionfish. After feeding at 1300 hrs for two weeks, there was a significant increase in activity at 1200 hrs.

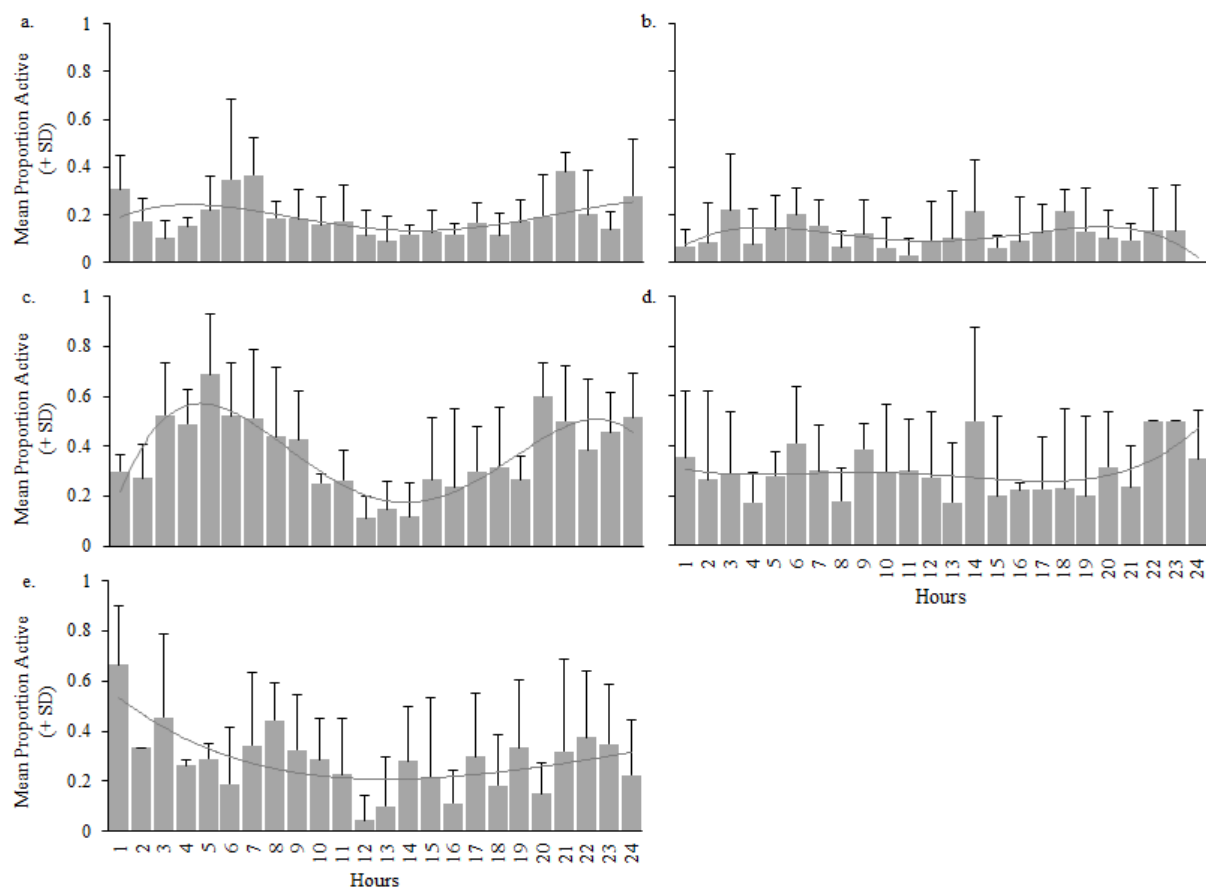


Fig. 2 Mean proportion of time spent active + SD over 24 h for five fish during weeks one and two. **a.** Activity patterns of Fish A, n = 550. **b.** Activity patterns of Fish B, n = 348. **c.** Activity patterns of Fish C, n = 330. **d.** Activity patterns of Fish D, n = 308. **e.** Activity patterns of Fish E, n = 310. *Lines* represent fourth order polynomial trends in data

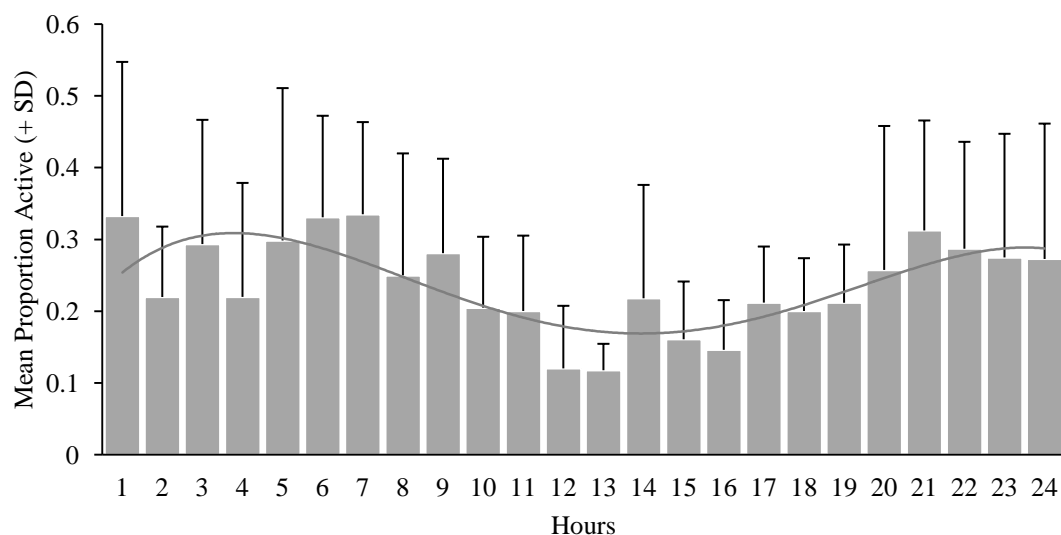


Fig. 3 Mean proportion of time spent active + SD for five fish over 24 h during weeks one and two (n = 1846). *Error bars* represent standard deviation. *Lines* represent fourth order polynomial trends in data

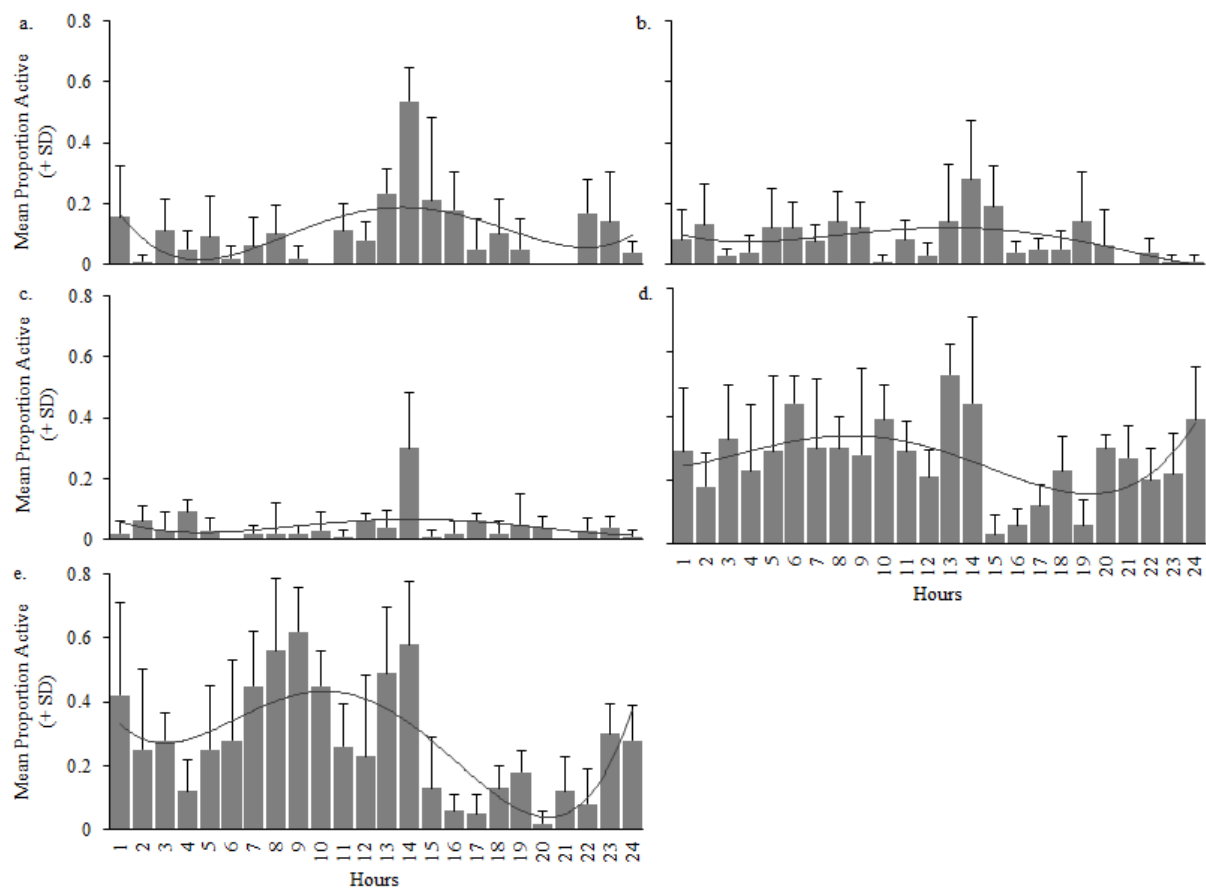


Fig. 4 Mean proportion of time spent active + SD over 24 h for five fish during weeks three and four. **a.** Activity patterns of Fish A, $n = 473$. **b.** Activity patterns of Fish B, $n = 484$. **c.** Activity patterns of Fish C, $n = 480$. **d.** Activity patterns of Fish D, $n = 480$. **e.** Activity patterns of Fish E, $n = 480$. Lines represent t fourth order polynomial trends in data

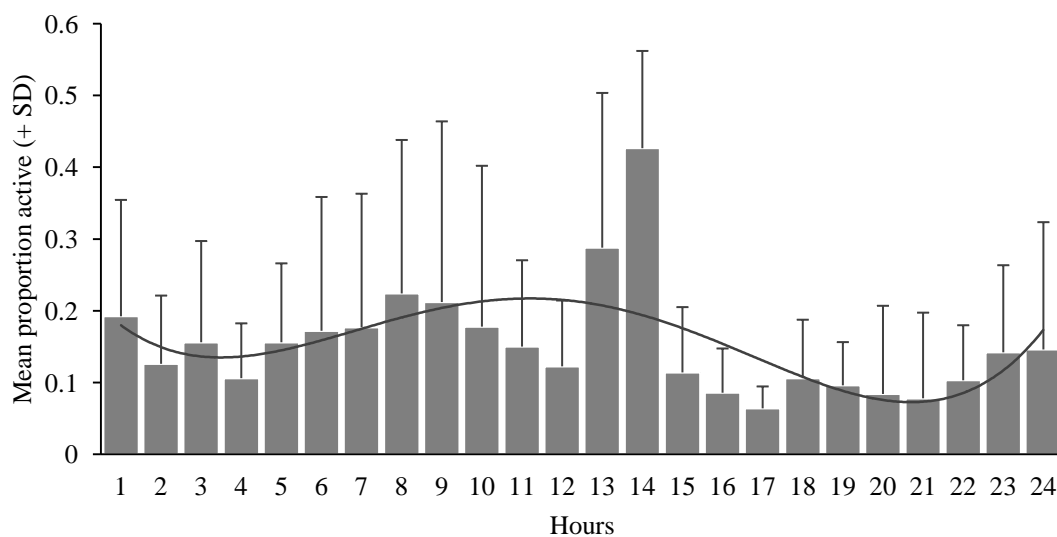


Fig. 5 Mean proportion of time spent active + SD for five fish over 24 h during weeks three and four ($n = 2397$). Error bars represent standard deviation. Lines represent fourth order polynomial trends in data

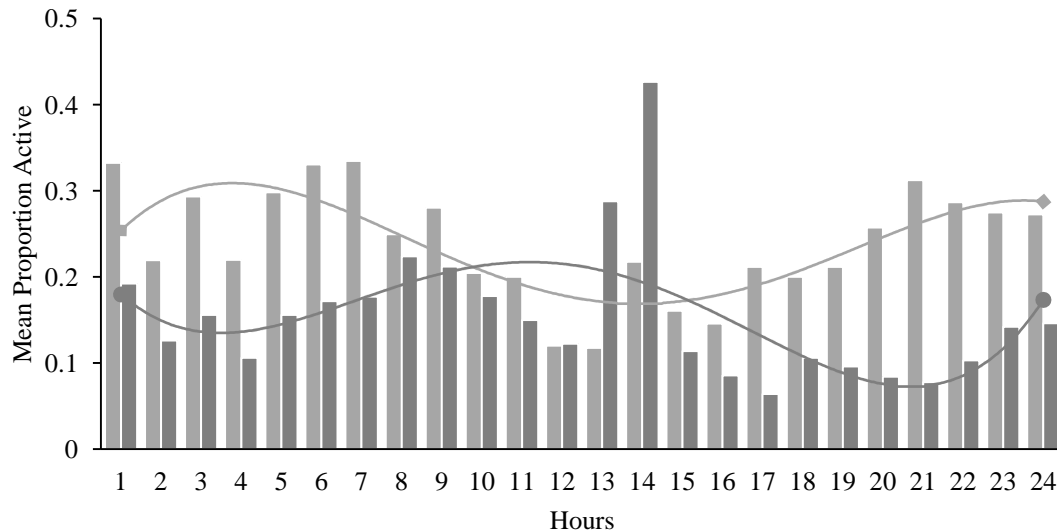


Fig. 6 Comparison of the mean proportion of time spent active over 24 h between weeks the first two weeks when prey were offered on a random time schedule and the second two weeks when prey were offered at 1300 hrs each day ($n = 4235$). *Light gray bars* represent weeks 1 and 2 and *dark gray bars* represent weeks 3 and 4. *Lines* represent fourth order polynomial trends in data

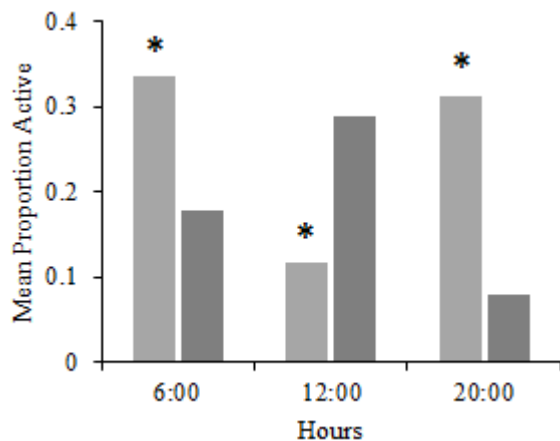


Fig. 7 Mean proportion of time spent active for five fish over four weeks. *Light gray bars* represent weeks 1 and 2 and *dark gray bars* represent weeks 3 and 4. *Asterisks* represent significant change ($p = 0.003$; $p < 0.001$)

As FAA is characterized by an increase in activity prior to the presentation of food, the data supports the hypothesis that lionfish can exhibit FAA. An ideal demonstration of FAA would have had the highest proportion at 1200 hrs and a sharp decline in activity from 1300 hrs onwards. Although the highest proportion of activity from weeks three and four was at 1300 hrs and not at 1200 hrs, this was likely a product of the limited time of entrainment. Similar studies of FAA, fish were entrained for 30 days. In this study, lionfish were entrained for 14

days. A longer experiment time may have produced more typical FAA patterns.

The fact that lionfish activity levels oriented around the presentation of food in just 14 days speaks to the capability of lionfish as an invasive species. Because the present study has demonstrated that lionfish are able to anticipate food, future studies might examine how long it takes for significant changes in activity levels to occur to get a more precise idea of how quickly lionfish can orient around prey availability.

This study is one of few studies on the circadian rhythms of fish and the first study on circadian rhythms in lionfish. Although the findings of the present study contribute to the existing literature on the chronobiology of fish and to what is known about lionfish ecology, knowledge of fish chronobiology is still lacking. Future studies are necessary to identify the Zeitgebers that affect lionfish activity levels. A better understanding of FAA in marine organisms may lead to novel, model organisms for use in studying the molecular aspect of circadian rhythms. And increased knowledge of lionfish ecology may provide insight into how lionfish, and other invasive marine organisms, have spread so successfully.

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The effects of *Stegastes planifrons* gardening on the prevalence of yellow band disease in the *Montastrea annularis* species complex

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Abstract

Yellow band disease (YBD) is a bacterial infection affecting corals of the *Montastrea annularis* species complex. Recent mortality rates of *M. annularis* spp. on Bonaire have risen due to YBD and other biotic and abiotic factors. The loss of staghorn coral, *Acropora cervicornis*, the preferred habitat of the threespot damselfish, *Stegastes planifrons*, has caused the damselfish to inhabit *M. annularis* spp. Unfortunately, *M. annularis* spp. are slower growing corals that take longer to reach reproductive maturity and are thus less able to withstand *S. planifrons* biting their tissues and creating algal gardens on exposed skeleton. This weakens the coral and makes it more susceptible to diseases like YBD. The objective of this study was to examine the relationship between *S. planifrons* gardening and YBD. Sample sites of healthy and diseased colonies of *M. annularis* spp. were established across depths at Yellow Sub dive site in Kralendijk, Bonaire. Sites were monitored for damselfish inhabitants and signs of coral biting. Pictures were taken of each site to chart the progress of the disease over the course of the study, and ImageJ was used to determine percent cover of healthy versus unhealthy coral. No significant relationship was found between *S. planifrons* activity and YBD, although *S. planifrons* seemed to select healthy colonies. The increase of damselfish populations and their detrimental effects on Bonaire's reef calls attention to the need for fishing regulations of predatory species and a heavier focus on the conservation of *A. cervicornis* thickets.

Introduction

The decline of Caribbean coral reefs, due to both anthropogenic and natural causes, has been a subject of ecological concern in recent years (Mora 2008). Bonaire, located in the Southern Caribbean, has long been regarded as having one of the healthiest reefs in the Caribbean; however, recent data show that this may no longer be the case (IUCN 2011). Stresses such as overfishing, nutrient runoff, rising sea temperatures, and disease have caused a decrease in live coral cover on many reefs and more recently on Bonaire's reef (Stokes et al. 2010). Coral coverage averages around 30% along the leeward side of Bonaire, which is less than half of what it was 30 years ago (Stokes et al. 2010). In this same study it was noted that up to 40% of the dominant coral species, *Montastrea annularis*, was diseased and dead at specific sites (Stokes et al. 2010).

Yellow band disease (YBD) was first

reported in the mid-to-late 1990s in many areas throughout the Caribbean. It has since spread rapidly and is now one of the most commonly reported coral diseases (Bruckner and Bruckner 2006a). YBD is a bacterial infection linked to a consortium of *Vibrio* pathogens that affects corals of the *M. annularis* species complex (Cervino et al. 2008; Dona et al. 2008). This species complex is comprised of three closely related species—*M. annularis*, *M. faveolata*, and *M. franksi*—and will be referred to as *M. annularis* spp. These corals represent a large proportion of the coral in Bonaire, and a disease that targets this complex may have major repercussions on the entire reef community. YBD begins as a small lesion affecting the zooxanthellae of a few polyps, and gradually spreads throughout the coral, creating a pale yellow ring between the healthy and diseased tissues. Since YBD directly attacks zooxanthellae, the host coral loses both its pigmentation and its primary

source of energy. This loss weakens the corals, causing mortality and subsequent algal overgrowth (Cervino et al. 2008; Dona et al. 2008). Little is known about the transmission of YBD; however, an increase in virulence has been linked to warmer sea surface temperatures (Cervino et al. 2008; Dona et al. 2008). At one site in Bonaire, 86% of *Montastrea* spp. was found to be infected with YBD, with a slightly higher percentage of disease at shallower depths, correlated with higher water temperatures. Along with the recent change in coral composition, there has also been a change in species assemblages on the reefs in Bonaire (Stokes et al. 2010).

The threespot damselfish, *Stegastes planifrons*, has historically inhabited thickets of staghorn coral, *Acropora cervicornis* (Precht et al. 2010). *S. planifrons* uses the complex branching structure of *A. cervicornis* to avoid predation and to cultivate algal gardens by biting live coral tissue and allowing algae to grow in its place. They maintain and defend these gardens, using them as a primary food source (Precht et al. 2010). Whilst *S. planifrons* gardening causes some mortality, the fast growth rate of *A. cervicornis* allows the coral to withstand this activity. However, in the 1980s, an outbreak of white band disease caused a reduction in *A. cervicornis* populations throughout the region, and *S. planifrons* now garden on alternative corals such as *M. annularis* spp., which grows more slowly and thus suffers higher mortality (Knowlton et al. 1990).

Like any other stress, the biting and subsequent algal gardens cultivated by the damselfish weakens the coral, potentially making it more susceptible to disease and death (Precht et al. 2010). Unlike *A. cervicornis*, which grows and reproduces quickly, members of the *M. annularis* species complex do not reach reproductive maturity for 7 to 10 yrs (Szmant 1991). Their rate of larval recruitment is low and

their tissue re-grows slowly, meaning they do not recover as readily from damselfish biting as *A. cervicornis* does (Bruckner et al. 2006b). In a study conducted in Curaçao, it was found that tissue re-growth after disease and destruction of *Montastrea* spp. was inhibited by damselfish biting and the creation of algal gardens on the exposed coral skeletons (Bruckner et al. 2006b). Algae gardens also stress the host corals in other ways (Potts 1977). The basal tissues underneath the algae gardens expend extra energy on continuously cleaning themselves (Potts 1977). Since the zooxanthellae are shaded by algal growth, the host coral receives less energy from photosynthesis, causing it to draw upon the rest of the colony for the energy needed to continue cleaning, further stressing and weakening the host coral (Potts 1977). Given the fact that damselfish gardening stresses the corals, it could be expected that *M. annularis* colonies with *S. planifrons* gardens are more likely to have YBD. Therefore, the following hypothesis was tested:

H₁: Algal gardening and coral biting of *S. planifrons* increases the prevalence of YBD in *M. annularis*.

Examining the relationship between the threespot damselfish and yellow band disease may illuminate the effects that these fish have on essential reef-building corals. This study may display the need for *A. cervicornis* conservation and highlight the importance of predatory fish species on the reef.

Materials and Methods

Study Site

This study took place at Yellow Submarine dive site in Kralendijk, Bonaire, Dutch Caribbean (Fig. 1). The coral colonies examined were within a depth range of 10-18 m, as this is the range at which most *M. annularis* corals are found (Szmant, 1991).

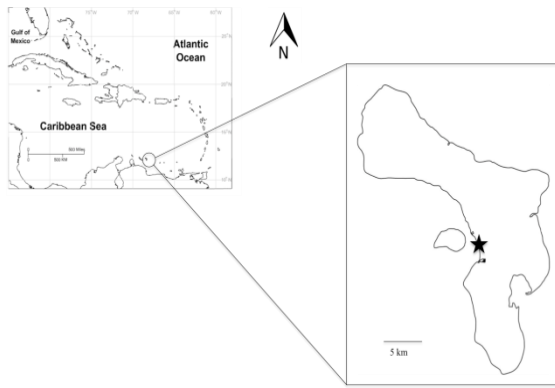


Fig. 1 Yellow Sub study site (*black star*) in Kralendijk, Bonaire, Dutch Caribbean

Methodology

This study focused on 30 colonies of the *M. annularis* species complex, five healthy and five unhealthy at each of three depth intervals (18, 14, and 10 m). For the purposes of this study, colonies were defined as a distinct separation of coral tissues, and healthy was defined as not having YBD. The percent of each colony infected with YBD was calculated from digital photographs using Image J (Collins 2007). The progression of disease was assessed by photographing each colony once a week over the course of a month using a half-meter stick in the photos as a scale for the size of the colony and the area of the disease present. The number of damselfish in association with each colony was assessed by observing colonies for one-min intervals, noting the number of damselfish present, number of times the damselfish bit the coral, and the number of bite marks already on the colony. This one-min timeframe was established prior to conducting observations. Density of the threespot damselfish was also calculated using data from 18 transects (30 x 2 m; Elmer unpublished data).

Data Analysis

The relationship between number of damselfish and the percent of disease on each colony was analyzed using linear regression. A linear regression was also used

to analyze number of damselfish and the number of bite marks on each colony. A two-tailed t-test was conducted to compare the number of damselfish found on diseased and healthy colonies.

Results

In total, 21 of the colonies were *M. annularis*, 6 were *M. franksi* and 3 were *M. faveolata*. *S. planifrons* was only found on 2 out of the 15 healthy colonies and 2 out of the 15 diseased colonies over the course of the study. Bite marks were found on 4 out of the 15 diseased colonies and 7 out of the 15 healthy colonies, none of which housed *S. planifrons*. The mean diseased area of the 15 *Montastrea* spp. colonies observed with YBD was $22.0\% \pm 19.2$ SD.

No significant relationship was found between percent diseased coral and number of *S. planifrons* on each colony ($r^2 = 0.006$, $df = 108$, $p = 0.403$; Fig. 2).

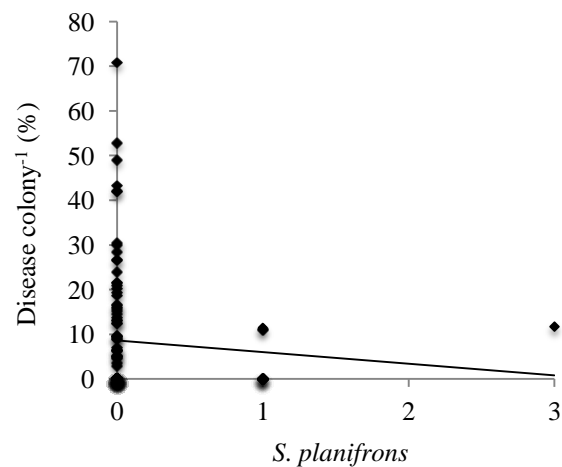


Fig. 2 Percent diseased vs. number of *S. planifrons* per colony of *M. annularis* spp. ($p > 0.05$)

No significant relationship was found between the number of damselfish and the number of bite marks on coral colonies ($r^2 = 0.022$, $df = 111$, $p = 0.113$; Fig. 3). The highest amount of damselfish marks was found on corals where no damselfish were present. No significant difference was found between the numbers of *S. planifrons* on diseased versus healthy colonies of *M.*

annularis ($t = -0.23$, $df = 111$, $p = 0.722$; Fig. 4).

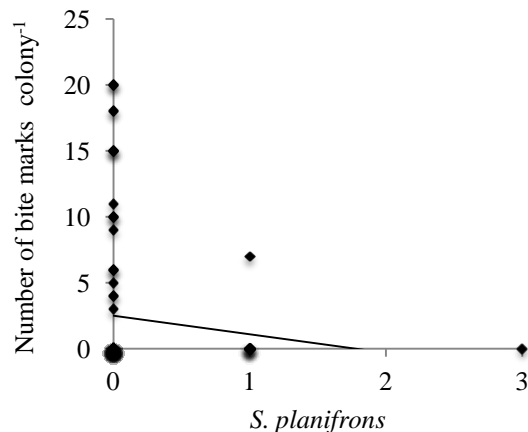


Fig. 3 Number of bite marks vs. number of *S. planifrons* on observed colonies of *M. annularis* spp. ($p > 0.05$)

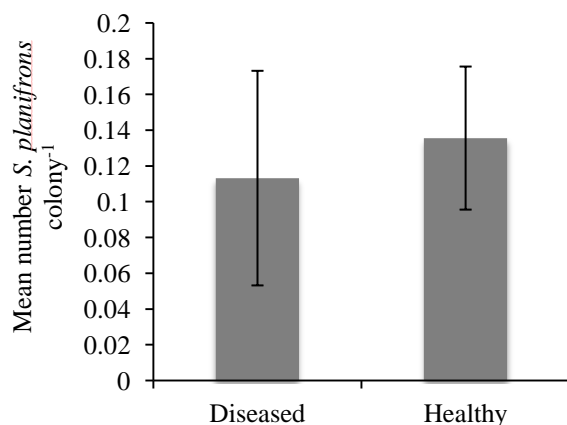


Fig. 4 Mean number of *S. planifrons* on diseased and healthy colonies of *M. annularis* spp. Error bars indicate standard deviation

Discussion

The data showed no significant relationship between biting and gardening of *S. planifrons* and prevalence of YBD.

Furthermore, no threespot damselfish were found on any of the coral colonies that had obvious bite marks. These results may suggest a number of different possibilities regarding the behavior of *S. planifrons*. The fact that *S. planifrons* seemed to select for healthy corals may exacerbate the degradation of a reef that is already in

decline from disease and anthropogenic factors (Mora 2008). Since damselfish populations, and thus the biting of healthy corals, are increasing in Bonaire (IUCN 2011), this demonstrates the importance of more predatory fish species on the reef and the need to revisit the fishing regulations of predatory species.

The data also suggest that damselfish are biting corals away from the colonies in which they live. This may imply that damselfish are straying further away from their garden territories to bite other corals, a behavior that should be examined more closely. In parrotfish, focused biting is used as a form of territorial marking, and further research into this behavior in damselfish might help to explain these results (Rotjan and Lewis 2006).

Along with these explanations, experimental error could have affected the results of the study. As the biting of the corals was not observed directly, the marks could be from other fish species, like parrotfish. This study took place over a short time period and was confined to a small site, meaning that site-specific factors could influence the behavior of the damselfish and thus the results of the study. Further research into the habitat preferences of damselfish may reveal a potential preference for more complex coral structures, such as the lobes of *M. annularis*, which resemble their natural habitat of *A. cervicornis* thickets more closely than the rounder structures of *M. franksi* and *M. faveolata*. The preference of damselfish for more rugose corals may necessitate a heavier focus on conservation of *A. cervicornis* thickets throughout the Caribbean.

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Comparison of fish assemblages of branching artificial reef habitat to adjacent habitats on the leeward coast of Bonaire, Dutch Caribbean

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Abstract

The implementation of artificial reefs is one effort used to mitigate the rate of decline of coral reefs and the deterioration of fish communities. Artificial reefs add support to struggling reefs habitats by providing additional or varied structural relief, sometimes mimicking specific coral structure types. The purpose of this study is to assess the effectiveness of branching artificial reef (BAR) habitat deployed in November 2011 by comparing the fish density and biomass, and species richness and diversity of the BAR to those of habitats in which it was placed. Three plots of BAR habitat were compared to three plots of rubble habitat and three plots of fore-reef habitat. BAR plots were found to have significantly lower fish density, fish biomass, and species richness than the fore-reef, but no statistical difference in species diversity. When compared to the rubble, BAR habitat showed significantly higher species richness, but no significant difference in density, biomass, or diversity. A comparison of family and fish phase community composition revealed that BAR habitat supports significantly more initial phase Scaridae than either adjacent habitat. It was concluded that BAR habitat adds little in the way of a complementary habitat to the terrace-fore-reef zone. The results from this study suggest that no further implementation of this form of artificial reef should be carried out along the rubble terraces of Bonaire. However, further monitoring of the BAR habitat and research into a branching structure with greater complexity, more interstitial matrix and constructed from calcareous material may be useful.

Introduction

The world's coral reefs have declined in live coral cover over the past three decades. In the Caribbean basin specifically, hard coral cover has decreased by 80% (Gardner et al. 2003). The island of Bonaire has experienced a 50% hard coral cover decrease since the 1980s (Stokes et al. 2010). Indirectly and directly, human activity has placed stress on coral reef environments (Mora 2008; Stokes 2010). Factors leading to coral loss include high nutrient levels, sedimentation, and prolonged thermal stress. Overfishing of predatory and herbivorous fish and regional losses of *Diadema antillarum* in 1983 have led to increased growth of algae which outcompetes corals and decreases available substrate for coral recruitment (Szmant 2002; Debrot and Nagelkerken 2006; Stokes et al. 2010; IUCN 2011).

Historically, *Acropora cervicornis* on the leeward coast of Bonaire was particularly

abundant, playing a significant ecological role in reef accretion and complexity, providing a crucial habitat supporting a high diversity of organisms (Precht 2002). Unfortunately, extensive *A. cervicornis* habitat has been lost throughout the Caribbean mainly due to effects of widespread white band disease and destruction by heavy storm events. The once densely populated shallow terrace between the shores and the fore-reef has now become sparsely inhabited sand and reef rubble (Aronson and Precht 2001; IUCN 2011).

The use of artificial reefs has been suggested as a solution to the problem of the loss of stony coral substrates (Seaman and Lindberg 2009). Artificial reefs have been used for centuries, mainly for artisanal and recreational fishing purposes, enhancing the aggregations and abundances of fish at specific sites (Seaman and Lindberg 2009). It is only recently that artificial reefs have been constructed for the purpose of protecting and

restoring components of marine ecosystems (Seaman and Lindberg 2009). Studies have been conducted to understand the effects of different materials, different shapes, and varying complexities on artificial reefs. There is a general agreement that increased rugosity in artificial structures increases species richness (Gratwicke and Speight 2005). However, currently, there is no broad consensus regarding the ideal shape, size, material, or placement of artificial reefs (Hixon and Beets 1989; Almany 2004; Gratwicke and Speight 2005).

A study in the Maldives found that one year after deployment of artificial reefs over destroyed and coral mined reefs, the artificial reef structures showed similar or greater species richness and density when compared to natural undamaged reef flats (Clark and Edwards 1999). In the Florida Keys artificial reef areas housed similar fish density and species composition to natural reef after 7 months (Stone et al. 1979). Use of such structures on barren or degraded substrate can provide the key structure and relief needed for the recruitment of juveniles and to support many fish species (Stone et al. 1979).

In Bonaire branching artificial reef (BAR) structures mimicking *A. cervicornis* habitat supported greater diversity but equal species richness and lower densities of fish when compared to live *A. cervicornis* (Nelson 2011). It was speculated the lower fish density in the artificial reef was due to the superior complexity of *A. cervicornis*, a factor that has been thought to increase richness, abundance, density, and biomass (Charbonnel et al. 2002; Sherman et al. 2002). To test the performance of an artificial reef, the structural and functional aspects of the fish community must be compared to natural reef habitat nearby (Carr and Hixon 1997). BAR provides a unique habitat structure and understanding its influence on reef fish assemblages is important for restoration efforts, especially if populations of *A. cervicornis* continue to decline. This study aims to evaluate how the fish assemblage supported by BAR habitat

compare to those found on adjacent rubble and fore-reef habitats.

The following hypotheses were tested:

- H₁: BAR habitat supports greater fish densities and fish biomass compared to natural adjacent habitats.
- H₂: BAR habitat supports greater species diversity and species richness of fish compared to natural adjacent habitats.
- H₃: BAR habitat supports a different fish community compared to adjacent natural habitats.

Materials and Methods

Study Site

The island of Bonaire lies in the south Caribbean and has narrow fringing reefs. Yellow Sub, the study site, is located on the leeward coast of Bonaire and is representative of the current state of the fringing reefs of Bonaire (Fig. 1); a shallow rubble terrace leads to a transition point at the fore-reef to a richly populated reef slope. Data were collected from BAR, rubble and fore-reef habitats located between 7 m and 10 m deep directly in front of Yellow Sub.

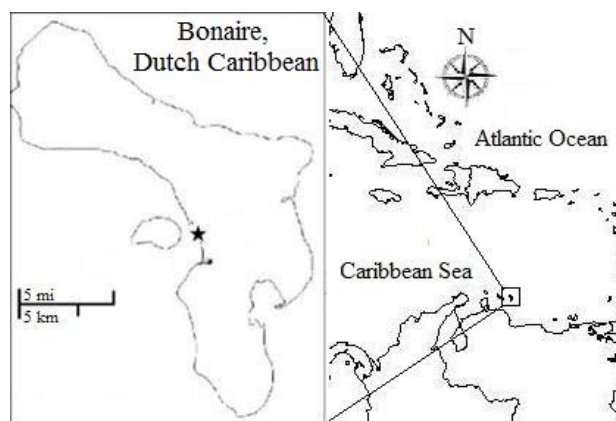


Fig. 1 Map of the Caribbean Sea with inset of Bonaire, Dutch Caribbean. The study site, Yellow Sub indicated by black star

Three separate plots of BAR habitat were placed 3 m from the fore-reef (Nelson 2011). Fore-reef and rubble plots were chosen randomly within 30 m north of the BAR plots along the reef crest.

The random selections excluded overlapping areas. The mean size of each plot was 2 x 1.7 m. The corner of each plot was marked using 0.5 m long rebar.

Fish Surveys

The time of day data were collected was restricted to around midday (between 1000 and 1400 hrs) to minimize the influence of species feeding patterns on behavior. All individuals were recorded for an 11 min period within each plot. The first 4 min were recorded from a 3 m distance to avoid disturbing fish; the next 5 min were recorded from a 1 m distance to see smaller fish; and the final 2 min were recorded from overhead to detect more cryptic, camouflaged or previously unrecorded fish. Observation times were chosen based on asymptotes of species accumulation curves (Sinkus, unpublished data).

Species, size category (Table 1) and stage (juvenile, intermediate/ initial, adult/terminal) of each individual was recorded. Each plot was sampled five times.

Table 1 Size categories of fish

Size	0-2	2-4	4-6	6-8	8-10	10-15
Category						
(cm)	15-20	20-25	25-30	30-40	40-50	>50

Fish density was calculated by averaging the total number of fish counted in each sample within a plot type and dividing by the mean plot area to get mean individuals m⁻². Fish biomass was calculated using a length weight relationship calculated for each fish species using the equation $W = a + L^b$, where W = weight of the fish in grams and L = total length of the fish in cm. values of a and b constants for each species provided by Fish Base (Froese and Pauly 2012). Mean fish biomass (g m⁻²) was calculated for each plot. Species diversity was calculated using the Shannon-Weiner Index, $H_s = -\sum_{i=1}^S (p_i \ln p_i)$, where p_i was the frequency of a species. The greater the

resulting number was, the higher the diversity of the fish assemblage. The proportion of the fish assemblage represented by each family was calculated by percent for each plot type.

Data Analyses

Single factor ANOVAs with post hoc tests were used to investigate whether (1) density, (2) biomass, (3) species richness and (4) species diversity differed between BAR, rubble and fore-reef habitats.

Results

A total of 2,958 individuals were observed in 495 min of survey time, representing 54 species from 19 families. Most individuals ($n = 1763$) were observed in fore-reef habitat, with fewer ($n = 664$) recorded in BAR and the least ($n = 531$) seen in rubble habitat plots.

Fish Density

The mean density of fish differed significantly between plot type (ANOVA; $F = 70.8$, $df = 2, 44$, $p < 0.001$). Tukey's post hoc test revealed that the fore-reef (mean = 34.5 ± 8.3 individuals m⁻²) density was significantly higher than the other habitats, but there was no difference between the density found in BAR (mean = 13.0 ± 3.8 individuals m⁻²) and rubble plots (mean = 10.4 ± 5.3 individuals m⁻²; Fig. 2a).

Fish Biomass

The biomass of fish differed significantly between plot types (ANOVA; $F = 18.9$, $df = 2, 44$, $p < 0.001$). Tukey's post hoc test revealed that the fore-reef (mean = 2522.4 ± 1002.9 g m⁻²) mean biomass was significantly greater than the other habitats, but there was no difference between the biomass found in BAR (mean = 1451 ± 700 gm⁻²) and rubble plots (mean = 819.4 ± 134.0 g m⁻²; Fig. 2b).

Species Richness

The species richness was significantly different between the three different habitat types (ANOVA; $F = 65.7$, $df = 2, 44$, $p < 0.001$) Tukey's post hoc test revealed that the fore-reef species richness (mean = 19.7 ± 4.7) was significantly higher than the other habitats, as well as significantly higher in the BAR (mean = 11.5 ± 2.29) than the rubble plots (mean = 7.5 ± 1.88 ; Fig. 2c).

Species Diversity

The species diversity differed significantly between plot types (ANOVA; $F = 18.9$, $df = 3, 33$, $p < 0.001$). Tukey's post hoc test revealed that the BAR ($H_s = 2.09 \pm 0.22$) was not significant different from the fore-reef ($H_s = 2.20 \pm 0.31$) or the rubble plots ($H_s = 1.61 \pm 0.30$; Fig. 3), but the fore-reef was significantly more diverse than the rubble (Fig. 2d).

Gobiidae were the most abundant family in all habitat types, representing 52%, 43% and 37% of individuals seen in rubble, BAR and fore-reef respectively (Fig. 3). The second most abundant family found in BAR were Scaridae, 31% (Fig. 3b). In the rubble, the second most abundant family was Labridae, 24% (Fig. 3a). In the fore-reef, the other major families were Lutjanidae and Haemildae, 28%, and Labridae, 19% (Fig. 3c).

Juvenile Scaridae were significantly more abundant in BAR plots ($2.63 \pm 2.01 \text{ m}^{-2}$) than fore-reef ($0.88 \pm 0.74 \text{ m}^{-2}$; Tukey's $p < 0.01$) and rubble plots ($0.10 \pm 0.24 \text{ m}^{-2}$; Tukey's $p < 0.001$).

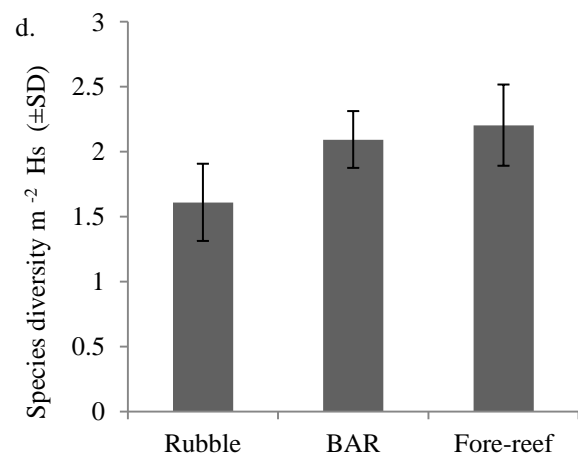
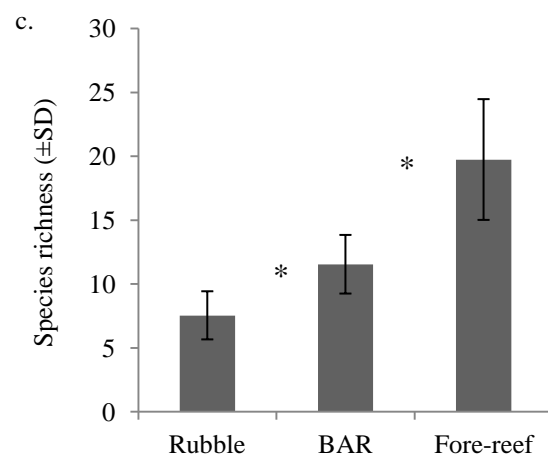
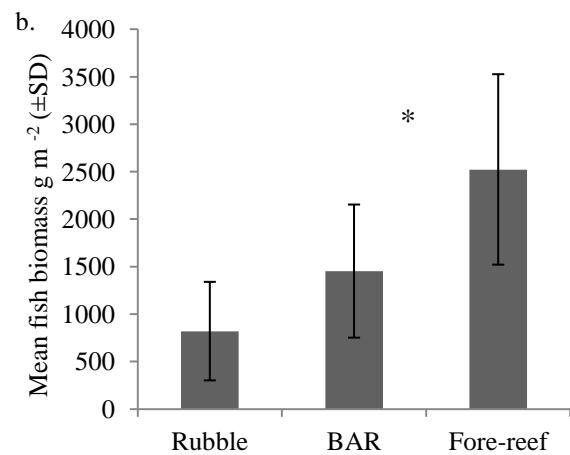
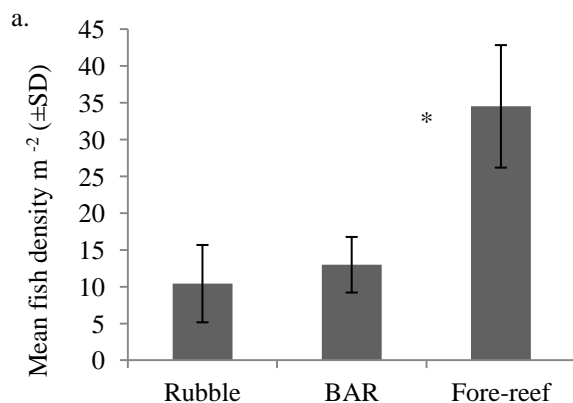


Fig. 2 Comparison of **a.** mean fish density individuals m^{-2} , **b.** mean fish biomass in g m^{-2} , **c.** mean species richness, **d.** mean species diversity found in rubble terrace plots, BAR plots and fore-reef plots. Asterisk represents a significant difference between BAR plot and adjacent plot type

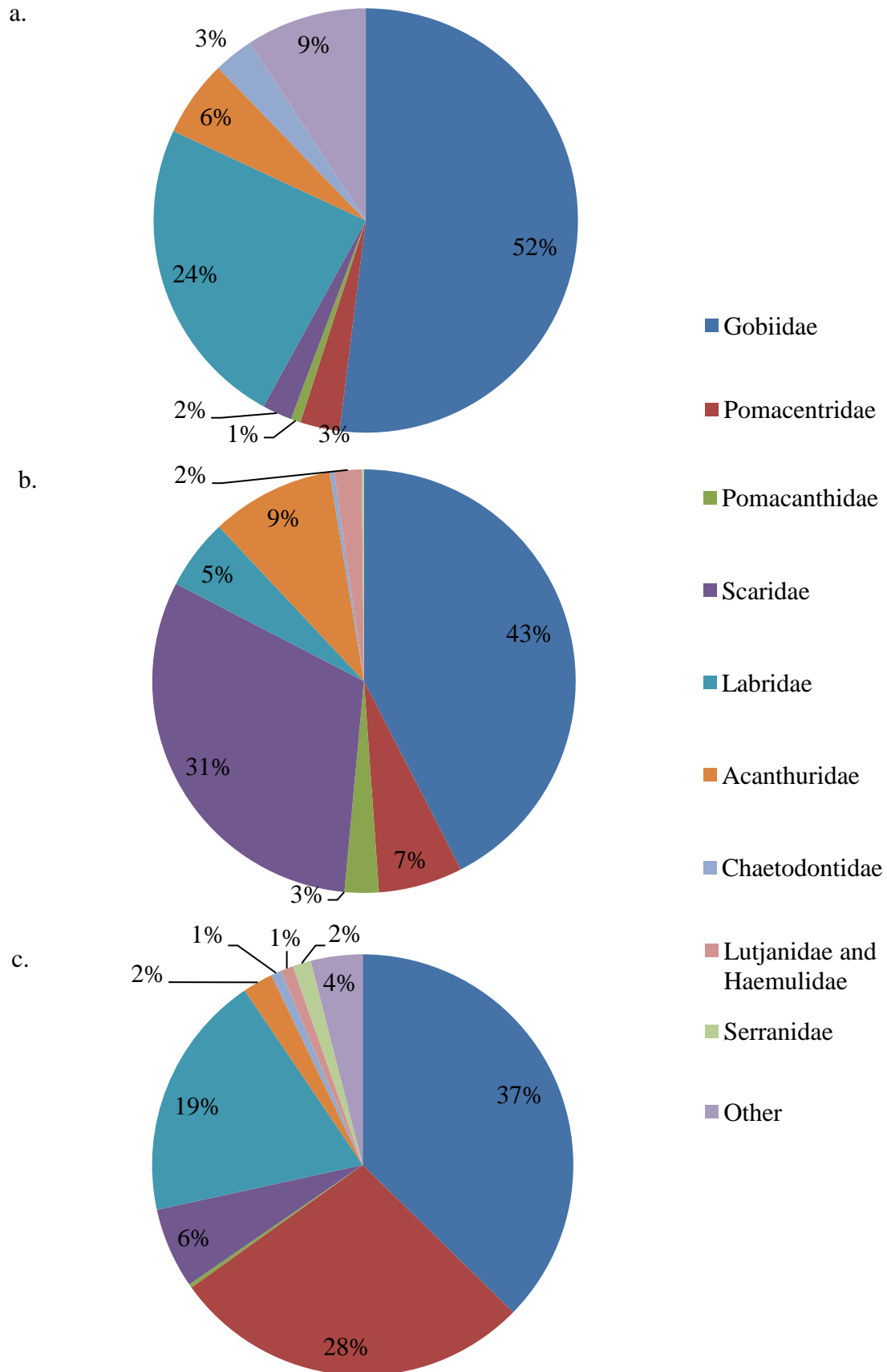


Fig. 3 Comparison of percent composition of fish families in **a.** rubble terrace plots, **b.** BAR plots and **c.** natural fore-reef

Discussion

The first hypothesis, that fish density and biomass were greater in the BAR habitat than the adjacent natural habitat was not supported by the data. This hypothesis was rejected because fish density and biomass were greater in the fore-reef than the BAR and no significant difference in density or biomass was evident between rubble and BAR habitats.

The species richness in the BAR habitat was significantly greater than in the rubble habitat. However, species richness in the BAR habitat was significantly less than in the fore-reef habitat. When combined with a lack of statistically significant difference in species diversity between BAR plots and the other plot types, the aforementioned data provide evidence for the rejection of the second hypothesis. These results show that habitats with greater complexity support greater species richness, which is consistent with the conclusions of Gratwicke and Speight (2005).

The BAR habitat had less density, biomass, richness and diversity of fish when compared to the natural reef because the BAR habitat is still in the first phase of its succession. The first phase of succession involves a rapid and homogenous colonization of fouling organisms. The wooden branches of the BAR were covered in a thick film of algae, which attracts a large abundance of algal grazers; Acanthuridae and initial stage Scaridae (personal observation). Artificial coral reefs experience four phases of reef development within an 11 y period: (1) the start-phase, (2) the preparation-phase with grazing-resistant algae, (3) the phase of pioneer frame-building with reef-building corals, and (4) the phase of frame-binding with new corals growing on dead corals (Schumacher 1977). The BAR habitat has not had enough time to transition from the first developmental phase to the second and thus supports less life than the natural reef. Furthermore, the BAR structures do not have sufficient complexity to provide a permanent living territory for

many species of fish, and currently only provide a foraging and resting habitat for transient fish (Marranzino 2012). High structural complexity, denoted by cracks, crevices and holes, influences the composition of a reef and rough or calcareous texture is necessary for the recruitment of corals (Bohnsack and Sutherland 1985).

It may be useful to continue monitoring the BAR habitat while making adjustments to its structure by adding more branches, making a denser and tighter framework of branching structures, better mimicking the lost branching habitat of *A. cervicornis*. A new artificial structure with branches made from a calcareous material could even be built to better promote larval coral recruitment and growth (Bohnsack and Southerland 1985).

More replications and more data points may minimize error, which currently overshadows the trends in the data that suggest the BAR habitat supports greater density, biomass and diversity than the rubble habitat.

Although the present study did not reveal greater or equal fish assemblages on artificial reefs placed in the rubble than the natural reef, elsewhere artificial reefs placed on damaged reef were found to have fish assemblages with greater richness and density than natural undamaged reef (Clark and Edwards 1999). This dissimilarity may be due to the difference in scale of artificial reef structures. The BAR habitat structures in place in Bonaire are small plots of branching reef close enough to the natural reef to provide a foraging ground for several species of herbivorous fish, but not complex or extensive enough to support greater density or richness than the natural reef (Bohnsack and Sutherland 1985).

Fish density, richness, and diversity can be compared to the results of the previous assessment of the BAR habitat conducted in the fall of 2011 (Nelson 2011). The assembly of fish families documented in the present study was similar to that recorded two weeks after the BAR was deployed, with

the majority of fish belonging to the Gobiidae and Scaridae families. There has been an approximate two-fold decrease in fish density and species richness in the BAR habitat, but substantial increase in species diversity in the BAR plots over the course of five months (Nelson 2011). These changes may be due to the effect of fish colonization of the new habitat. A study in the Red Sea supports this by showing an initial rapid increase in fish abundance and number of species in the first four months, followed by a decrease in the number of individuals observed per survey (Golani and Diamant 1999). This decrease in observed individuals, led to an increase in evenness and subsequently species diversity (Golani and Diamant 1999). However, the changes observed may be due to slight variation in procedure or observer bias.

As predicted, the BAR habitat supported a different fish community compared to the other two habitats. BAR habitat supported significantly greater numbers of Scaridae than both rubble and fore-reef habitat and 7% more Acanthuridae than the fore-reef. Habitat complexity and substrate type are important factors in structuring fish assemblages (Friedlander and Parrish 1997). The BAR has greatly differing structural characteristics from the two other habitats studied and would therefore be expected to support differing fish assemblages. The BAR plots showed significantly more initial stage parrotfish than both the fore-reef and rubble, which suggests that the BAR habitat is offering a complimentary habitat to the area around it.

Conclusions from this study are mixed: the BAR supported a different assemblage of fishes than adjacent habitats and greater species richness than the rubble habitat, but did not support a greater density, biomass or species diversity of fishes than either two habitats. These results suggest that the BAR habitat does provide a complimentary ecological function to the natural adjacent habitats, but are not conclusive enough to justify further application of this form of artificial reefs in

Bonaire. Deploying an alternative artificial structure that better replicates *A. cervicornis* in degraded shallow terrace areas may still be an effective restoration strategy.

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Habitat preference of coral dwelling gobies, and the effects of coral disease

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Abstract

Coral reefs are one of the most diverse ecosystems found on earth, and are home to many habitat-specific fish. The Gobiidae family is known to be one of the most habitat-specific groups. Two common gobies found in the Caribbean are *Coryphopterus lipernes* and *Gobiosoma evelynae*, and both species rest on live coral heads. This study was conducted to determine if *C. lipernes* and *G. evelynae* show a preference for certain coral species and if the presence of disease affects this selection. A benthic survey was performed using video transects and CPC data analysis, allowing calculation of percent frequency for each coral species and frequency of diseased corals. Goby searches were conducted using SCUBA within a depth range of 10 - 15 m along the reef, recording the coral of choice and its disease status. The results showed that *C. lipernes* selected for 3 coral species and against 5, favoring *Colpophyllia natans* and *Montastraea cavernosa*. *G. evelynae* selected for 3 coral species and against 5, favoring *M. cavernosa* and *Stephanocoenia* spp. Both goby species selected significantly against coral disease, *C. lipernes* had a mean disease selection ratio of 0.39, and *G. evelynae* showed a complete selection against disease. Coral reefs are important ecosystems that are currently under significant abiotic and biotic stressors. It is important to understand the influence that an increase in disease and reduction in coral abundance may have on habitat-specific fish.

Introduction

Coral reefs are complex ecosystems that provide food, shelter, and protection for a variety of organisms. Many fish species found on reefs are habitat specific, requiring particular environmental factors in order to survive (Dirnwöber and Herler 2007; Munday et al. 1997; Wilson et al. 2006). Live coral cover, coral availability, abundance and disease are shown to influence the assemblages of these fish (Bell and Galzin 1984; Chabanet et al. 1997; Munday et al. 1997; Schiemer et al. 2009). There are two types of habitat-specificity displayed by coral reef fishes, generalists and specialists. Generalists prefer a specific habitat, but the species is readily able to relocate to a more suitable location whereas, specialists require a specific habitat (i.e. coral species, sand, rubble) and are less likely to relocate if disturbances in the habitat occur (Dirnwöber and Herler 2007; Schiemer et al. 2009). Generalist species are more common than specialist species, but the

abundance of each depends on the abundance and distribution of the preferred habitat (Dirnwöber and Herler 2007; Munday et al. 1997).

Gobiidae are some of the most specialized reef fishes found on coral reefs (Dirnwöber and Herler 2007; Munday et al. 1997; Munday 2000; Robins et al. 1986; Schiemer et al. 2009), including generalist and specialist species (Dirnwöber and Herler 2007; Feary 2007; Munday et al. 1997; Schiemer et al. 2009). Coral species preference is known to differ depending on goby species, and the majority of studies on *Gobiodon* spp. have concluded that gobies prefer certain coral species (Dirnwöber and Herler 2007; Feary 2007; Schiemer et al. 2009). In the Indo-Pacific, coral diversity and the abundance of host corals have been shown to have a direct impact on goby populations (Nanami et al. 2004), as coral populations decline, so do Gobiidae populations (Munday et al. 1997). Coral reefs are also experiencing widespread disease and the presence of coral disease has influenced

habitat preference of some goby species (Weil 2001). Gobies found in the Red Sea were observed more frequently on coral colonies with lower partial mortality compared to corals with high partial mortality (Schiemer et al. 2009), indicating the importance of habitat to these benthic reef fish (Dirnwöber and Herler 2007; Munday et al. 1997; Munday 2000; Schiemer et al. 2009).

Coryphopterus lipernes and *Gobiosoma evelynae*, are common coral associated benthic fish found on reefs in the southern Caribbean (Robins et al. 1986), but little is known about specificity in either species. *C. lipernes* are one of the few western Atlantic fish species to live in close proximity to live coral (Smith and Tyler 1977) and are found in grooved and drop-off habitats, resting directly on coral heads (Greenfield and Johnson 1999; Robins et al. 1986). *G. evelynae* are one of the most abundant cleaning gobies found in the Caribbean and are found in grooved, spur, and patched reefs with a depth range from 1.2 – 27.1 m (Greenfield and Johnson 1999; Robins et al. 1986). Studies conducted in the Caribbean found that around the Virgin Islands, *C. lipernes* was observed solely on *Diploria labyrinthiformis*, however, in Jamaica the species preferred *M. annularis*, *M. cavernosa*, *Diploria* spp., *Agaricia* spp., and *P. astreoides* (Smith and Tyler 1977). *C. lipernes* and *G. evelynae* have been reported to live on various species of live coral, but there are no reports on preference for specific coral species (Smith and Tyler 1977).

The purpose of the study is to determine habitat specificity of *C. lipernes* and *G. evelynae* on the reefs of Bonaire and determine if the presence of disease affects habitat preference. The following hypotheses were tested:

- H₁: *C. lipernes* and *G. evelynae* will prefer certain coral species living on the reef.
- H₂: *C. lipernes* and *G. evelynae* prefer corals without disease.

Little is known about habitat specialization of gobies on the global scale (Dirnwöber and Herler 2007; Greenfield and Johnson 1999; Munday et al. 1997) and although gobies are difficult to study because of their size, an ecological study will provide a better understanding of how goby populations will be affected if live coral cover continues to degrade.

Materials and Methods

Study Site

The study was conducted on the leeward coast of Bonaire, Dutch Caribbean, located in the Caribbean Sea, 80 km off the coast of Venezuela (Fig. 1). Bonaire is surrounded by a fringing reef, which is located close to shore. The reef crest begins around 5 - 15 m deep and slopes down to depths of 30 - 80 m. Data were collected during the months of February and March 2012 at Yellow Sub (N 12° 09' 36.5" W 068° 16' 54.9") (Fig. 1) where the reef is located 45 m off shore and begins at a depth of 9 m; sloping down to around 30 m. The site was chosen because live coral and disease are present and gobies are found in high abundances.



Fig. 1 Bonaire, Dutch Caribbean located in the Caribbean Sea. Asterisk denotes Yellow Sub dive site

Benthic Survey

Data was collected using an underwater video transect method at depths of 10 m, 12 m, and 15 m along the reef isobath. Transect lines (30 m) were laid out, two at

each depth in front of Yellow Sub, three to the right and three to the left of the entry point. Thirty still frames were randomly selected from each video transect. The frames were analyzed using Coral Point Count (CPC), with 30 random points generated on each frame. The identity of the substrate under each point was identified to species level for corals, and all the other points were labeled as other. Percent frequency of diseased coral colonies were calculated using the same 30 still frames used for CPC. Total coral species colonies were counted and individual colonies were identified as diseased or non-diseased.

Goby Search

Across the same area as surveyed in the benthic survey a U-search pattern was used to find *C. lipernes* and *G. evelynae*. Five distinct non-overlapping searches were completed to cover the study area. Each goby was identified, the coral species on which the goby was residing, and presence of coral disease was noted.

Data Analysis

Percent coral cover of each species as generated from CPC analysis was converted to percent frequency ($a_i = C_i/TC * 100$, where a_i = percent frequency of coral species i , C_i = % coral cover of species i , and TC = % total coral cover). Percent frequency of disease was also calculated for each coral species ($t_i = D_i/TCC_i * 100$, where t_i = percent frequency of coral disease for species i , D_i = number of diseased colonies of species i , and TCC_i = total number of coral colonies of species i).

Goby species were analyzed separately and percent frequencies for gobies on each coral species were calculated ($o_i = G_i/TG * 100$, where o_i = percent frequency of gobies on coral species i , G_i = number of gobies seen on coral species

i , and TG = total number of gobies seen). Percent frequency of gobies on diseased coral colonies ($r_i = GD_i/TG_i * 100$, where r_i = percent frequency of gobies on diseased coral species i , GD_i = number of gobies found on diseased colonies of coral species i , and TG_i = total number of gobies found on coral species i) was also calculated. Selection ratios (w_i) for selection for or against a specific coral species were calculated using the formula:

$$w_i = \frac{o_i}{a_i}$$

where o_i = percent frequency of *C. lipernes* or *G. evelynae* found on coral species i , and a_i = percent frequency of coral species i (Munday 2000). Selection ratios were taken as significant when ± 1 goby changed the direction of preference, either for or against the coral species.

Selection ratios for diseased corals (z_i) were calculated with the formula:

$$z_i = \frac{r_i}{t_i}$$

where r_i = percent frequency of *C. lipernes* or *G. evelynae* found on diseased coral species i , and t_i = the percent frequency of diseased coral species i . The mean of disease selection ratio across all species was calculated and a 1-sample t-test was performed with a hypothesis test of less than 1. The 1-tailed t-test was used to test for a significant selection against coral disease.

Results

Benthos

A total of 18 coral species were identified at the dive site with *M. annularis* (27.87%) and *Agaricia* spp. (26.56%) as the most abundant species (Fig. 2 and Fig. 3). Coral disease was found on 6 coral species (Fig. 4) with *Stephanocoenia* spp. having the highest percentage (60%).

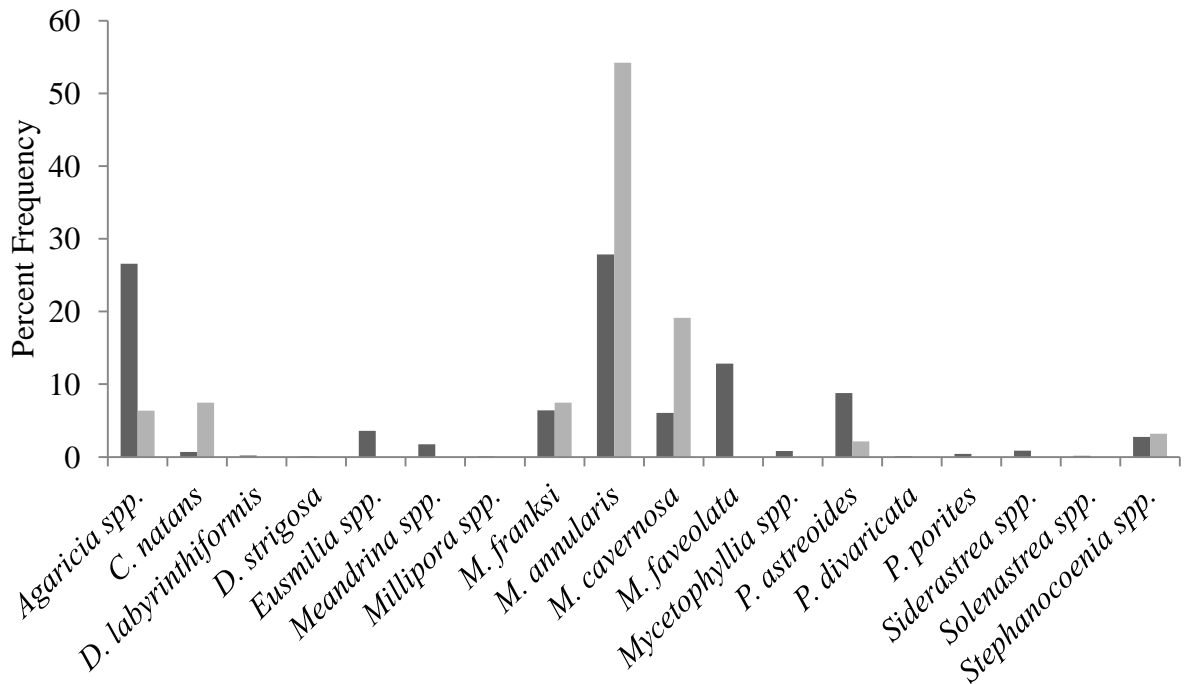


Fig. 2 Comparison among percent frequency cover of each coral species and percent frequency of coral species chosen by the goby species *C. lipernes*. Dark gray bars indicate total coral cover and light gray bars indicate *C. lipernes* found on coral species

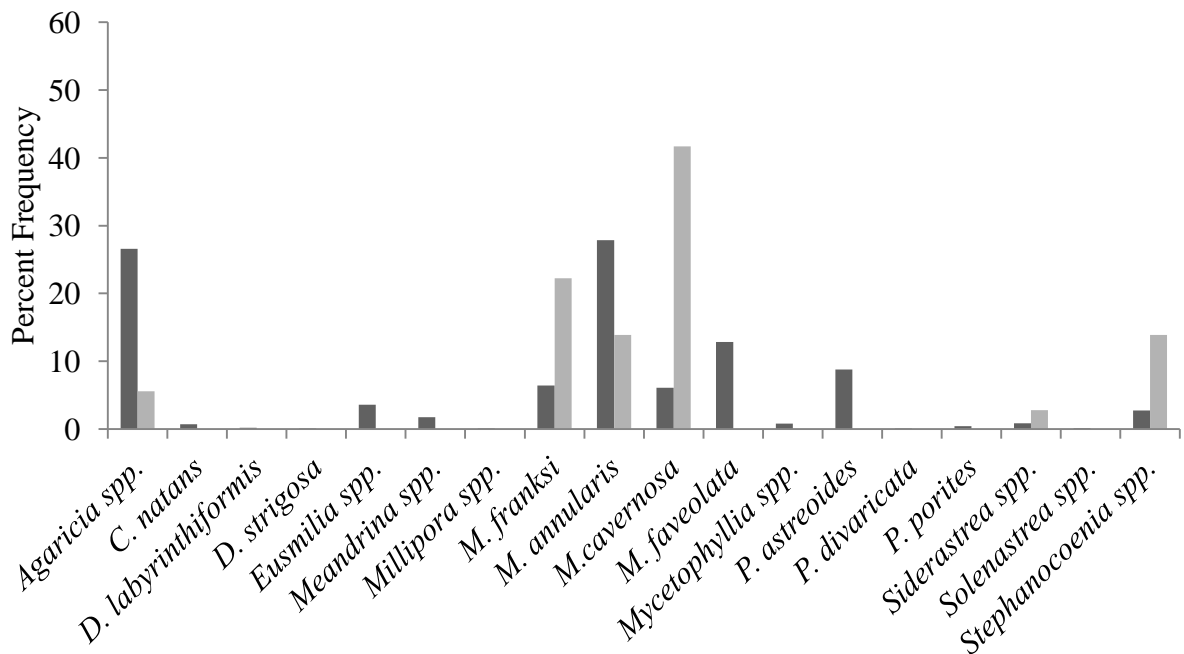


Fig. 3 Comparison among percent frequency cover of each coral species and percent frequency of coral species chosen by the goby species *G. evelynae*. Dark gray bars indicate total coral cover and light gray bars indicate *G. evelynae* found on coral species

Gobies

A total of 130 gobies were identified, 94 *C. lipernes*, found resting on 7 coral species

and 36 *G. evelynae*, found resting on 6 species. *C. lipernes* were most commonly observed on *M. annularis* (54.26%; Fig. 2) and *G. evelynae* were observed most

commonly on *M. cavernosa* (41.6%; Fig. 3).

C. lipernes selected for ($w > 1$) three coral species and against ($w < 1$) five (Table 1). The strongest selection was for *C. natans* ($w = 10.81$) and *M. cavernosa* ($w = 3.15$) and against *M. faveolata* ($w = 0$) and *Eusmilia* spp. ($w = 0$). *G. evelynae* showed a selection for 3 coral species and against 5 (Table 1). The strongest selection was towards *M. cavernosa* ($w = 6.86$) and *Stephanocoenia* spp. ($w = 5.06$) and against *M. faveolata* ($w = 0$) and *Eusmilia* spp. ($w = 0$).

Of the 94 *C. lipernes* individuals found, 12 were observed on 3 diseased coral species (Fig. 4). A positive and negative selection for disease was found varying among coral species (Table 2). The mean selection ratio of *C. lipernes* for diseased coral (0.39) was significantly less than 1 ($t = -2.27$, $df = 5$, $p = 0.036$), illustrating a significant selection against disease. A t-test was not performed on *G. evelynae* because no variation was seen among coral species, and the goby showed a complete selection against disease, with a mean selection ratio of 0 (Table 2).

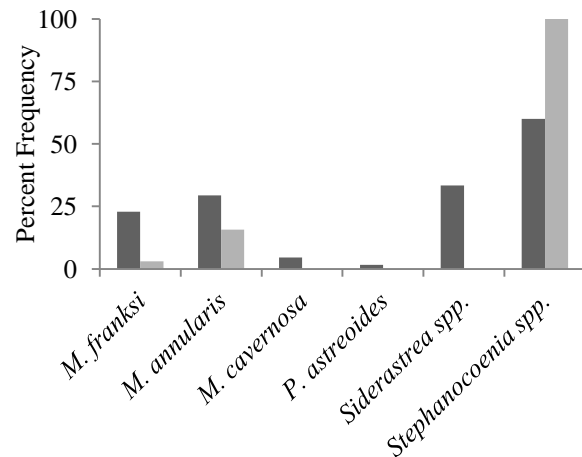


Fig. 4 Comparison between percent frequency of diseased colonies and percent frequency of diseased coral chosen by *C. lipernes*. Dark gray bars denote diseased coral and light gray bars denote *C. lipernes*

Table 2 Selection ratio of *C. lipernes* and *G. evelynae* for diseased coral species found at Yellow Sub. Asterisk indicates a mean ratio significantly less than one. N/A indicates that *G. evelynae* were not observed on coral species *P. astreoides* found on diseased coral

Coral	<i>C. lipernes</i>	<i>G. evelynae</i>
	Selection ratio (<i>w</i>)	Selection ratio (<i>w</i>)
<i>M. franksi</i>	0.13	0.00
<i>M. annularis</i>	0.53	0.00
<i>M. cavernosa</i>	0.00	0.00
<i>P. astreoides</i>	0.00	N/A
<i>Siderastrea</i> spp.	0.00	0.00
<i>Stephanocoenia</i> spp.	1.67	0.00
Mean	0.39 *	0.00

Table 1 Selection ratio of *C. lipernes* and *G. evelynae* for coral species found at Yellow Sub. Asterisk indicates when significance was found following the protocol outlined in the methods

Coral	<i>C. lipernes</i>		<i>G. evelynae</i>	
	Selection ratio (<i>w</i>)	Significance	Selection ratio (<i>w</i>)	Significance
<i>Agaricia</i> spp.	0.24	*	0.21	*
<i>C. natans</i>	10.81	*	0.00	
<i>D. labyrinthiformis</i>	0.00		0.00	
<i>D. strigosa</i>	0.00		0.00	
<i>Eusmilia</i> spp.	0.00	*	0.00	*
<i>Meandrina</i> spp.	0.00	*	0.00	
<i>Millipora</i> spp.	0.00		0.00	
<i>M. franksi</i>	1.16		3.47	*
<i>M. annularis</i>	1.95	*	0.50	*
<i>M. cavernosa</i>	3.15	*	6.86	*
<i>M. faveolata</i>	0.00	*	0.00	*
<i>Mycetophyllia</i>	0.00		0.00	
<i>P. astreoides</i>	0.24	*	0.00	*
<i>P. divaricata</i>	0.00		0.00	
<i>P. porites</i>	0.00		0.00	
<i>Siderastrea</i> spp.	0.00		3.21	
<i>Solenastrea</i> spp.	0.00		0.00	
<i>Stephanocoenia</i> spp.	1.16		5.06	*

Discussion

In this study, each goby selected for and against certain coral species found on the reef at the study site, and selected against coral disease. *C. lipernes* showed a selection for three species of the 18 corals identified (*C. natans*, *M. annularis*, *M. cavernosa*). *G. evelynae* selected for three coral species, different than those selected by *C. lipernes* (*M. franksi*, *M. cavernosa*, *Stephanocoenia* spp.). Six coral species were identified with disease with both goby species selected against the presence of disease. The results support both hypotheses, showing that *C. lipernes* and *G. evelynae* are habitat-specific, preferring certain coral species and non-diseased colonies. *C. lipernes* and *G. evelynae* were observed on some of the same coral species as seen in Jamaica (Smith and Tyler 1977), which shows that both goby species display strong habitat specificity.

Coral reefs all over the world are experiencing stressors that are reducing live coral cover (Schiemer et al. 2009; Sutherland et al. 2004; Weil 2001) and many coral dependent fishes are significantly affected by live coral abundance. Many studies have found that when coral cover decreases, fish assemblages decline as a result (Bell and Galzin 1984; Bobin et al. 2009; Chabanet et al 1997; Coker et al. 2012; Feary 2007; Jones et al. 2004; Munday et al 1997; Wilson et al. 2006). Reefs in the Caribbean have been declining over the last few decades, decreasing in live coral cover by 80% (Weil 2001). The increase in widespread disease is thought to be the major cause (Sutherland et al. 2004; Weil 2001). The Caribbean is known as the disease “hot spot,” where 70% of all coral diseases can be found on up to 82% of the coral species. Some species are affected by disease more than others; *Colpophyllia* spp. and *Montastraea* spp. have low resilience and are susceptible to four different types (Sutherland et al. 2004; Weil 2001). Low resilience increases the risk of contracting a disease and decreases the abundance of preferred coral colonies of *C. lipernes* and *G. evelynae*. As habitat

availability decreases, goby populations may begin to decline (Jones et al. 2004).

For this study, more replicate goby searches would have been desired, and conducting research at sites that varied in coral cover and disease would have offered a better understanding of habitat selection of *C. lipernes* and *G. evelynae*. Habitat-specific fish are vulnerable to environmental changes that affect their preferred habitat. Being as dependent on live coral as the Gobiidae family, population abundance can be significantly affected as changes occur. Environmental stressors continue to rise and coral reefs continue to degrade, goby species that rely on live coral for shelter and food may be at risk of regional extinctions (Jones et al. 2004). Knowledge of habitat preference of reef dependent fish species provides an understanding of the importance connectivity has on the reef.

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