Physis

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Journal of Marine Science

CIEE Research Station Bonaire
Tropical Marine Ecology &
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"There is no end in nature, but every ending is a beginning; that there is always another dawn risen on mid-noon and under every deep a lower deep opens …."

-Ralph Waldo Emerson

In *Heal the Ocean*, *Physis* is defined as "allowing nature to heal itself." We, as humans, often revel in the beauty of nature, but a significant part of nature's beauty is its ability to stand alone—it begs no human interference. Indeed, throughout our history we have separated ourselves from nature. Our capacity for conscious decision-making and our ability to manipulate tools have given us a distinction among animals: rather than melding with the environment around us in mutualistic relationships, we have become an opposing force, interrupting and often harming the natural flow of planet earth's biology. Without us, nature weaves an intricate web of species and ecosystems that have evolved with precision to withstand disease and destruction. Our involvement has resulted in the degradation of our home's finest attributes. It may sound harsh, but in many respects we have become the patrons of disease and destruction.

Physis is the way the earth is, the way the earth heals itself, and the way the earth progresses over time. It is the interconnectedness of every ecosystem on earth, the way the forests of Mali affect the worldwide climate, or how the dust from Africa spreads and has effects on the other side of the globe. At this point in time we must realize that we have disrupted *Physis*, and we must begin the delicate process of re-instilling it into the earth to protect our home for generations to come.

Throughout the semester at CIEE we have gained a great deal of knowledge on conservation and protection efforts taking place on Bonaire. As students, we have taken part in this effort toward making the community of Bonaire more aware of the fragile ecosystem by which they are constantly surrounded. Our projects involving numerous chemical, nutrient and visual sampling have not only aided conservation efforts but also provided new knowledge that may be built upon by future CIEE students and members of the community near and far.

CIEE Bonaire, Spring 2011

FOREWORD

The Council on International Educational Exchange (CIEE) is an American non-profit organization with over 100 study abroad programs in 41 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 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 6th of April 2011 at the 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 Eva Toth, PhD. In addition to faculty advisors, each student had CIEE Interns that were directly involved in logistics, weekly meetings and editing student papers.

CIEE FACULTY AND STAFF

RITA PEACHEY, PH.D.
Resident Director

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 coral reef ecology and she is particularly interested in larval ecology and effects of UV radiation on larval stages of marine invertebrates. At CIEE, Dr. Peachey teaches Independent Research and the Cultural and Environmental History of Bonaire. Her advisees for Independent Research this semester were Grace Giampietro, Meaghan Harty, Leah Harper, Kyle McBurnie and Charlotte McCleery.

EVA TOTH, PH.D.
Tropical Marine Conservation Biology Faculty

Dr. Eva Toth has always been fascinated with the tropics. She started out as a terrestrial ecologist, studying social stingless bees in Costa Rica M.S (Utrecht University, NL) and Brazil Ph.D (Rice Univeristy, TX). She became interested in marine science by studying social sponge-dwelling shrimp (Virginia Institute of Marine Science, Smithsonian Tropical Research Institute) in Belize and Panama, and fell in love with marine environments. She is interested in terrestrial and marine conservation and teaches Independent Research and Tropical Marine Conservation Biology. Her advisees for Independent Research this semester were Christopher Sundby, MaliaKelly White, Trevor Poole, Lori Sako and Ashton Williams.

CAREN ECKRICH
Coral Reef Ecology Faculty, Dive Safety Officer

Professor Caren Eckrich is the Coral Reef Ecology Faculty and the Dive Safety Officer for CIEE. She holds a B.S. in Wildlife and Fisheries from Texas A&M University and a M.S. in Biological Oceanography at the University of Puerto Rico in Mayaguez. Caren is the instructor for Coral Reef Ecology, Marine Ecology Field Research Methods and Advanced SCUBA. She manages dive planning for the student independent research projects and has a wealth of local experience on the reefs that contributes to the success of student projects. Caren's research interests include fish behavior, seagrass and algal ecology, sea turtle ecology, and coral disease.

AMY WILDE
Administrative Assistant

Amy Wilde is the Administrative Assistant for CIEE. She holds a B.S. degree in Business Administration, as well as, a Masters of Science in Management Administrative Sciences in Organizational Behavior, from the University of Texas at Dallas. She has worked in call center management for the insurance industry and accounting for long term care while living in Texas. Amy currently provides accounting and administrative support for staff and students at CIEE.

ANOUSCHKA VAN DE VEN
Assistant Resident Director

Anouschka van de Ven is the Assistant Resident Director for CIEE. She is a PADI Dive Instructor and underwater videographer and she assists with Advanced SCUBA and the Cultural and Environmental History of Bonaire courses. Anouschka has a B.A., First Class Honours Degree in Communications Studies, from the London Metropolitan University and worked in television and advertising in Amsterdam before moving to Bonaire. She provides administrative support for the research station and is responsible for the website and public relations. She is also a volunteer operator at the hyperbaric chamber.

CIEE INTERNS

CAMERRON CROWDER
Coral Reef Ecology Intern

Camerron assisted Professor Eckrich with the Coral Reef Ecology course, assisted Dr. Toth with Independent Research, and served as the dive master for the Advanced SCUBA and Marine Ecology Field Research Methods courses. While on Bonaire, Camerron worked as a research assistant investigating the presence of lionfish in Bonaire's Lac Bay. Although originally from Birmingham, AL she received her B.S. in Marine and Molecular Biology from The Evergreen State College in Olympia, WA and her M.S. in environmental microbiology from Northern Arizona University. She is thrilled to be starting her Ph.D in marine zoology beginning fall 2011 at Oregon State University and feels that her time in Bonaire was invaluable in preparing her for her doctorate work.

JENNIFER BLAINE
Conservation Biology Intern

Jen assisted Dr. Eva Toth with Tropical Marine Conservation Biology and Dr. Rita Peachey with Independent Research and Cultural and Environmental History of Bonaire. Jen is originally from Ohio, where she earned her B.S. in Biology and Marine Science at Wittenberg University. In December 2010, she finished her M.S. in Environmental Science at Washington State University in Vancouver, WA, where she worked on the baseline surveys of the central CA Marine Protected Areas. In Bonaire, Jen assisted in the research of lionfish presence in Lac Bay. After CIEE, she hopes to work at the intersection of science, policy, and the public on issues of marine conservation and sustainable fisheries…and will forever be eager to share her passion for sea cucumbers!

STUDENT PROFILES

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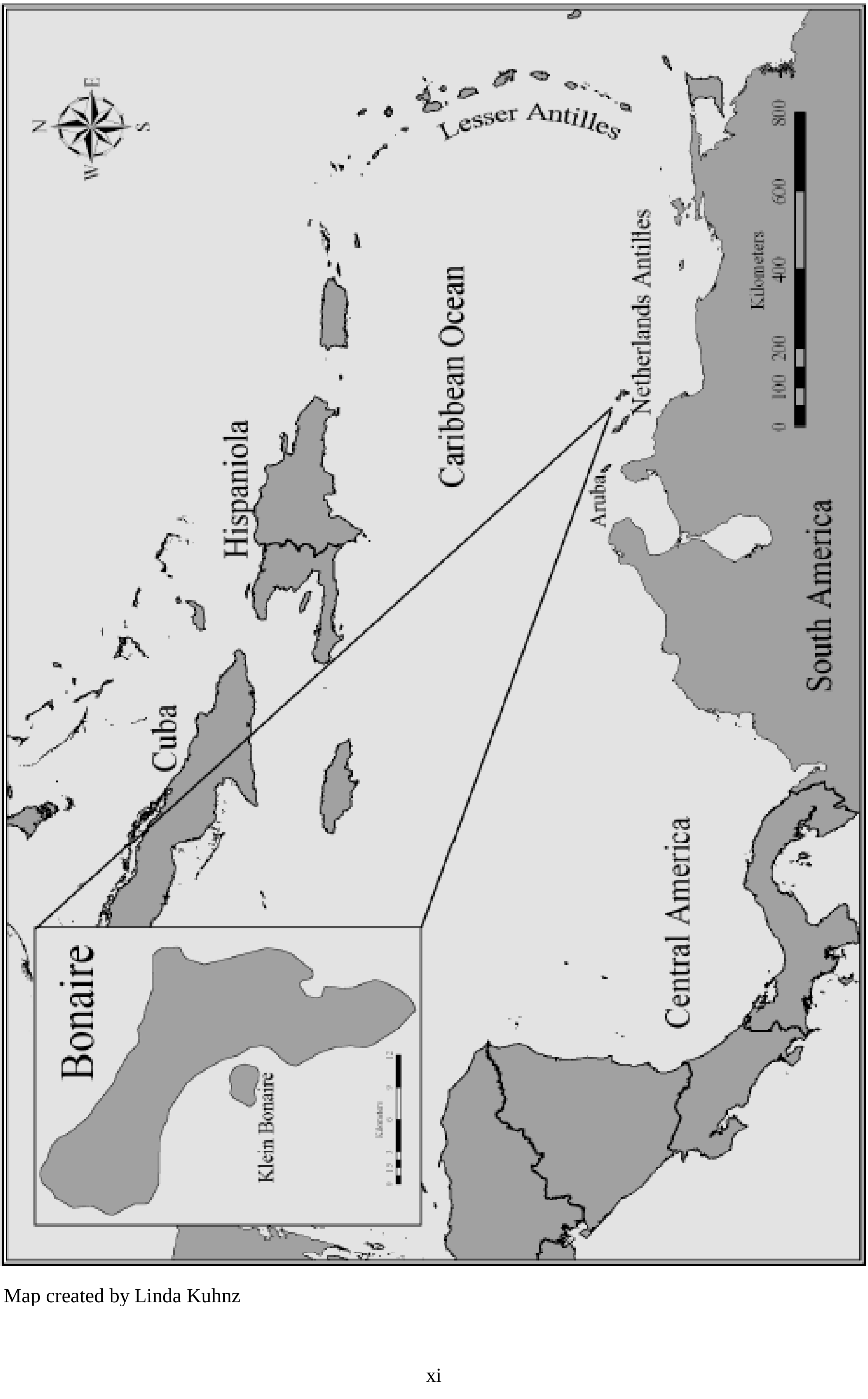


Map created by Linda Kuhnz

Effects of Nutrient Enrichment and Water Quality on Coral Disease Prevalence in Bonaire, Dutch Caribbean

Grace Giampetro

University of Minnesota, Twin Cities

Abstract

Trying to understand the extent to which anthropogenic stressors impact coral reefs globally has led to an increase in studies which analyze the effects of nutrient enrichment on the frequency and severity of coral disease. Bonaire, Dutch Caribbean currently has no sewage treatment facility in place, resulting in the percolation of wastewater to the surrounding coastal marine environment. On the reefs near resorts, there is a large volume of groundwater used and subsequently discharged into the ocean. As a result, the reefs directly in front of major resorts are likely to have higher disease levels than reefs without resorts nearby. The goal of this study was to evaluate the difference in prevalence of coral disease between sites located in close proximity to groundwater discharge and sites located further away. In order to achieve this objective, six sites with varying gradients of exposure to sewage discharge were surveyed by laying down 1 m x 30 m transect belts parallel to the shore at 6 m, 12 m and 18 m depths. During each survey, nutrient enrichment, macroalgal cover, water depth and coral colonies displaying signs of disease were recorded. Water quality was assessed using a number of parameters including nutrients (ammonium, ammonia, phosphate and dissolved oxygen), *Enterococci* bacteria and sedimentation. At sites closer to resorts there were higher nutrient levels and percent cover macroalgae, however sedimentation rates and mean percent coral disease frequency were highest at medium impacted sites. Low impacted sites had a greater presence of coral disease at shallower depths, compared to high impacted sites. This data will be used to illustrate a relationship between coral disease and anthropogenic stressors and provide a baseline for future studies.

Introduction

The biological diversity of coral reefs provides humans with a multitude of ecosystem services including fisheries, coastal protection, building materials and tourism (Hoegh-Guldberg et al. 2007). However, the rapid increase of human populations along the coastlines have led to new and more intense anthropogenic pressures on ocean systems, such as untreated wastewater and terrestrial runoff which are discharged into the ocean and increase the amount of nutrients present in the ocean (Voss et al. 2006). In the tropics, such increases have lead to harmful impacts on coral reefs decreasing coral recruitment and resilience to disturbances (Smith et al. 2008). Sutherland et al. (2004) suggests that there has been a culmination of evidence linking human activity in the watershed and coral decline resulting from increases in the amount of new disease and species affected.

There are multiple anthropogenic factors that increase the prevalence of organic matter which increases susceptibility of corals to disease in coastal regions (Kline et al. 2006). Organic carbon and sewage discharge have been linked to disease in corals (Kline et al. 2006). Organic carbon treatments lead to pathologies similar to ones reported for band diseases on *Montastraea annularis* (Kline et al. 2006). There is a greater prevalence of disease near sites within close proximity to sewage discharge where there was a higher presence of *Enterococci* and fecal coliform levels (Kaczmarsky et al. 2005). The most common factor identified is nutrient enrichment resulting from increasing human populations (Nixon 1995) and many tropical islands that have coral reefs have no waste treatment (Kaczmarsky et al. 2005).

There are a large number of studies indicating that water eutrophication alters growth and reproduction rates which affects the tight knit symbiotic relationship between coral and zooxanthellae that support coral reef growth (Szmant 2002). Under nutrient enriched conditions, pathogen fitness and virulence are able to become more successful which makes coral more susceptible to diseases (Bruno et al. 2003). As a result, coral disease has become increasingly common in the world's oceans (Bruno et al. 2003). Voss and Richardson (2006) suggest that higher nitrate concentrations can double the migration rate of black band disease. Furthermore, Bruno et al. (2003) indicated that small increases in nutrient concentrations had a large effect on the abundance of coral disease and coral death after conducting in *situ* field experiments focusing on two important Caribbean coral epizootics, *Aspergillus sydowii*, which affects the common gorgonian sea fan *Gorgonia ventalina* and yellow band disease, which often affects *Montastraea* species. Eutrophication of water systems with gradually increased distance from shoreline development and other land-based activities lead to run off and pollution, the effects of which have been observed predominantly in shallower waters (Bak et al. 2005). Influxes of nutrients have led to shifts from coral dominated to algal dominated communities. McManus et al. (2004), in a review of coral-algal phase-shifts, implies that shifts are a result of increases in stress from increased nutrient loading and herbivore reductions; however there is a tendency for major shifts to occur after large scale disasters such as hurricanes, coral disease outbreaks, or widespread bleaching.

Since the 1970s, Bonaire, Dutch Caribbean, has experienced degradation of its coral reefs, which has been associated with anthropogenic disturbances such as shoreline development and land-base changes which lower the water quality and create an influx of nutrients. (Bak et al. 2005). Therefore, increasing coastal and sewage discharge are a possible cause of increased nutrient, sediment and bacterial loads, especially along the leeward coast of the island where residential areas and resorts are concentrated. (Bak et al. 2005). Due to the lack of a wastewater treatment plant on the island, the release of sewage into the ground by the use of cesspits and unlined septic tanks has negatively impacted the groundwater quality in the region (van Sambeek et al. 2000). Kralendijk, Bonaire's capital, lies on limestone bedrock. The porous nature of limestone allows groundwater and sewage to easily percolate through, which then leaches into the surrounding marine environment (van Sambeek et al. 2000). Van Kekem et al. (2006) sampled septic tanks near resorts along Kralendijk's west coast and found that nitrogen in the form of ammonia (NH_4^+) was as high as 70 mgL^{-1}, whereas values of NH_4^+, on the east coast were undetectable. However, the effects of the untreated sewage on coral reefs and disease levels in Bonaire remain unclear.

The objective of this study is to compare the effects of water sewage discharge on the surrounding coral reef environment in relation to increasing distance from the source to see if there is a relationship between coral disease and nutrient enrichment. A gradient of sites (low impacted to high impacted) will be included in this study. Low impacted will be sites located further away from sewage discharge. High impacted sites will be located in close proximity to sewage discharge. At each site, frequency of diseased coral, levels of nutrient concentrations, percent cover of macroalgae, levels of *Enterococci* bacteria and sedimentation rates will be measured. It is expected that contaminated groundwater will contain elevated nutrient levels, increased macroalgal cover and fecal bacteria. The following hypothesis will be tested:

H_1: There will be higher nutrient levels, percent cover of macroalgae, sedimentation rates, bacteria and frequency of coral disease at high impact sites compared to low impact development sites.

H_2: Coral disease will be more prevalent at shallower depths.

This study will add a greater body of information on the effects of eutrophication on Bonaire's coral reef ecosystem.

Methods

Site selection

This study was conducted during February through March on the island of Bonaire, Dutch Caribbean (Fig. 1). A total of six sites were selected based on their proximity to major resorts, where greater release of groundwater discharge is likely (van Kekem et al. 2006). Two reefs that were selected where major resorts are located include 18th Palm Reef (12.13861°N, 068.27644°W) and Bari Reef (12.16768°N 068.28634°W). Other sites are located at intermediate distances from a major resort including Cha Cha Cha Reef (12.1420°N 068.27652°W), 0.5 km away from a major resort and Kas di Arte (12.0922°N 068.1653°W), 0.9 km away from a major resort. Two reefs furthest away from resorts and groundwater discharge are Jerry's Reef (12.0847°N 068.1749°W) on Klein Bonaire, 2.7 km away from the closest resort and Witches Hut (12.1217°N 68.1842°W), 3.8 km away from the closest resort.

Fig. 1 Map of Bonaire, D.C. Witches Hut and Jerrys are less impacted, Kas Di Arte and ChaChaCha Reef are medium impacted, Bari Reef and 18th Palm are more impacted. (STINAPA)

Identification of coral disease

Surveys to identify coral disease were conducted using SCUBA and a 30 m transect tape and slate to record coral disease. At each site, 30 m x 1 m transects were laid down on the reef following the depth contour conducted at 6 m, 12 m, 18 m depths. For the first 10 m of each transect, a diver recorded the species of the coral head >10 cm and, if diseases were present, identified the type of coral disease. On the next 10 m, the diver followed the Atlantic and Gulf Rapid Reef Assessment (AGRRA) Benthic procedure to measure the percentage of live coral, dead coral, turf algae, macro algae, rubble, sand and other. For the final 10m, the diver followed the same procedure used for the first 10 m.

Nutrient levels

Nutrient samples were collected before conducting the AGRRA methodology at each site samples were taken at the three depths surv. One 250 mL water bottle sample was collected at each depth. In order to do so, empty bottles were first filled at the surface with water. Once at depth, bottles were opened, turned upside down, filled with air and turned right side up. This procedure was repeated three times. Water samples were placed on ice immediately after surfacing to ensure nutrient levels remained intact. Nutrient levels that were measured include nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_4^+). Nutrients were analyzed using the LaMotte Salt Water Aquaculture test kit within two hours of collection. Phosphate (PO_4^{3-}) levels were analyzed using the Hanna Instruments phosphate analysis kit.

Bacteria samples

Water samples for bacterial analysis were collected at the start of each 30 m transect. Before each dive, three sterile 100 mL bottles were filled with sterile water to reduce buoyancy. Once at sampling depth, bottles were opened, turned upside down, filled with air from the diver's second stage to release the sterile water then turned right side up to fill the bottle. To test for the presence of *Enterococci* bacteria, a common fecal indicator bacteria that is used to identify sewage contamination (Kaczmarsky et al. 2005) the Enterolert testing system (IDEXX, Philadelphia, PA) was employed. This was completed once at each depth.

Sedimentation analysis

At each site and depth, sediments were collected using PVC traps (7.5 cm diameter, 15 cm long). Collection and analysis of sediment, including particle size, were modified from Gleason's (1998) methodology. Traps were closed off at the base and left open on the top. This allowed sediments to settle inside. Above the substrate, PVC traps were attached ~10 m above in an upright position to a rebar stake (1m). At 5 m, the traps were capped, collected and replaced using SCUBA on a weekly basis. At 12 m, the traps were capped, collected and replaced using SCUBA on a biweekly basis. Once collected, the traps were brought to the laboratory and sediments were left to settle for ~1 h until surface saltwater was decanted off. Following this, sediments were gently rinsed three times using tap water (between each rinse water was decanted), to dissolve any remaining salts, poured into a pre-weighed aluminum foil container, and placed in a drying oven (~ 40 °C) for 48 h. When sediments were dry, remaining visible organic material was removed using forceps. The dry sample was re-weighed to calculate sedimentation rate (mg cm^{-2} day^{-1}).

Statistical Analyses

A series of 2-factor ANOVAs were used to assess interactions between and within depth, site impact level and water chemistry. Fisher's Protected Least-Significant Difference (PLSD) post hoc analyses for pair-wise comparisons were used to determine significance between factors tested at $\alpha = 0.05$. A one-factor ANOVA test was used to test sedimentation rates between site gradients.

Results

Identification of Coral Disease

A significant effect of site type on live coral cover was detected (Table 1a). Percent live coral was significantly affected by depth specifically between 6 m and 12 m, and between 6 m and 18 m (Table 1b). However, such differences in depth were not significant between 12 m and 18 m (Table 1b). Live coral cover was significantly higher at low impacted than medium and high impacted sites (Table 1c). Percent coral cover did not significantly differ between medium and high impacted sites (Fig. 2c).

Percent cover of macroalgae was not significantly affected by site impact level (Table 2a). However, there was a significant difference by depth, specifically between 6 m and 18 m, and between 12 m and 18 m (Table 2b). Such differences in depth were not found between 6 m and 12 m. Although insignificant, lower impacted sites experienced higher percentage of macroalgae cover compared to medium impacted sites.

Additionally, there were no significant differences in the mean frequency of disease found between site impact and depth (Table 3a, b, c) (Fig. 2c). At high impacted sites, mean frequency of disease decreased as depth decreased. At low impacted sites, mean frequency of disease increased as depth decreased. This was not the case for medium impacted sites. There was a higher mean frequency of disease present overall at medium impacted sites at 6 m, 12 m and 18 m (36.11, 45.09 and 36.36 ± 5.114 SD**,** respectively).

Nutrients

There were no significant differences between site impact and depth for ammonia (NH_4^+) (Table 5a, b and c). Although insignificant results were found, ammonia slightly increased between low to high site gradients (Fig. 3a). Nitrite (NO_2^-) and nitrate (NO_3^-), levels were consistent between site gradients (0.05 and 0.25 ppm, respectively) (Fig. 3a). Phosphate (PO_4^{3-}) showed no significant trend between site gradients (Fig. 3b). Although insignificant, there was more phosphate present at high impacted sites. Significant results were found between 12 m and 18 m but not between 6 m and 12 m or 6 m and 18 m.

Bacteria Samples

Five of the 18 (28%) water samples collected tested positive for *Enterococci*. Presence occurred two times at high impacted sites, two times at medium impacted sites, and once at low impacted sites. For these samples, ranges were from 1.0 to 7.5 colony-forming units (cfu). Witches Hut was one of the least impacted sites, yet had the highest colony-forming units recorded (7.5 cfu) at 18 m. Cha Cha Cha, a medium impacted site, had *Enterococci* presence (1.0 cfu) at both 6 m and 12 m. Finally, 18th palm, a highly impacted site, had 1.0 cfu at 18 m and 2.0 cfu at 6 m.

Sedimentation

Mean sedimentation rates were slightly higher (Fig. 4), but not significantly higher at medium impacted sites (0.089 ± 0.013 SD mg cm^{-2} day^{-1}) compared to low impacted sites (0.059 ± 0.012 SD mg cm^{-2} day^{-1}). High impacted sites also had a lower sedimentation rate than medium impacted

Table 1 (a) Two-way ANOVA testing the effects of (6, 12, 18 m) and site type (low, med and high) and the percent of live coral present. **(b, c)** Fisher's PLSD post-hoc comparisons within factors (depth and site type, α = 0.05, S = significant).

(a)

Source of Variation	DF	SS	MS	F	P
Site	2	2,716.62	1,358.31	10.687	0.0004
Depth	2	2,742.04	1,371.02	10.787	0.0004
Site x Depth	4	637.98	159.494	1.255	0.3118
Type	27	3,431.62	127.097		

(b)

Depth	Mean Diff	Crit Diff	P-Value	
6m, 12m	12.45	9.444	0.0117	S
6m, 18m	21.275	9.444	<.0001	S
12m, 18m	-8.825	9.444	0.0658	

(c)

Site	Mean Diff	Crit Diff	P-Value	
low, med	20.95	9.444	0.0001	S
low, high	13.7	9.444	0.0061	S
med, high	-7.25	9.444	0.1268	

Table 2. Two-way ANOVA testing the effects of depth (6, 12, 18 m) and site type (low, med and high) and the percent of macroalgae present. **(b, c)** Fisher's PLSD post-hoc comparisons within factors (depth and site type, α = 0.05, S = significant).

(a)

Source of Variation	DF	SS	MS	F	P
Site	2	556.727	278.363	2.09	0.38
Depth	2	2,245.30	1,122.65	8.43	0.955
Site x Depth	4	1,056.11	264.028	1.983	0.512
Type	27	3,595.71	113.175		

(b)

Depth	Mean Diff	Crit Diff	P-Value	
6m, 12m	-6.75	9.667	0.1634	
6m, 18m	-19.075	9.667	0.0004	S
12m, 18m	-12.325	9.667	0.0144	S

(c)

Site	Mean Diff	Crit Diff	P-Value
low, med	-0.01093	9.667	0.6468
low, high	-0.04147	9.667	0.0608
med, high	-7.033	9.667	0.1417

Table 3. **(a)** Two-way ANOVA testing the effects of (6, 12, 18 m) and site type (low, med and high) and the percent of disease present. **(b, c)** Fisher's PLSD post-hoc comparisons within factors (depth and site type, α = 0.05, S = significant).

(a)

Source of Variation	DF	SS	MS	F	P
Site	2	918.804	459.4	1.114	0.3695
Depth	2	43.66	21.83	0.053	0.9487
Site x Depth	4	337.39	84.348	0.205	0.9295
Type	9	3,709.92	412.213		

(b)

Depth	Mean Diff	Crit Diff	P-Value
6m, 12m	-2.801	26.517	0.8165
6m, 18m	0.842	26.517	0.9443
12m, 18m	3.643	26.517	0.763

(c)

Site	Mean Diff	Crit Diff	P-Value
low, med	-16.092	26.517	0.203
low, high	-2.09	26.517	0.8625
med, high	14.003	26.517	0.2628

Table 4 **(a)** Two-way ANOVA testing the effects of (6, 12, 18 m) and site type (low, med and high) and the concentration of phosphate (ppm). **(b, c)** Fisher's PLSD post-hoc comparisons within factors (depth and site type, α = 0.05, S = significant).

(a)

Source of Variation	DF	SS	MS	F	P
Site	2	0	0	0.289	0.7559
Depth	2	0.004	0	3.383	0.0802
Site x Depth	4	0.01	0.002	3.284	0.0604
Type	9	0.01	0.001		

(b)

Depth	Mean Diff	Crit Diff	P-Value	
6m, 12m	-0.01	0.033	0.5159	
6m, 18m	0.842	0.033	0.0994	
12m, 18m	3.643	0.033	0.0331	S

(c)

Site	Mean Diff	Crit Diff	P-Value
low, med	0.005	0.033	0.7678
low, high	0.011	0.033	0.4695
med, high	0.007	0.033	0.6628

Table 5 (a) Two-way ANOVA testing the effects of (6, 12, 18 m) and site type (low, med and high) and the concentration of ammonia (ppm). **(b, c)** Fisher's PLSD post-hoc comparisons within factors (depth and site type, α= 0.05, S = significant).

(a)

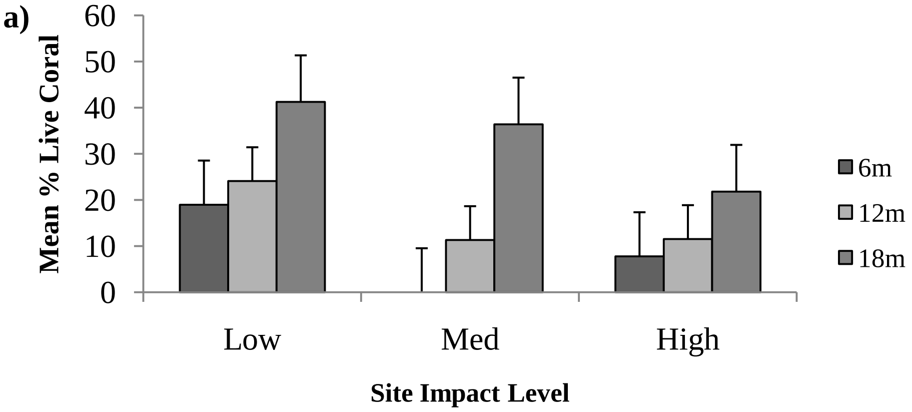
Source of Variation	DF	SS	MS	F	P
Site	2	1.149	0.58	1.308	0.3172
Depth	2	0.302	0.15	0.344	0.718
Site x Depth	4	0.37	0.923	2.101	0.1632
Type	9	0.4	0.438		

(b)

Depth	Mean Diff	Crit Diff	P-Value
6m, 12m	-0.2	0.866	0.6139
6m, 18m	-0.313	0.866	0.4341
12m, 18m	-0.113	0.866	0.7738

(c)

Depth	Mean Diff	Crit Diff	P-Value
low, med	-0.427	0.866	0.2938
low, high	-0.602	0.866	0.1504
med, high	-0.175	0.866	0.6583



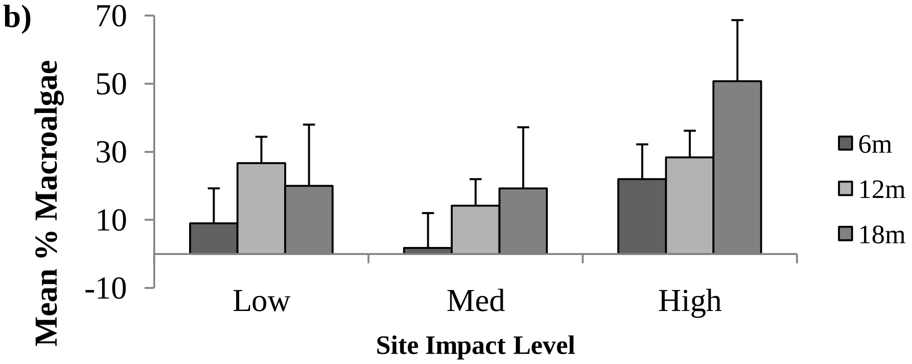


Fig. 2 a) Mean percentage of live coral (± SD) at 6m, 12m and 18m between low, medium and high impacted sites. **b)** Mean percentage of macroalgal cover (± SD) between low, medium and high impacted sites. **c)** Mean percentage of coral disease (± SD) between low, medium, and high impacted sites.

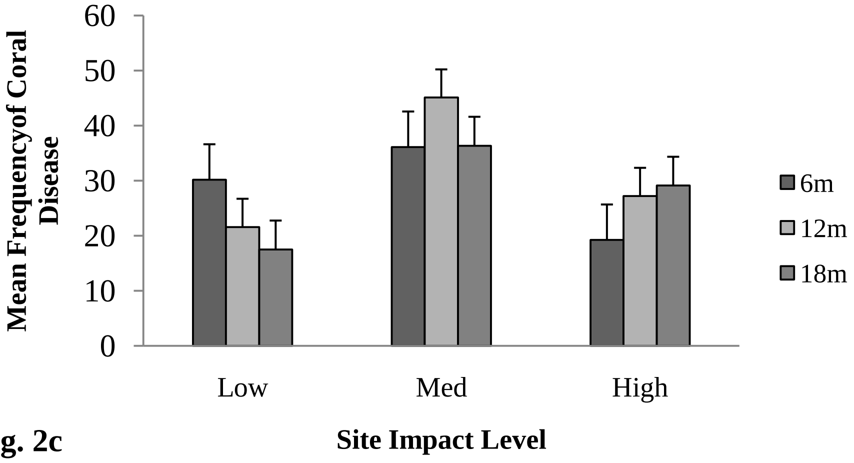


Fig. 2c

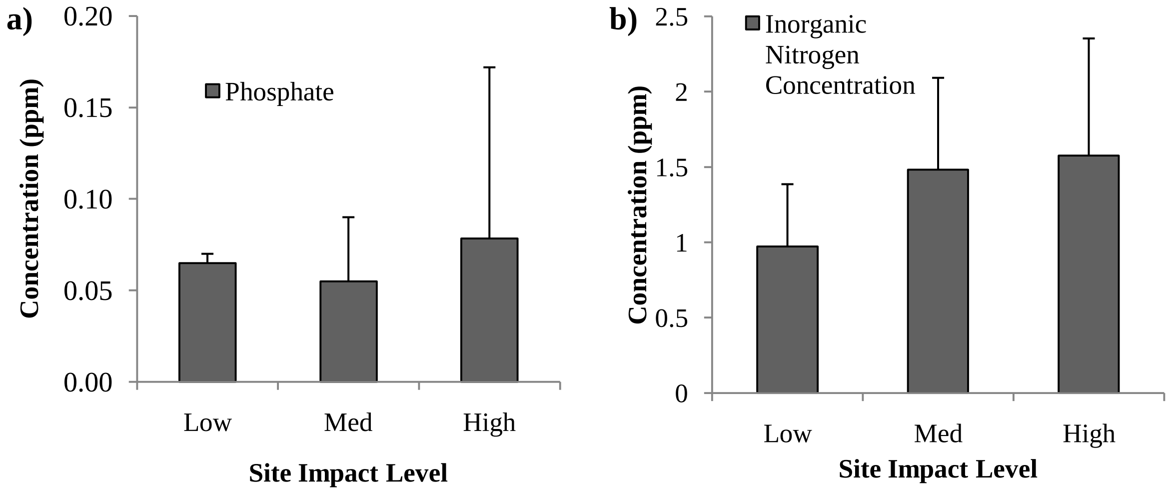


Fig. 3 a) Mean NO3-, NO2- and NH4-+ (± SD ppm) at high, medium and low impacted sites.
b) Mean PO43-(± SD ppm) at high, medium and low impacted sites.

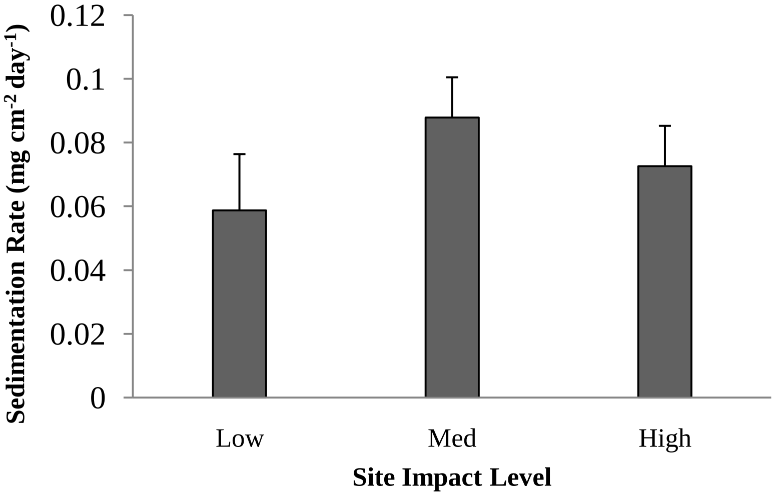


Fig. 4 Mean sedimentation rates (± SD mg cm^{-2} day^{-1}) for low, medium and high impacted sites.

Discussion

The purpose of this study was to see if higher nutrient levels, percent of macroalgae, sedimentation rates and frequency of coral disease are affected by sewage water discharge and depth. At sites closer to resorts there were higher nutrient levels and percent cover macroalgae. Sedimentation rates and mean percent coral disease frequency were highest at medium impacted sites. This study also analyzed coral disease prevalence at shallower depths and deeper depths. It was found that low impacted sites had a greater presence of coral disease at shallower depths, compared to high impacted sites which had greater presence of coral disease at deeper depths.

The minimal levels of PO_4^{3-}, NO_3^-, NO_2^- and NH_4^+ could imply that these have a lesser impact on the current condition of Bonaire's reefs during the time this study was conducted. However, the equipment used to test for nutrients was a hindering factor of this study since it could have produced inaccurate results. Even though no significant differences were found in nutrient levels between site impact levels there were still higher levels of NH_4^+ and PO_4^{3-} at higher impacted sites, which is important to relate to the higher percentage of macroalgae found at higher impacted sites, which could have resulted from eutrophication. Inorganic nitrogen levels above 1.0 ppm and phosphate levels above 0.3 ppm are thresholds that if exceeded, could lead to eutrophication of marine systems (Bell 1992). Results from this study found inorganic nitrogen levels for medium and high impacted sites to be above the 1.0 ppm level (Fig. 2a). Low impacted sites had levels of 0.97 ppm. This is a reason to monitor the levels and have a greater indepth analysis of how compounds affect the reefs of Bonaire. Phosphate levels were under the threshold for all three site impact levels.

The fringing reefs surrounding Bonaire are a protected marine park which has helped slow down the degradation process other reefs throughout the Caribbean have experienced. Stokes et al. (2010) found that the leeward side of Bonaire had 30% live coral cover on average compared to this study which found 29.15% live coral coverage on the sites along the leeward side of Bonaire. Stokes et al. (2010) noted that in the 1980's the leeward live coral average was greater than 80%. It's important to note that if Bonaire does not take care if its reefs, they could easily transition into the degraded reefs that are found throughout the rest of the Caribbean. In this study, there were significant differences found between live coral cover at 6 m and 12 m, and 6 m and 18 m which support the findings of Bak et al. (2005). Also, low impacted sites had a higher percentage of live coral cover than high impacted sites. Bak et al. (2005) found that over the last three decades there has been a sharp decrease in live coral on shallower Caribbean reefs and only a slight decrease in live coral on deeper Caribbean reefs. Bak et al. (2005) also suggested that shallower reefs are more susceptible degradation from anthropogenic factors because they are not connected to the resilient environment to which deep coral reefs are connected. Additionally, higher impacted sites had the greatest percentage of macroalgae overall. Low impacted sites had a greater percentage of macroalgae than medium impacted sites. This could be due to the currents of the groundwater flow which facilitates the movement of nutrients toward sites further away from where the groundwater was discharged.

There was no significant difference between high impacted sights and low impacted sites in sedimentation rate analysis. Although, medium impacted sites had a higher sedimentation rate than low and high impacted sites. The area where the medium impacted sites are located is right in front of Kralendijk, the capital of Bonaire. There have been new construction developments taking place along the road which sedimentation runoff would enter the ocean. Currently, developers are not using the best construction practices to retain sediments. New development sites involve breaking up soil and concrete mix into fine particles which increases the amount of dust present in the atmosphere resulting in sedimentation runoff into the ocean during rainfall events (BNMP 2006; Rini 2008). This could be one reason why medium impacted sites had higher sedimentation rates than the other site gradients. Higher sedimentation rates increase turbidity which could block out the light needed by zooxanthellae to maintain their symbiotic relationship with coral. Susceptibility to disease in corals could be increased by such a stressor. Cha Cha Cha Reef, a medium impacted site, had the greatest prevalence of Dark Spot Disease. Additionally, Kas di Arte, a medium impacted site, had the second highest mean frequency of coral disease. Sutherland et al. (2004) suggests that nutrient and sediment loading have the ability to transport pathogenic organisms to the surrounding marine environment.

In addition to high nutrient levels and increased sedimentation, *Enterococci*, a common fecal indicator, has been used to identify regions of sewage contamination (Kaczmarsky et al. 2005). High nutrient levels and increased sedimentation were also indicators of sewage contamination (Kaczmarsky et al. 2005). There is a great deal of evidence which suggests that the health of coral reef ecosystems is hindered by sewage pollution (Sutherland et al. 2004). Sites that were chosen for this study, although presented on a gradient level, had some flaws. The medium impacted sites are located in an inlet, so the sewage effluent percolating from resorts such as 18th Palm go through that region and can create degrade the coral reef ecosystem more than sites deemed higher impacted. Data from this study suggests that all site gradients have *Enterococci* bacteria present. Witches hut had the highest amount of presence (7.5 cfu) at 18 m. Also, at Cha Cha Cha reef (medium impacted site) *Enterococci* bacteria was present at 6 m and 12 m but not at the deepest depth, 18 m. This could be due to the currents at that particular time. Additionally, when *Enterococci* is persistent in the environment the current direction could have impacted Witches Hut where it occurred at the deeper depths. At 18th palm (high impacted site) *Enterococci* bacteria was present at the deepest and shallowest depths. However, it is important to note that limitations from these results, since sites that were chosen for this study, although presented on a gradient level, had some flaws. If groundwater percolation is the source of *Enterococci* bacteria then there should be a greater tendency for its presence at shallower depths since freshwater is less dense than sea water. Once mixing occurs, there can be *Enterococci* presence at any depth. This could be why *Enterococci* were present at both 6 m and 18 m at 18th Palm. Even though levels were not significant, the fact that *Enterococci* are present is important. Human waste has been connected with increased coral mortality (Kline et al 2006). Also, *Enterococci* on Bonaire's reefs can create negative public health effects due to the increase in fecal toxins which can be ingested as well as a decrease in fish populations and a decrease in tourism due to lower visibility levels (Bonkosky 2009).

As supported by this study, septic tank usage on Bonaire has been connected to degradation of water quality which led to the installation of organic content monitoring programs such as the Light and Motion Sensor Program (LMSP) (Jones et al. 2008). These influxes of nutrients increase the percent of macroalgae cover and create phase shifts where live coral is no longer dominant (Szmant 2002). Based on the current study, the link cannot be established between nutrients and coral disease, macroalgae cover, bacteria levels and sedimentation. However, the lack of evidence for establishing a clear link could be due to the low sensitivity of analytical testing available at. For more information on how human fecal waste specifically affects corals a study could be undertaken to find out how increasing organic matter affects the reefs along the west coast of Bonaire. The information produced by this study may be useful for BNMP and the government of Bonaire to facilitate the development of its wastewater treatment plant. Also, I would recommend expanding this study by including sites that have a larger gradient, take into account groundwater direction and use more sensitive testing gear to detect important differences.

Acknowledgements

I would like to thank Dr. Rita Peachey for her motivation and guidance in every way possible, Scott Hausmann for taking Meaghan Harty and I over to our study sites on Klein and Cha Cha Cha Reef, Professor Caren Eckrich for always being supportive, Jennifer Blaine and Cammie Crowder for their guidance and dedication throughout the whole research process, and Meaghan Harty for accompanying me on every dive, spending countless hours with me in the lab and always lending a helping hand. I would also like to thank BNMP for allowing CIEE to conduct research within their limits.

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Nest site selection of sergeant majors (*Abudefduf saxatilis*): artificial vs. natural reef structures

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Abstract

Nesting animals select sites for reproduction based on adaptive behaviors that have evolved to improve reproductive success and the survival rates of offspring. *Abudefduf saxatilis*, commonly known as the sergeant major, is a pomacentrid fish that exhibits nesting and nest-guarding behaviors. This study in the coastal waters of Bonaire, Dutch Caribbean, evaluates whether *A. saxatilis* selects rough or smooth substrates for nesting, and whether there is a vertical relief requirement for the nesting site using a reef survey and a field experiment. Nests on the reef were surveyed to determine if trends exist in the number and area of nests as height and rugosity of substrates increase, and a field experiment was designed to present the fish with a choice between rough or smooth surfaces and between units of varying heights. It was hypothesized that *A. saxatilis* would lay more and larger nests on smooth substrates with a vertical relief >20cm in both the experimental and reef environments. A trend towards more nests on smoother surfaces was observed in both the block experiment and in the reef survey, but no trends in number or area of nests were consistent between the experiment and the reef survey. Understanding the patterns of nest selection of *A. saxatilis* will provide important insight into the reproductive success of this highly abundant species, and because availability of nesting sites is a bottom-up control that can influence coral reef trophic structures.

Introduction

The selection of nesting sites by animals is an adaptive behavior that contributes to fitness by increasing the rates of offspring survival (Møller 1989). Nest sites may be chosen based on proximity to resources (Møller 1989) or structural features that potentially prevent predation (Candolin and Voigt 1998). For example, in a study performed on sticklebacks, Candolin and Voigt (1998) demonstrated that if predation risk is high, sticklebacks will nest in highly vegetated areas rather than open spaces. While open spaces would give the male sticklebacks an advantage in finding females, nesting in open spaces makes mating sticklebacks and their young more vulnerable to predation (Candolin and Voigt 1998). Studies such as this support the notion of nesting patterns as adaptive mechanisms contributing to the survival of an animal and its offspring. In order to improve their fitness, animals may seek preferable nesting sites or remain faithful to safe ones; these behaviors may be genetic or learned (Clark and Shutler 1999).

Pomacentridae, commonly known as damselfishes, utilize the spawning strategy of benthic egg laying, in which males prepare nest areas by removing live organisms and debris, then entice the female to lay a clutch of eggs on the surface which the male then fertilizes (Deloach and Humann 1999a). Eggs hatch approximately three days later (Deloach and Humann 1999a). The behavior of benthic egg laying is adaptive when its benefits, which include avoiding hazardous spawning ascents, outweigh the energy costs of preparing and guarding the nest, and some species of benthic egg layers are discerning when selecting nesting sites (Deloach and Humann 1999a).

Nesting behaviors of the tropical fish *Abudefduf saxatilis* are readily observed in the coastal waters of Bonaire, D.C. *Abudefduf saxatilis*, commonly known as the sergeant major, is a species of Pomacentridae that is highly abundant and widely distributed on reefs throughout the world (Fishelson 1970). When mating, *A. saxatilis* seeks "bare and eroded" surfaces, often on walls, as nesting sites (Fishelson 1970). Males stake out nesting sites on hard substrates and clean and prepare an area where females will deposit eggs in large masses that are spread out across the nesting site (Fishelson 1970). During nest preparation and reproductive behaviors, *A. saxatilis* swims very close to the substrate and presses its abdomen to the surface (Fishelson 1970). *A. saxatilis* are very territorial, competing for egg-laying areas and fiercely guarding the nests, which appear as distinct reddish-purple patches (Fishelson 1970). When guarding, *A. saxatilis* take on darker coloration and adopt aggressive behaviors, including biting and chasing away all other fish, often members of the same species (Fishelson 1970).

For these omnivorous fish, access to food is readily available in most reef habitats. *A. saxatilis* feeds opportunistically on zooplankton, algae, eggs, and even small fish (Deloach and Humann 1999b). Thus, it is unlikely that proximity to resources is a critical factor in *A. saxatilis* nest site selection. Both casual observations and specific studies have suggested that *A. saxatilis* aggregate in large groups on mooring blocks and other artificial substrates (Rooker et al. 1997; Rilov and Benayahu 2000). The fish travel between mooring blocks and nearby natural reefs (Robertson 1988) and have been observed to exhibit aggressive territorial behaviors more often on artificial substrates than on the reef (Rooker et al. 1997; Rilov and Benayahu 2000). It is unknown, however, whether they prefer artificial substrates for egg-laying, rather than the rough surfaces typical of natural coral structures. If a preference for smooth substrates exists, it could be due to increased ability to prepare a nest site, lay eggs, and protect the nest.

The objective of this study was to determine whether *A. saxatilis* exhibits particular requirements when selecting nesting sites, including vertical height and roughness of the substrate. Both reef observations and a field experiment were used to test the following hypotheses:

H_1: Number and area of *A. saxatilis* nests will be larger on smooth substrates compared with rough substrates, both on natural reefs and on experimental block units.

H_2: Number and area of *A. saxatilis* nests will be larger on substrates of greater vertical height (> 20cm) than areas of low vertical relief (< 20cm), both on natural reefs and on experimental block units.

As Caribbean reefs decrease in structural complexity (Alvarez-Filip et al. 2009), understanding the reproductive patterns of the animals that live there becomes critical to maintaining populations within the ecosystem. If *A. saxatilis* selects nest sites on artificial substrates due to a lack of suitable substrates on the reef, it may be a result of the declining health of Caribbean reefs. Understanding the nesting preferences of *A. saxatilis* will provide both insight into the ecology of a fish that is highly abundant on the coral reefs of Bonaire and an attractive model for testing nesting choices of a tropical fish.

Methods

Site selection

To evaluate *A. saxatilis'* preferences for egg-laying substrates, experimental block units were deployed near three boat moorings near the Yellow Submarine dive shop (12˚10'31.80" N, 068˚17'30.30" W) on the west coast of Bonaire, Dutch Caribbean. At the site, established mooring blocks—popular nesting sites for *A. saxatilis*— are located about 50 m from shore at a depth of about 5 m. Bottom cover is mostly sand with some rubble, and the reef begins about 5 m seaward of the mooring blocks. Because *A. saxatilis* aggregate at the blocks to lay eggs, it was presumed that egg laying would also occur on cement blocks placed in the same location.

In addition to the field experiment, surveys of the natural reef were conducted along the west coast of Bonaire to search for *A. saxatilis* nests. Surveys were conducted at the following sites, listed from north to south: Witches Hut (12˚12’30.82” N, 068˚19’09.80” W), The Cliff (12˚10’25.41” N, 068˚17’19.93” W), Bari Reef (12˚10’12.52” N, 068˚17’14.97” W), Something Special (12˚09’41.10” N, 068˚17’00.96” W), Yellow Sub site (12˚10’31.80” N, 068˚17’30.30” W), and Kas di Arte (12˚09’19.34” N, 068˚16’46.94” W).

Experimental block units

Experimental block units were constructed to compare heights and rugosities of egg-laying substrates selected by *A. saxatilis* in an artificial setting with substrates on the natural reef. At each of the three mooring block sites, four experimental block units were positioned approximately 2 m from the existing mooring blocks and 2 m apart from each other (Fig. 1). One unit was one cinderblock high, one was two, one was three, and one was four. Dead coral fragments were attached with marine epoxy to two adjacent sides of each unit to make them “rough.” These fragments had an average rugosity of about 2 cm from the surface of the cinderblock. The remaining surfaces of the units were smooth. At two of the sites, the rough sides were positioned facing the current; at the third site, the rough sides were facing away from the current, to control for any effect current may have on egg-laying.

The blocks were visited every other day via snorkeling or SCUBA. The area (cm^2) of each nest was calculated by measuring the greatest width and length and multiplying the values. Each nest was only recorded once. Sides on which no nests were observed were included in average area calculations as zeros.

Reef survey

Six reef locations were surveyed using SCUBA to locate *A. saxatilis* on natural substrates. Surveys began at a depth of 20 m and proceeded up the reef using a U-shaped search pattern 50 m across, ending at 5 m. Nests were measured and area calculated in the same manner as on the experimental block units. Vertical height of the substrate on which the eggs mass was laid was measured to the nearest 10 cm, and the heights were classified into five categories. The first category was “20 cm,” encompassed all nesting substrates 20 cm or shorter, and was comparable to the height of the shortest experimental block unit. The second category was “40 cm” and encompassed substrates 21 – 40 cm high, the third was “60 cm” and encompassed substrates 41 – 60 cm high, the fourth was “80 cm” and encompassed substrates 61 – 80 cm high, and the fifth category was “> 80cm” and encompassed all

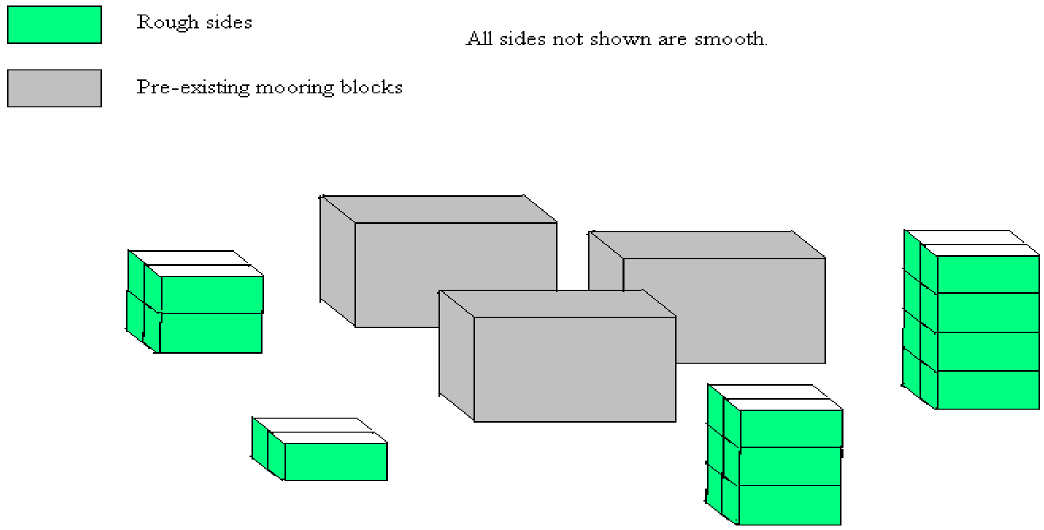

Fig. 1 Diagram of layout of experimental block units. Rough sides of the experimental block units are shown in green and are depicted as they were placed underwater surrounding the pre-existing mooring blocks.

higher nesting substrates. Rugosity of the substrate was graded subjectively on a scale of RG1 to RG4 (abbreviation of "rugosity grade"): RG1 = smooth, RG2 = < 2 cm rugosity, RG3 = 2 cm (comparable with rough sides of experimental block units), and RG4 = > 2 cm.

Results

Experimental block units

Smooth vs. rough sides: number of nests

Within three days of the placement of experimental block units, two nests appeared at the site. During the first nesting period, which lasted one week from the time territories were established to the time the eggs hatched, ten nests were recorded on experimental block units. All of these nests were on the smooth surfaces. After the first set of nests hatched another nesting period began and ten more nests were found on the blocks. Three of these nests were found on rough block surfaces. During the third nesting period only two sets of blocks were observed. Five nests were recorded; one was on a rough surface. Of 25 nests recorded, 21 were laid on smooth sides of block units and four were on rough sides (Fig 2).

Smooth vs. rough sides: area of nests

A trend was observed towards larger nests on smooth surfaces (Fig. 3). The average area of nests laid (± SD) on smooth sides was 441.9 ± 275.4 cm^2 and the average area of nests laid on rough sides was 328.5 ± 321.8 cm^2.

Block height: number of nests

Number of nests increased as block height increased (Fig. 4). Of 25 recorded nests across the three experimental setups, one was observed on a 20 cm block, five were observed on 40 cm blocks, nine were observed on 60 cm blocks, and ten were observed on 80 cm blocks.

Block height: area of nests

No trend was observed in the area of nests as block height increased (Fig. 5). The largest average nest area was found on the 60 cm blocks. The area of each nest was divided by the area of the block side to determine average ratio of area of nests (cm^2) to block side area (cm^2). Average ratio of nest area to side area (± SD) on smooth sides of 20 cm blocks was 0.023 ± 0.090; for rough sides it was 0. Average nest area ratio (± SD) on smooth sides of 40 cm blocks was 0.067 ± 0.102, rough sides 0; average ratio (± SD) on smooth sides of 60 cm blocks was 0.120 ± 0.159, rough sides was 0.025 ± 0.095; and average ratio (± SD) on smooth sides of 80 cm blocks was 0.067 ± 0.088, rough sides was 0.0090 ± 0.019.

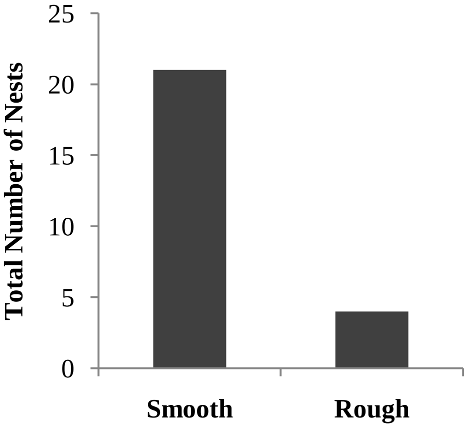

Fig. 2 Total number of nests observed on smooth and rough sides of experimental blocks is shown.

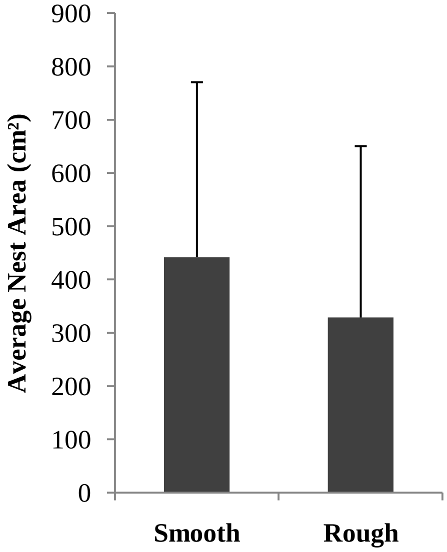

Fig. 3 Average nest area for smooth and rough experimental block sides is shown. Error bars represent standard deviation.

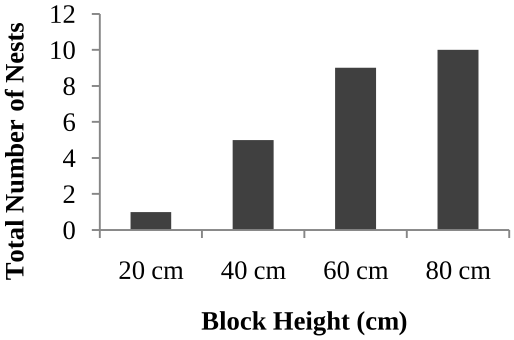

Fig. 4 Total number of nests for each height of block (20 cm, 40 cm, 60 cm, and 80 cm tall) is shown.

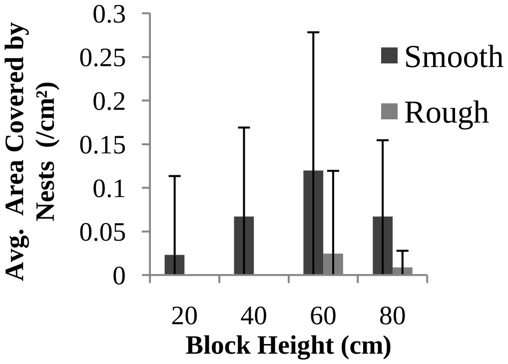

Fig. 5 Average nest area is shown for each height of block (20 cm. 40 cm, 60 cm, and 80 cm tall) is shown. Error bars represent standard deviation. The area of each nest was divided by the area of the block side to determine average ratio of area of nests (cm^2)

Reef survey

Rugosity grade: number of nests

Of 30 nests recorded on the reef, ten were on RG1 substrates, 13 were on RG2 substrates, four were on RG3 substrates, and three were on RG4 substrates. RG1 substrates are smooth and RG2 substrates are of lesser rugosity than the rough sides of experimental blocks, so a trend is shown towards more nests on smooth or almost smooth substrates (Fig. 6).

Rugosity grade: area of nests

No trend was observed in the average nest areas over the four rugosity grades (Fig. 7). The average area of nests (± SD) on RG1 substrates is 952.8 ± 581.1 cm^2, on RG2 substrates is 715.7 ± 308.7 cm^2, on RG3 substrates is 584.5 ± 407.8 cm^2 and on RG4 substrates is 1757.3 ± 1054.538 cm^2.

Height of substrate: number of nests

There was no pattern observed between vertical relief and number of nests (Fig. 8). Of 30 nests observed, eight were on substrates ≤ 20 cm tall, four were on substrates in the “40 cm” category, eight were on substrates in the “60 cm” category, three were on substrates in the “80 cm” category, and seven were on substrates in the “> 80 cm” category.

Height of substrate: area of nests

No pattern was observed in the average area of nest as vertical height of substrates increased (Fig. 9). Of nests found on substrates ≤ 20 cm, the average nest area was 1184.9 ± 916.2 cm^2, on substrates in the “40 cm” category it was 608.8 ± 349.8 cm^2, on substrates in the “60 m” category it was 810.1 ± 470.6 cm^2, on substrates in the “80 cm” category it was 622.7cm^2 ± 242.6, and on substrates in the “> 80 cm” category it was 822.7 ± 348.0 cm^2.

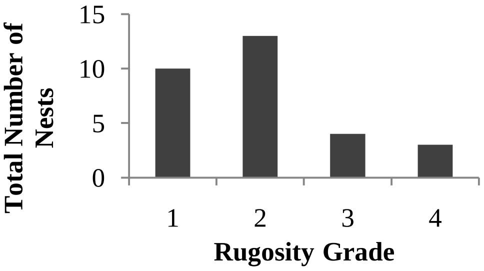

Fig. 6 Total number of nests found on substrates of each rugosity grade is shown.

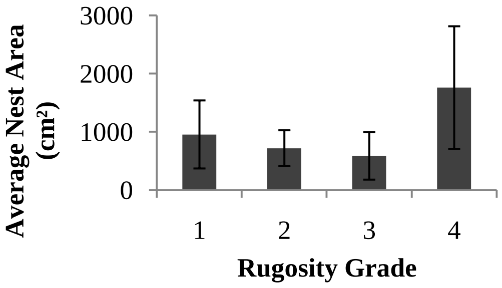

Fig. 7 Average nest area for each rugosity grade on the reef is shown. Error bars represent standard deviation.

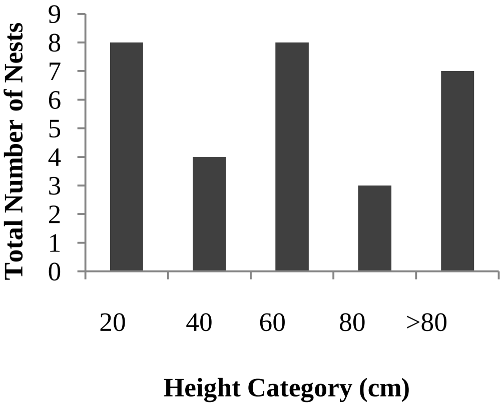

Fig. 8 Total number of nests for each height category on the reef is shown. There was no pattern observed between vertical relief and number of nests.

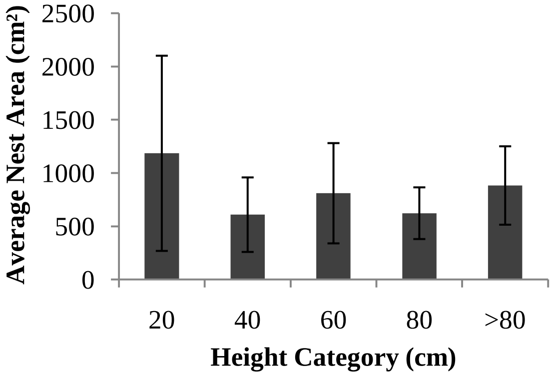

Fig. 9 Average nest area is shown for each height category observed on the reef. Error bars represent standard deviation.

Discussion

The first hypothesis stated that number and area of nests would be larger on smooth substrates compared with rough substrates, both in the experiment and on the reef. This hypothesis is partially supported. Average area of nests on the experimental blocks was larger on smooth surfaces than rough surfaces; however, no trend in area as rugosity increased on the reef substrates was observed. However, in both the experiment and the reef survey a larger number of nests were found on smooth surfaces than rough surfaces. More nests were found on RG2 substrates than RG1 substrates, but all RG1 substrates were manmade objects such as submerged pipes and other debris, because surfaces naturally found on the reef are rarely (if ever) smooth. Because there are a limited number of smooth substrates available on the reef, it would follow that *A. saxatilis* also frequently lays eggs on old dead coral that is the smoothest substrate available. This may be related to the nest preparation and guarding behaviors of *A. saxatilis.* As *A. saxatilis* stakes out a territory, it circles above the substrate, rubbing its stomach over the surface and cleaning the substrate. When spawning, females also press their abdomens to the surface as they lay their eggs. The bumps that were attached to the experimental block units, as well as the bumps that occur naturally on highly rugous old dead coral, could make it difficult for *A. saxatilis* to effectively practice these behaviors.

The second hypothesis stated that number and area of nests would be larger on tall substrates (> 20 cm high) compared with substrates of low vertical relief (≤ 20 cm), both in the experiment and on the reef. In the experiment, a trend was observed in which the number of nests increased as block height increased. However, the average area of nests was not largest on the tallest block. Meanwhile, height did not seem to impact the nest choices of *A. saxatilis* on the reef. No trends were observed in number of nests or in area as substrate heights increased. Because a pattern was observed in the experiment but not in the reef survey, it may be that differing pressures influence nest site selection of *A. saxatilis* in the natural reef and in the “patch reef” environment created by mooring blocks in shallow waters. Perhaps it is advantageous in the open “patch reef” environment, where the majority of the substrate is sand, for *A. saxatilis* to establish territories on tall substrates where the territories are easily visible to females, which then come to the territories to lay their eggs. On the reef, however, the spaces are not as open and the substrate is comprised of mostly dead and live coral. Rather than the height of the particular substrate determining nesting selection, structural complexity of a particular area could enhance its appeal as a nest site. As the survey progressed, it was observed that many of the largest nests (those close to or over 2000 cm^2) were found on the undersides of substrates, making them highly sheltered and easy to protect. A subsequent study could determine a method for quantifying protectedness of nests and evaluate whether a correlation exists between level of protectedness and area of a nest.

Because this study produced little evidence of a relationship between the physical characteristics of a substrate and the area of nests that *A. saxatilis* lays, it may be that the area of nests laid is influenced by different variables or a wider variety of variables than substrate type. Factors that could influence nest size could include the size and the health of the fish. (Deloach and Humann 1999a).

A limitation of the reef survey was the limited amount of data collected. Only four nests were found on RG3 substrates and only three were found on RG4 substrates. The small sample size made assessing the relationship between rugosity grade and nest area difficult.

With the world's reefs in a crippled state, *A. saxatilis* remains more abundant than most other reef fishes. The success of the species may in part be a product of the abundance of old dead coral and manmade debris. While dead coral heads and submerged objects indicate the poor health of reefs, *A. saxatilis* utilizes them by laying eggs on the smooth surfaces they provide. The proliferation of old dead coral and manmade debris on present day reefs could be contributing to the abundance of *A. saxatilis* by creating viable nesting sites. Perhaps rather than the degradation of reefs forcing *A. saxatilis* to nest on unnatural substrates, as was postulated in the introduction, degraded reefs may be a factor in *A. saxatilis*' reproductive success. Further research should be conducted to investigate whether other species benefit from degraded reef composition. A study that examines whether there is a positive correlation between percent old dead coral cover and *A. saxatilis* population could help to support the hypothesis that *A. saxatilis* benefits from degraded reefs. This information could provide insight into the changing trophic structures of coral reefs.

Studies have shown that present day reefs support relatively greater populations of lower trophic level fishes compared with higher trophic level fishes (Stevenson et al. 2007). The study conducted by Stevenson et al. (2007) examines the affect of overfishing, a top-down control, on the biomasses of predators and herbivores on the coral reefs of three central Pacific islands. Areas exposed to high fishing pressures show a reduction of predator biomass; however, lower-level carnivore biomass was not significantly reduced. Lower-level carnivores are defined by Stevenson et al. (2007) as "benthic invertebrate feeders, planktivores, and corallivores," similar to the diet of *A. saxatilis.* While examining the effects of top-down controls on coral reef trophic structures is essential, bottom-up controls also play a role in determining which organisms proliferate in today's degraded reef habitat. Nest site availability is an example of a bottom-up control that could be contributing to the success of certain fish, including *A. saxatilis*, on reefs comprised of high percentages of dead coral and manmade debris.

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I would like to thank Dr. Rita Peachey for her guidance in transforming this study from a vague idea to a feasible experiment, Jennifer Blaine for her advice and diligent editing, and all of CIEE for their support throughout the project. I would also like to thank Kyle McBurnie for his commitment in assisting me with the project, particularly with the demanding and at times tedious labor of installing experimental block units. Finally I would like to thank the Yellow Submarine Dive Shop for their patience as experimental block units were installed and for their ongoing support of CIEE.

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Christmas tree worms (*Spirobranchus giganteus*) as a potential bioindicator species of sedimentation stress in coral reef environments of Bonaire, Dutch Caribbean

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Abstract

The effect of land-based pollution on Bonaire's coral reef ecosystem has not been well-quantified. Observations of the coral reefs of Bonaire show a great abundance of the polychaete *Spirobranchus giganteus*. This study investigated whether *S. giganteus* is sensitive to the environmental stress caused by wastewater pollution and therefore could be used as a bioindicator species of pollution in coral reef health assessments. Pollution indicators were assessed through the analysis of water samples, concentrating on the levels of ammonia, nitrate, nitrite and phosphate, sediment levels, and fecal contamination. To allow for comparison between differing levels of water pollution, six different sites were chosen with various levels of expected pollution impact by their proximity to resorts. Abundance of *S. giganteus* and coral reef cover at these sites were analyzed through transects at different depths. No significance was found between *S. giganteus* density and nutrient levels or fecal contamination. This study found a significant positive correlation ($r^2 = 0.936$) between *S. giganteus* density and sedimentation rates, suggesting the possible use of *S. giganteus* as a bioindicator of sedimentation stress on coral reefs.

Introduction

Nearly two-thirds of coral reefs in the Caribbean are threatened by at least one form of human activity, including water-based activities like overfishing and land-based activities such as coastal development and pollution (Burke and Maidens 2004). Land-based pollution is often responsible for localized damage to coral reefs caused by nutrient enrichment, sedimentation, and toxicity (Pastorok and Bilyard 1985). Localized damage is compounded by degradation to coral reef health caused by global warming that exacerbates coral bleaching and diseases (Burke and Maidens 2004). Localized land-based impacts can be assessed using four main water quality parameters: dissolved inorganic nutrients, suspended particulate organic matter, light reduction from turbidity and sedimentation (Fabricius 2005).

To address anthropogenic impacts on coral reef health, conservation management plans are essential. The efficiency of management plans rely on identifying, assessing, and monitoring the effects of pollution on the reef. While it is possible to test water samples directly for certain chemical pollutants, chemical-based analyses may not adequately quantify the effect of chemicals on the overall biological system (Lam 2009). Additionally, chemicals can often have negative interactions, causing harmful additive, antagonistic, or synergistic effects (Lam 2009). Due to such complications, there has been a recent shift from chemical-based monitoring to biological-based monitoring, in which a contaminant's effects on organisms within a potentially polluted area are measured (Lam 2009). As monitoring the effects on each organism in an ecosystem is not practical, the use of a single species that is sensitive to certain environmental stresses, including pollution and runoff, can be implemented (Linton and Warner 2003; Scaps et al. 2008). Using such 'bioindicator' species has been recommended to monitor anthropogenic influences in the coral reef ecosystem (Linton and Warner 2003; Scaps et al. 2008).

In the case of coral reef communities, nutrient enrichment is a particularly stressful form of pollution since coral reefs are usually low nutrient environments (Fabricius 2005). As nutrients increase, coral reef communities change from predominantly nutrient-recycling symbiotic organisms such as corals, to a dominance of macroalgae, resulting in a surge in the population of heterotrophic suspension feeders (Risk et al. 2001). In a review of reef evaluation techniques, Risk et al. (2001) cited two studies as examples of the change in community structure that can be caused by pollution including a study that reported increased numbers of heterotrophic suspension feeders on reefs with high nutrient levels (Birkeland 1977), and a study in Hawaii that showed community change from autotrophic to heterotrophic organisms under sewage stress (Maragos et al. 1985).

Studies have already demonstrated the use of other polychaetes as key taxa for the monitoring of the quality of marine environments, specifically in relation to plankton growth and nutrient over enrichment (Pocklington and Wells 1992). There are several species of polychaetes that are already known as pollution indicators, such as *Capitella capitata* and *Malacoceros fuliginosus* in Western Europe and *Streblospio benedicti* in North America; if these species are found in disproportionate abundance, far outranking other common reef species, they are accepted as indicators of pollution (Pocklington and Wells 1992).

A previous study in Bonaire showed that there was a greater abundance of *Spirobranchus giganteus*, a suspension feeder that feeds on small plankton and that is common on the coral reefs of Bonaire, Dutch Caribbean, at sites with higher suspected risk of wastewater contamination (Williams 2009). Nutrient enriched areas are known to have increased amounts of phytoplankton (Risk et al. 2001); thus, with a stable abundance of food for *S. giganteus* in nutrient enriched areas such as nearby major resorts, it follows that populations of *S. giganteus* would be greater in such areas. It has yet to be shown whether it is an increase in plankton that leads to the higher population of *S. giganteus* or a higher concentration of preferred coral hosts in certain areas.

Some previous studies indicate that *S. giganteus* prefers certain coral species but results have varied on the specific preferences (Marsden and Meewuig1990; Nygaard 2008). In Barbados, *Porites asteroides* and *Millepora complanatu* were found to be heavily colonized, *Siderastrea siderea*, *Monastrea cavernosa*, and *Dendro cylindrus* are rarely or never colonized and no preference was found for *Agaricia agarcities* (Marsden and Meeuwig 1990). In Bonaire, *S. giganteus* exhibited a settlement preference for *Montastraea annularis* with 60.9% surveyed *S. giganteus*, followed by *P. astreoides* (17.9%) and *A.agaricites* (11.9%); the other coral species accounted for less than 2% of the total number of *S. giganteus* surveyed (Nygaard 2008).

The use of *S. giganteus* as a bioindicator species was investigated using sites with increasing distances from major resorts on coral reefs in Bonaire. By comparing nutrient levels, sedimentation rates, *S. giganteus* coral species preferences and fecal contamination at six sites of increasing distances from resorts, a prominent source of nutrient enrichment, this study determined whether *S. giganteus* abundance was positively related to indicators of eutrophication. In order to identify whether concentration of preferred coral is related to the abundance of *S. giganteus*, this study included an analysis of coral preference in Bonaire in relationship to high nutrient levels. Prior to this study, it was hypothesized that,

H_1: Polluted sites, as defined by their proximity to a resort, will have higher nutrient levels, increased sedimentation, and evidence of fecal contamination.

H_2: Higher densities of *S. giganteus* will be more positively related to higher sedimentation rates than concentration of preferred coral hosts.

H_3 : There will be a higher density of *S. giganteus* at sites near polluting sources.

If *S. giganteus* density is greater in nutrient enriched areas, this would support their use as a localized bioindicator of nutrient enrichment. It is important to further study this relationship since nutrient enrichment can be the most significant pressure on coral reefs on a local scale (Fabricius 2005).

Methods

Study site

This study was conducted in the waters surrounding the island of Bonaire, Dutch Caribbean, within the limits of the Bonaire National Marine Park (BNMP) during the months of February and March of 2011 (Fig. 1). Six study sites were chosen by their varying proximity to areas of suspected ground water discharge as a way to establish a continuous gradient from highly impacted to lesser impacted sites (Fig. 1). Shorter distances to major resorts were considered as potentially more exposed to human sewage and human disturbances. Eighteenth Palm Reef (12° 8'18.06"N, 068°16'36.65"W) was chosen due to its location in front of the largest dive resort on Bonaire, Plaza Resort. Bari Reef (12°10'3.74"N, 068°17'13.87"W) was then chosen due to its location in front of smaller resort of condominiums, Sand Dollar Condominiums. Cha Cha Cha Reef (12° 8'44.59"N, 068°16'36.62"W) was the next site selected, located approximately 150m from a major resort, Divi Flamingo Resort. Kas di Arte (12° 9'22.37"N, 068°16'45.76"W) was chosen as the next impacted site, at 1250 m from any major resorts. The next study site selected was on the uninhabited island of Klein Bonaire off the western coast of Bonaire. This site, Jerry's Reef (12° 9'20.57"N, 068°17'32.06"W) is located approximately 2000 m across the bay from any resorts in Kralendijk, the capital of the island. The site, expected to be least impacted. is Witches Hut (12°12'23.22"N, 068°18'59.40"W), north of Kralendijk and approximately 4000 m north of the nearest large resort.

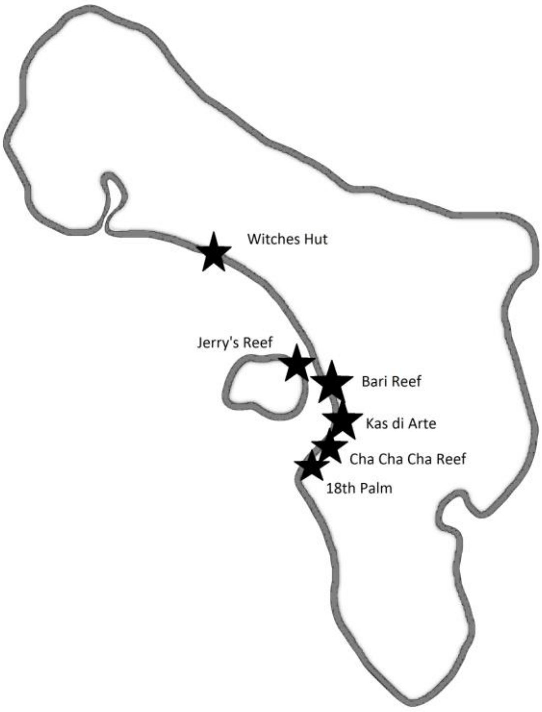

Fig. 1 Map indicating dive sites along the western coast of Bonaire.

Benthic community and *S. giganteus* abundance

This study was conducted using SCUBA gear at each study site. At each site, coral health, determined by the benthic community structure, and *S. giganteus* abundance at 6 m, 12 m, and 18 m depths were assessed using a 30 m x 1 m belt transect. Benthic community composition was determined by a point line method recording percent dead coral, percent live coral, and other cover constituents such as macroalgae or sand cover on the middle 10 m section of the 30 m belt transect. In addition, the first 10 m section and the last 10 m section of the 30 m transect at each depth were assessed in greater detail by also recording coral species and size using the point line method. Each *S. giganteus* found within the belt transect was counted and its coral host identified. An adjusted density of *S. giganteus* for each transect was determined by dividing the total number of individual worms observed by the centimeters of live and dead coral on each transect. A relationship between depth and density of *S. giganteus* as well as a relationship between sites and density of *S.giganteus* was assessed through a one-way analysis of variance (ANOVA, $\alpha = 0.05$). Percent composition of coral species on each transect was determined by dividing the number of coral heads of one species divided by the total number of coral heads found on that transect. An indication of the impact of *S. giganteus* preference for different coral species on their density in a given transect was determined using regression analysis of the density of the polychaete found on a species to the percent composition of that coral species in the transect.

Nutrient analysis

Water samples for nutrient analysis were collected at each site. Acid-washed 250 ml bottles were initially filled with surface water before each dive to reduce buoyancy. At each sampling depth, 6 m,12 m, and 18 m, a bottle was opened upside down, filled with air from the diver's alternate air source, and turned right side up to refill with water; this was repeated three times before each bottle was capped. Immediately after the dive, the sample from each depth was put on ice and returned to the CIEE Research Station for laboratory analysis. Within 2 hours, these samples were analyzed using a LaMotte Salt Water Aquaculture test kit (Model AQ-4) and protocol (LaMotte Company 2008) to determine the levels of ammonia, nitrite and nitrate. A Hanna instruments low level phosphate analysis kit was also used to determine phosphate levels. Relationships between levels of total inorganic nitrogen as well as phosphate levels for both depth and sites were assessed using a one-way ANOVA.

Sedimentation analysis

Particle size data from each site collected from February 2009 to March 2010 by staff from CIEE Research Station in Bonaire were used to assess differences in sediment levels among study sites. Sediment traps were made from PVC pipes (7.5 cm diameter, 15 cm long) with an open top and a closed bottom. The trap was placed vertically about 10 cm from the substrate. Traps were collected approximately every 14 days; shorter or longer periods were accounted for in the data analysis. After collection, the sediments were allowed to settle for one hour. The sea water was then decanted and the remaining sediments were rinsed 3X with sterile water to remove any excess salt. The sediments were placed in an oven at 60°C for 48 h. Organic matter was later removed with forceps and the rest of the sample was weighed. Sedimentation rate was calculated by dividing the final dried sediment weigh by number of days that trap was left in the water (Gleason 1998). Relationships between sedimentation rates and sites were assessed through a one-way ANOVA.

Particle size composition was determined by re-suspending the dried sample in 25 mL of tap water. One mL of the re-suspended solution was placed on a 200 cell slide to be viewed under a compound microscope. One out of 200 cells was haphazardly chosen and the next 199 particles encountered in a left to right orientation were counted and categorized by size classes; only the number of particles <10 µm were considered in this study. Three subsamples were analyzed for each site. Relationships between number of particles <10 µm and sites were assessed using a one-way ANOVA.

***Entercoccus* presence**

At every site, a sample was taken at the depths of 18 m, 12 m, and 6 m. New sterile 125 mL bottles were filled with sterile water and labeled before the samples were collected. At each sampling depth, the respective bottle was opened upside down and filled with air from the alternate air source of the scuba diver. The bottle was then flipped right side up, filled with sea water, and sealed with the cap. Within two hours following each dive, the water samples for each depth were separately analyzed. The samples were diluted by putting 10 mL of the sample water into a sterile 125 mL bottle and filling the rest of the bottle with sterile water. The Enterolert system (IDEXX, Philadelphia, PA) was used to estimate the density of *Enterococci* bacteria in sea water samples. First, the Enterolert reagent was added to the diluted bottle and shaken with the cap on. Then, the prepared sample was poured into an Enterolet tray and put through the tray press, being careful to remove any bubbles in the tray beforehand. The trays were put in an oven set at 41^{o} C (+/- 0.5^{o}C) for 24 h. After the incubation period, the trays were examined underneath a black light to count the number of luminescent cells, large and small counted separately. The most-probable-number-chart provided by Enterolert was then used to determine the quantity of bacteria times the volume of 10 mL to calculate the colony forming units (cfu) per 100 mL.

Results

Benthic Community and S. giganteus abundance

Available substrate of live and dead coral for *S. giganteus* did not differ between sites (ANOVA, df = 5, p = 0.1247) but differed by depth between 6m and 12m (ANOVA, df = 2, p = 0.0025) and between 6 m and 18 m (ANOVA, df = 2 p = 0.0030). There was no difference in available substrate between 12 m and 18 m (ANOVA, df = 2, p = 0.9432). There was no significant difference between the number of *S. giganteus* found at any depth (ANOVA, df = 2, p = 0.0526). *S. giganteus* abundance per suitable substrate was found to vary significantly between sites (ANOVA, df = 5, p = 0.0044) (Fig. 2), namely between Cha Cha Cha and the five other sites (Table 1). No significant variation was found between *S. giganteus* density (live and dead coral was accounted for) and depth (ANOVA, df = 2, p = 0.1561).

Nutrient analysis

No significant relationships were found between any of the nutrients analyzed and the sites or depth (ANOVA, df = 5 and df = 2 respectively, always > 0.05) (Fig. 3a and 3b). The concentration of nitrate and nitrite were consistent over every site at 0.25 ppm and 0.05 ppm respectively for a summation of 0.30 ppm of nitrates and nitrites (Fig. 3a).

Sedimentation rates and particle size distribution

Sedimentation rates were shown to significantly differ between sites (ANOVA, df = 5, p < 0.0001) (Table 1). Cha Cha Cha was found to have significantly higher sedimentation rates than all other sites (Fig. 3c). Furthermore, 18th Palm and Jerry's Reef were also found to have significantly higher rates of sedimentation than Kas di Arte (Table 1, Fig. 3c). Particle distributions of particles less than 10 µm did not vary significantly between sites (ANOVA, df = 5, p always > 0.05).

A strong correlation was found between calculated mean sedimentation rates and mean density of *S. giganteus* per live and dead coral (regression analysis, r^2 = 0.936, Fig. 4). In contrast, no significant correlations were found between the most common *S. giganteus* hosts, *M. annularis*, *A. agaricites*, and *M.* c*avernosa*, and the total percent composition of that host at each site (Figs. 5, 6, 7).

Entercoccus presence

Five of 18 (27.78%) water samples tested positive for *Enterococci* presence; Cha Cha Cha had 2.0 total colony-forming units (cfu) from two separate water samples at depths of 6 m and 12 m. Eighteen Palm had 3.0 total cfu from two separate water samples at 6 m and 18 m. Witches Hut showed 7.5 cfu in one water sample from 18 m. Kas di Arte, Bari Reef, and Jerry's Reef did not test positive for *Enterococcus* at any depth.

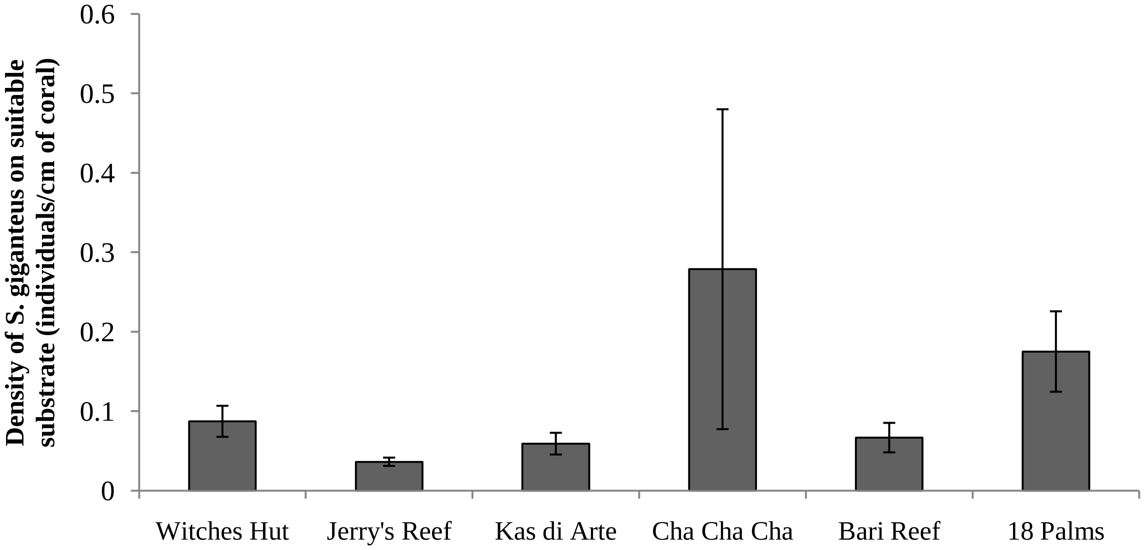

Fig. 2 Mean density of S.giganteus (individuals/cm of coral) ± SD shown by site in order from left to right of least to most impacted. p=.0044 Cha Cha Cha reef exhibited a significantly higher mean density (see Table 1).

Table 1. Fisher's PLSD post-hoc comparison between site and sedimentation rates(site, α = 0.05, S = significant)

Site	Mean Diff.	Crit. Diff.	P-Value	
Bari Reef, Cha Cha Cha	-0.067	0.027	< 0.0001	S
Bari Reef, 18th Palm	0.009	0.024	0.4518	
Bari Reef, Jerry's Reef	0.017	0.027	0.2104	
Bari Reef, Kas di Arte	-0.018	0.027	0.1985	
Bari Reef, Witches Hut	-0.008	0.027	0.586	
Cha Cha Cha, 18th Palm	0.076	0.024	< 0.0001	S
Cha Cha Cha, Jerry's Reef	0.084	0.027	< 0.0001	S
Cha Cha Cha, Kas di Arte	0.049	0.027	0.0006	S
Cha Cha Cha, Witches Hut	0.059	0.027	< 0.0001	S
18th Palm, Jerry's Reef	0.008	0.024	0.4865	
18th Palm, Kas di Arte	-0.027	0.024	0.0261	S
18th Palm, Witches Hut	-0.017	0.024	0.1681	
Jerry's Reef, Kas di Arte	-0.035	0.027	0.0118	S
Jerry's Reef, Witches Hut	-0.025	0.027	0.0732	
Kas di Arte, Witches Hut	0.01	0.027	0.4572	

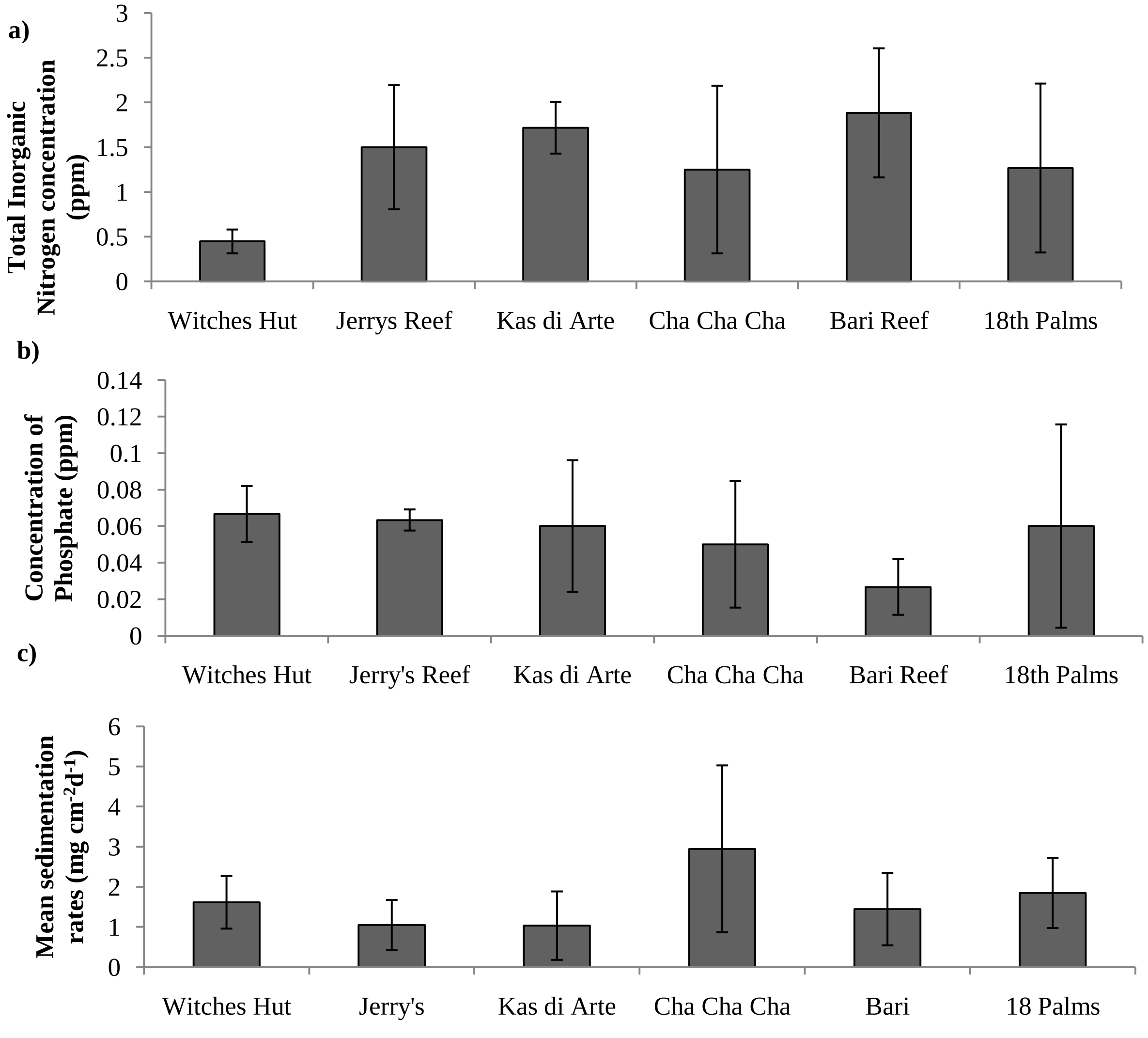

Fig. 3 Mean densities of a) total inorganic nitrogen (ANOVA, df = 5, p = 0.1648), b) phosphates (ANOVA, df = 5, p = 0.5417) and c) sedimentation (± SD) shown by site in order from left to right of least to most impacted. (ANOVA, df = 5, p < 0.0001)

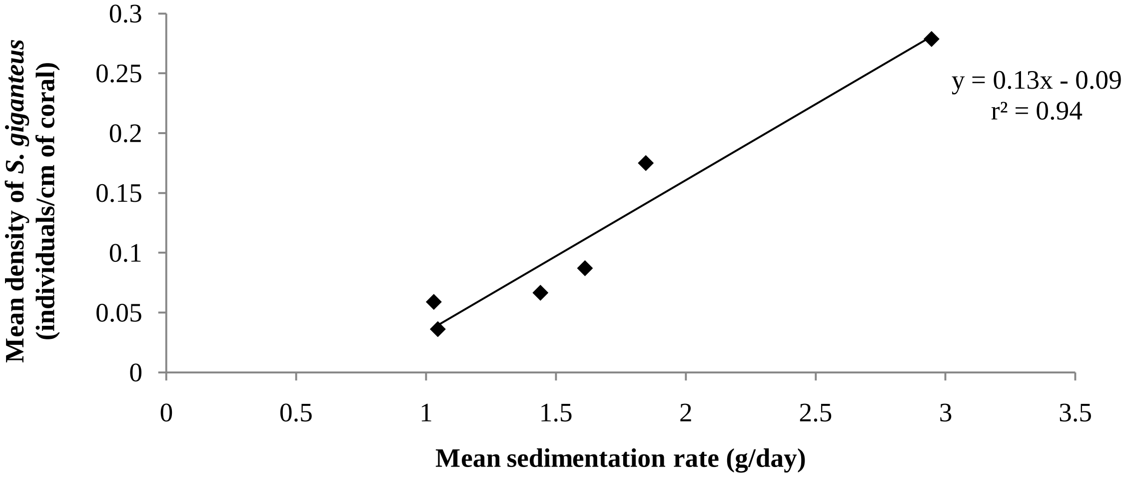

Fig. 4 Graphic representation of the regression analysis results between mean density of *S.giganteus* and the mean sedimentation rate at each site.

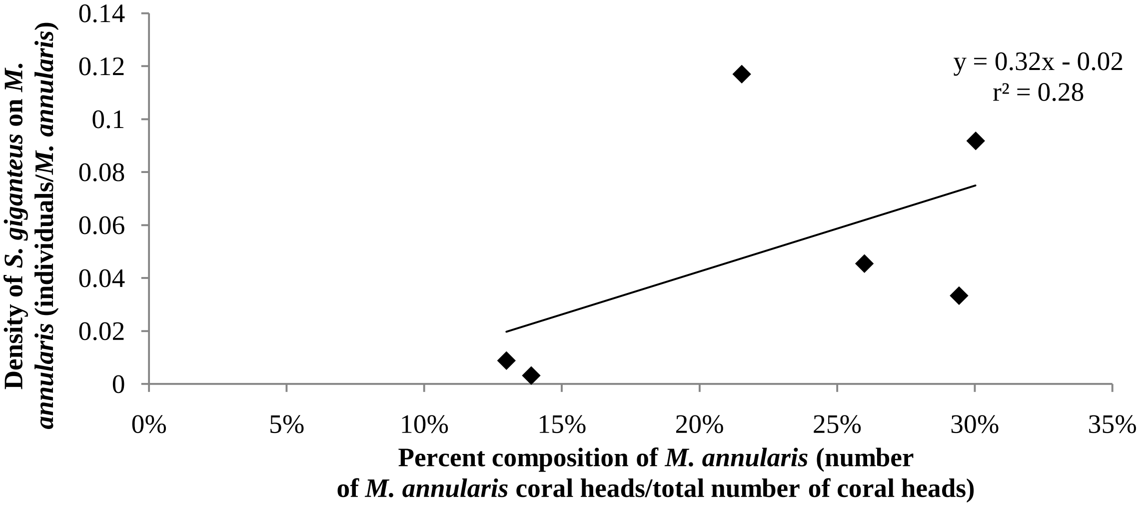

Fig. 5 Graphic representation of the significant correlation analysis between percent composition of the most common coral of these transects, *M. annularis*, and the density of *S. giganteus* found only over *M. annularis*.

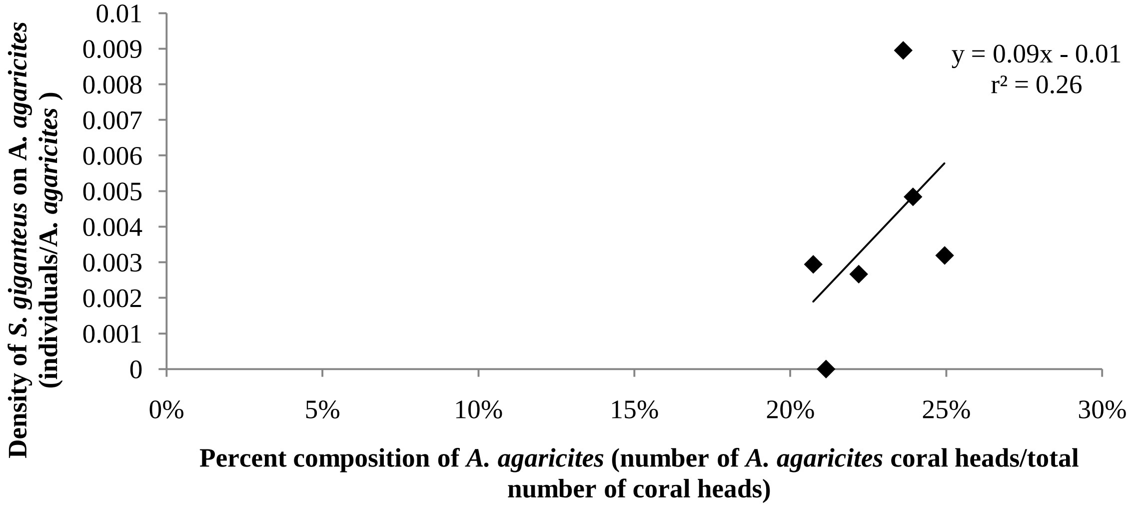

Fig. 6 Graphic representation of the significant correlation between *A. agaricites*, second most common coral of these transects, and the density of *S. giganteus* found only on *A.agarcites*.

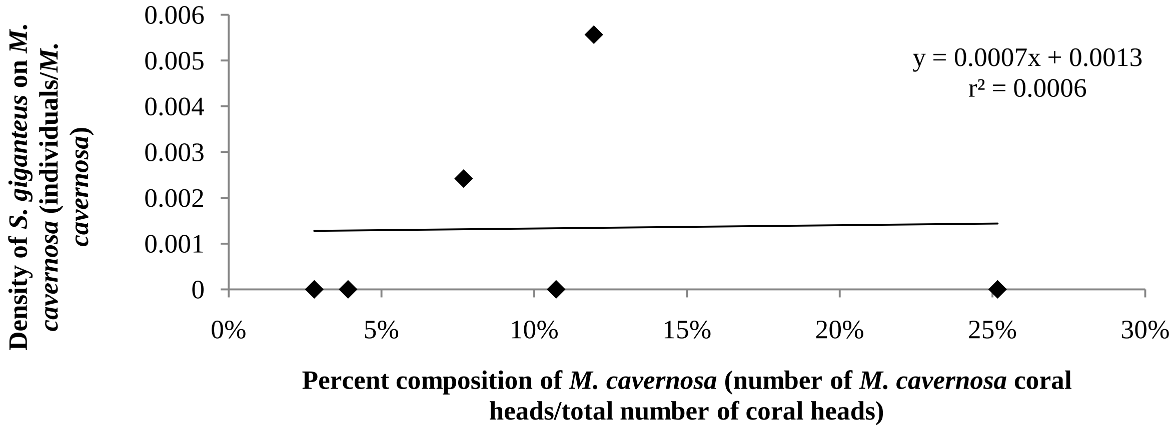

Fig. 7 Percent composition of the third most common coral, *M. cavernosa*, was compared to the density *of S. giganteus* found only on M. cavernosa. No correlation between a high percent composition of *M. annularis* and a high density of *S. giganteus* was not found ($r^2 = 0.0006$).

Discussion

This study did not find any significant relationship between increasing proximity to resorts and levels of nutrients, *Enterococci*, or sedimentation rates. This study also did not find any significant relationship between percent composition of preferred coral host and *S. giganteus* density. However, this study found a strong positive relationship between the density of *S. giganteus* and sedimentation rates at each site.

Relationships between benthic community and *S. giganteus* abundance

Cha Cha Cha was the only site to show a significant difference from the other sites in number of *S. giganteus* per live and dead coral. Since the amount of live and dead coral did not differ significantly between any sites nor did percent composition of preferred coral hosts, it is unclear as to why Cha Cha Cha was the only site to show a significant difference supporting the original hypothesis that the density of *S. giganteus* would increase with proximity to polluting sources. The site expected to be the most polluted, 18th Palm, showed a higher density of *S. giganteus* than other sites but it was not a significant increase. A previous study in Bonaire (Williams 2009) found that *S. giganteus* numbers were significantly increased at a depth of 12 m but utilized shorter (10 m) transects and did not look at *S. giganteus* numbers per area of suitable host substrate. Given the larger, yet still limited, sample size of this study, it is possible that the previous study may have suffered from sampling limitations. The simple survey method used in this study to calculate polychaete density has been shown to parallel data obtained by laborious and time-consuming methods (Risk et al. 2001).

Effects of nutrients on *S. giganteus* densities

In contrast to the expected results that nutrient levels would increase as proximity to a resort increased, no significant differences between nutrient levels were found at any site suggesting that the sites might not be significantly affected by varying nutrient levels. It is also possible that the length of time of this study as well as the low resolution of the data, due to only one water sample analyzed per site as well as the use of a limited resolution laboratory kit, was insufficient to show any significant differences in nutrient levels. Future studies of nutrient levels across these sites should consider collecting data for longer periods of time as well as performing more sophisticated nutrient analysis techniques that provide a higher resolution of data than a LaMotte kit can provide. However, the results of this study found that the levels of phosphate at all sites were below the threshold of 0.3 µm established by another study (Bell 1992), which suggests that the levels of pollution in Bonaire are not nearly as high as in other locations; this could simply be related to the smaller population density of Bonaire. However, this study also found that the levels of total inorganic nitrogen was above the threshold of 1 µm (Bell 1992), suggesting that Nitrogen is a pollution factor on the coral reefs of Bonaire, perhaps related to the lack of wastewater treatment and the use of septic tanks on the island.

Effects of sedimentation rates and particle size distribution on *S. giganteus* densities

Cha Cha Cha was also the only site to have significant sedimentation rates in comparison to the other five sites. Cha Cha Cha had the highest *S. giganteus* density as well as the highest sedimentation rate which supports the hypothesis that the two factors are positively correlated. Although the simplified classification of sites from least impacted to most impacted by proximity to a major resort may have been flawed, the mean sedimentation rates correlated with the mean *S. giganteus* densities (Fig. 4) also supported the positive correlation.

Particle size distribution data was insignificant among the different sites, suggesting that it is not an increase in smaller size particles that *S. giganteus* can feed upon that leads to an increase in the density of the polychaete but rather an overall increase in the sedimentation rate. In particular, the number of sediment fragments less than 10 µm, as a representation of a range of 2 to12 µm of phytoplankton that *S. giganteus* are suspected to selectively feed on (Ostroumov 2005), were not found to differ among sites. Interestingly, the opposite findings were found previously in Bonaire in which there was no significant difference in sedimentation rates between sites but there was a significant difference in particle size distribution (Willams 2009). This study used data collected from over a year rather than a few months as in the Williams study, which strengthens the results obtained in this study. The trend in the differences between this study and the Williams study suggests that pollution indicators such as sedimentation may be significantly influenced by temporal factors.

Effects of *Enterococcus* contamination on *S. giganteus* densities

In contrast to the hypothesis of this study that *Enterococcus* contamination would increase among higher impacted sites, there were no significant findings among sites for *Enterococcus* contamination. Fecal contamination was found at Cha Cha Cha and 18th Palm, two sites with high sedimentation rates and a close proximity to resorts, but the findings were not significant. Future studies should look at more depths since the sources of fecal contamination may have been overlooked at these sites, especially shallow depths given their proximity to the polluting source. An odd outlier, at 18 m at Witches Hut, could have been related to the northward current and location of Witches Hut north of the more populated area of Kralendijk; this finding was unexpected but also not significant. The mere presence of *Enterococcus* at any of these sites is cause for concern due to the tendency of the bacteria to sustain itself over time once levels are established; this bacteria has been linked to increases in coral mortality, which,combined with other threats to coral reef health, is concerning (Kline et al. 2006).

Coral host preference by *S. giganteus* and coral composition

The coral composition of each transect broken down by species was not related to the density of *S. giganteus* on any specific coral host. This suggests that *S. giganteus* density at any given site is not related to differences in preferred host, supporting the original hypothesis that there would be no positive correlation between the presence of preferred hosts and the density of *S. giganteus*. The most common coral host preferences were found to be *M. annularis, A. agaricites,* and *M. cavernosa* when looked at over all sites this supports data found in another study on Bonaire (Williams 2009). While this study supports that *S. giganteus* has preferences for coral hosts, the results suggests that the hypothesis that host preference did not affect the other findings is correct.

Future studies and applications

The oversimplification of classifying the impact of pollution on a reef by its proximity to a major resort may have affected the ability of this study to support the hypothesis that proximity to resorts will reflect increased pollution. The short time period of this study negated the ability to assess temporal fluctuations in the parameters of this study. Geographic features (such as bays or other areas that may trap nutrients or sediments) and the physical oceanography, current strength and direction, as well as other anthropogenic disturbances such as the recent land development may be equally important in quantifying the true impact of pollution on certain reefs. In this study in Bonaire, a strong northward current flows up the west side of the island where the study sites were located. It is possible that this current affects the distribution of nutrients along the coast, causing dispersal of pollution from resorts to be distributed northward of the source. In addition, Witches Hut was more impacted than expected by the pollution indicators assessed in this study given that is the farthest distance from any resort; this unexpected result could be related to both the strong northward current bringing nutrients up the coast and the bay-like topography of the island at this site which prevents continuing flow of nutrients further north along the coast.

The strong positive relationship of *S. giganteus* density to sedimentation rates is an important and significant finding that could be utilized in monitoring applications. While this study did not sufficiently establish *S. giganteus* as an indicator of anthropogenic pollution, the findings of this study supported that *S. giganteus* could potentially be used as an indicator of increased sedimentation. Sedimentation is a serious threat to coral reefs as it reduces both survival and growth rates of many coral species as well as smothers coral recruits; settlement rates were found to be near-zero on surfaces covered with sediments (Fabricius 2005). Since *S.giganteus*'s range expands from the Caribbean to the Indo-Pacific, it is possible that it could be used as a bio-indicator of increased sedimentation in other coral reef systems beyond Bonaire. This study justifies further research into the use of *S. giganteus* as a rapid assessment for increased sedimentation in coral reef ecosystems that can integrated into other assessment programs, in particular programs involving community volunteers given the simplicity of assessing the density of *S. giganteus*.

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A comparison of cleaning stations operated by the cleaner shrimp *Periclemenes pedersoni* on host anemones *Condylactis gigantea* and *Bartholomea annulata*

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Abstract

Cleaner shrimp are commonly found throughout Caribbean coral reefs and can effectively reduce parasite loads on reef fish resulting in increased fitness of local reef fish populations. The marine cleaner shrimp, *Periclemenes pedersoni*, most commonly inhabits two coral reef anemones, *Condylactis gigantea* or *Bartholomea annulata*, which have different distribution patterns on reefs. *C. gigantea* resides on hard or rocky substrates with greater relief, whereas *B. annulata* lives on muddy, sandy substrates or crevices with less relief. Past studies have been done on cleaning by *P. pedersoni*, yet no research has been done on the effects the differing host anemones may have on cleaning interactions. Using 12 min observation periods between 13:00 -17:00 h on the fringing reef in Bonaire, number of *P. pedersoni* shrimp, number of clients, species of clients, and time cleaned per client were recorded for *C. gigantea* and *B. annulata* anemone cleaning stations. When compared to *B. annulata*, *C. gigantea* had significantly more *P. pedersoni* shrimp, which cleaned a significantly greater number of client fishes. Greater species richness of clients was observed visiting *C. gigantea* than *B. annulata* cleaning stations, however there was no difference in time spent cleaning per client. Although protected in Bonaire, aquarium trade collection of *C. gigantea* throughout the rest of the Caribbean may result in an overall reduction in the number of cleaning interactions occurring on the reef, potentially having detrimental effects on the health of local fish populations.

Introduction

Mutualistic interactions are encounters between organisms that benefit both while causing harm to neither (Boucher 1982). Over the past two decades ecologists have come to realize that mutualistic interactions between organisms are equally as important as negative effects and physical factors when determining distribution and abundance of species (Stachowicz 2001). Mutualistic interactions can be observed worldwide, from ants that protect and distribute fungi while using it for nourishment, to bioluminescent microorganisms living mutualistically with pelagic marine jellyfish (Boucher 1982). Mutualistic interactions are also apparent in coral reefs, such as the relationship between scleractinian corals and dinoflagellate zooxanthellae. The scleractinian corals provide protection and a place for the zooxanthellae to live, and in return the zooxanthellae provide greater calcification rates for the corals to grow (Pearse 1971). The foundation created by the mutualism between corals and zooxanthellae provide a backdrop for other mutualistic interactions to occur, including cleaning.

Mutualistic cleaning associations in the marine environment involve organisms, often shrimp or fish, which remove parasites, mucus, scales and skin from cooperating client fish (Feder 1966). The interactions between clients and cleaners range from simple associations such as large groupers that pass by a goby cleaning station and stop to be cleaned, to highly complex interactions such as reef fish that learn and remember the location of cleaning shrimp which dance and wave their antennae to attract clients (Feder 1966). Mutualistic cleaning interactions can be observed in both tropical and temperate areas of the marine environment, and provide mutually beneficial partnerships in which client fish parasite loads are reduced and the cleaner species gains nutrition (Becker and Grutter 2004). Although it has been debated in the past that cleaner species were possibly parasitic and had little effect on actual parasite loads of clients, Grutter (1999) and Becker and Grutter (2004) analyzed gut contents of cleaner fish and shrimp and determined that they actually do clean – specifically cleaner shrimp reduced client parasite loads by 74.5% (Becker and Grutter 2004).

The Caribbean marine shrimp *Periclemenes pedersoni* has been documented on multiple occasions to exhibit cleaning behavior, both in field (Feder 1966, Mahnken 1972, Wicksten 1995, Velasquez 2008, McCammon 2010) and laboratory settings (Bunkley-Williams and Williams 1998). *P. pedersoni* may even be controlling parasite loads on reef fish (Bunkley-Williams and Williams 1998, Mccammon et al. 2010). When the parasite loads of fish with and without access to *P. pedersoni* were compared, the fish without access to the cleaner shrimp averaged a parasitic load 4.4-times-higher than fish with access to the shrimp (McCammon et al. 2010).

P. pedersoni is found primarily on two species of anemones, *Condylactis gigantea* and *Bartholomea annulata*, although it is occasionally found on other invertebrate species as well (Williams and Bunkley-Williams 2000). *B. annulata* inhabits muddy, sandy areas in crevices, on generally flatter substrates then does *C. gigantea,* which is often found on rocky substrates with more relief (Mahnken 1972). Once settled, *P. pedersoni* rarely leave the host anemone (Mahnken 1972) and if it leaves the host, it is much more likely to re-settle on the same species of anemone (Williams and Bunkley-Williams 2000). Upon further investigation by Silbiger and Childress (2008), *P. pedersoni* appeared to have no particular host specificity as the abundance of the shrimp was related to the abundance of anemones. In an earlier study (Mahnken 1972) there were more *P. pedersoni* on anemones that were located near high fish traffic zones. Therefore, the distribution of *P. pedersoni* on reefs is influenced by location rather than host species (Mahnken 1972, Silbiger and Childress 2008). In addition, Mahnken (1972) suggests that the transparent color of *B. annulata* helps to better broadcast the availability of cleaner shrimp, but in his study there was a greater abundance of *B. annulata* when compared to *C. gigantea*, and nothing is said regarding the effect of the more brightly colored *C. gigantea* on distinguishing *P. pedersoni* by reef fish.

P. pedersoni has been reported as the most common and prevalent cleaner species on reefs in Bonaire, Dutch Caribbean (Wicksten 1995). During observations of *P. pedersoni* cleaning stations in Bonaire, Wicksten (1995) identified the most common client fishes to be groupers, parrotfishes, tangs, and goatfishes. However, although the author noted that the host anemones were either *C. gigantea* or *B. annulata*, Wicksten (1995) did not differentiate between the two anemone species in regards to the cleaning frequency or client composition of the cleaning stations. The varying physical characteristics and habitat distributions of the host anemones may affect cleaning interactions, but no previous studies have yet to compare the differences between *B. annulata* and *C. gigantea* cleaning stations.

The purpose of this study was thus to compare characteristics of *P. pedersoni* cleaning stations on *C. gigantea* and *B. annulata* in Bonaire through field observations, testing the following hypotheses:

H_1: As in the study by Silbiger and Childress (2008), there will be no difference in the mean number of shrimp on the host anemones *C. gigantea* and *B. annulata*.

H_2: There will be no difference in the mean number of clients visiting shrimp on *C. gigantea* and *B. annulata* during the observation period.

H_3*:* *C. gigantea* will have greater mean species richness of visiting clients than *B. annulata* due to *B. annulata*'s habitat distribution on the reef, which may be less central to reef fish traffic (Mahnken 1972).

H_4: There will be no difference in the mean length of cleaning time per client at the host anemones *C. gigantea* and *B. annulata*, because time spent cleaning depends on client parasite load rather than species of anemone (Feder 1966).

Methods

Study Site

Between the months of February and March 2011, SCUBA was used to find and make behavioral observations on anemone cleaning stations at several dive sites on the west coast of Bonaire (listed in order from North to South): Witches Hut (12˚12’30.82” N, 068˚19’09.80” W), Cliff (12˚10’25.41” N, 068˚17’19.93” W), Bari Reef (12˚10’12.52” N, 068˚17’14.97” W), Something Special (12˚09’41.10” N, 068˚17’00.96” W), and Red Slave (12˚1’52.59” N, 068˚15’24.93” W). All study sites had fringing reefs with shallow, sandy flats − both areas where cleaning stations are commonly found. The reef crests were between 5 - 12 m deep and gradually sloped down to deeper depths, with the exception of Cliff at which the reef dropped off at 10 m.

Field observations

Research dives were conducted between the hours of 13:00 and 17:00, as Velasquez (2008) observed that there tended to be a high intensity of cleaning done by *P. pedersoni* on reefs in Bonaire during the afternoon. A U-shaped search pattern was used at each study site to search for *C. gigantea* or *B. annulata* beginning at the max depth of 20 m. Divers swam for 60 m along the depth contour then moved shoreward two meters and returned along the new depth contour in the opposite direction. This pattern was continued until either *C. gigantea* or *B. annulata* housing *P. pedersoni* shrimp were found. Once an anemone was located, the number of resident *P. pedersoni* shrimp was recorded. An acclimation period of two minutes was used to allow the reef community to acclimate to the presence of the observers, during which the observer would retreat approximately three meters from the anemone. During 12 min. observations the following data were recorded: the species of fish visiting the cleaning station and the amount of time cleaned per visit. At the end of the observation period, the U-search pattern was resumed until the next anemone was located and the observational process was repeated. From the data collected, number of shrimp per anemone, number of clients per anemone, and time spent cleaning per client were calculated for both *C. gigantea* and *B. annulata* anemones to use in statistical comparisons.

Data analysis

A two-tailed t-test (α = 0.05) was conducted to compare the mean number of *P. pedersoni* shrimp living on *C. gigantea* to *B. annulata*, compare the mean number of clients visiting *C. gigantea* and *B. annulata* during the observation period, compare the mean species richness of client fish visiting *C. gigantea* and *B. annulata*, and compare mean number of time spent cleaning per client at *C. gigantea* and *B. annulata.*

Results

Results were based on observations on 14 *C. gigantea* found at seven sites at depths ranging from 7 - 18 m, and 15 *B. annulata* found at seven sites at depths ranging from 6 - 19 m.

Number of shrimp

The density of *P. pedersoni* shrimp residing on *C. gigantea* varied from 1 - 10 individuals per anemone, whereas the density of *P. pedersoni* shrimp residing on *B. annulata* varied from 1 - 6 shrimp per anemone. There were significantly more *P. pedersoni* on *C. gigantea* than *B. annulata* (Fig. 1) (two-tailed t-test, p = 0.039, df = 21).

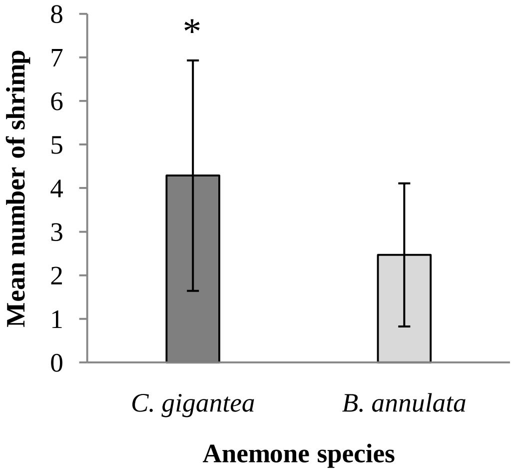

Fig. 1 Comparison of mean number of P. pedersoni shrimp residing in host C. gigantea (n = 14) and B. annulata (n = 15) anemones (two-tailed t-test, p = 0.039, df = 21) observed between 13:00-17:00 h on the fringing reef in Bonaire.

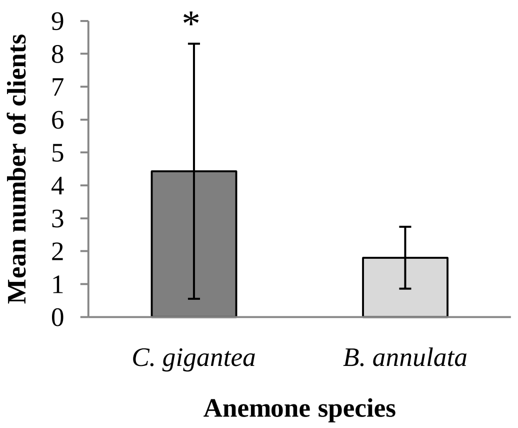

Fig. 2 Comparison of mean number of cleaning interactions observed during 12min observation periods for C. gigantea (n = 14) and B. annulata (n = 15) (two-tailed t-test, p = 0.027, df = 14) during observations between 13:00 - 17:00 h on the fringing reef in Bonaire.

Number of clients

Sixty-two clients were observed being cleaned at the 14 *C. gigantea* anemones, with the amount of clients per anemone varying from 0 - 13 individuals during the observation period. Twenty-six clients were observed being cleaned at 15 *B. annulata,* while individual anemones varied from 0 - 3 clients. There were significantly more clients per 12 min. observation period visiting *C. gigantea* anemones than *B. annulata* anemones (Fig. 2) (two-tailed t-test, p = 0.027, df = 14).

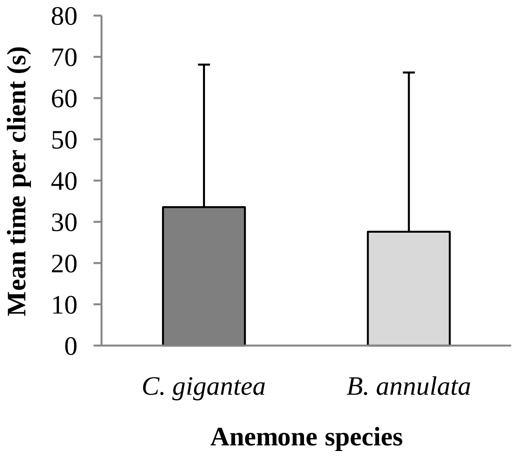

Fig. 3 Comparison of mean species richness of visiting client fishes observed during 12min observation periods for C. gigantea (n = 14) and B. annulata (n = 15) (one-tailed t-test, p = 0.031, df = 17), during observations between 13:00-17:00 h

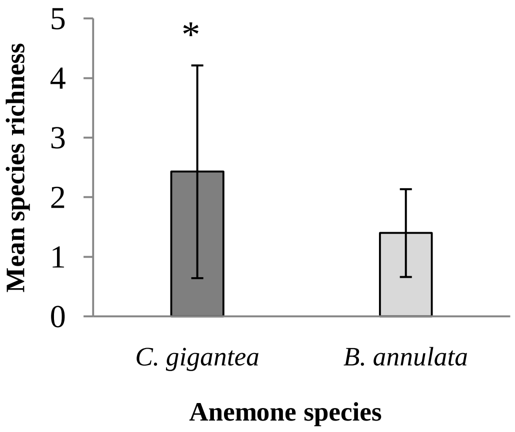

Fig. 4 Comparison of mean amount of time spent cleaning per client for C. gigantea (n = 62) and B. annulata (n = 27) (two-tailed t-test, p = 0.491, df = 45) during observations between 13:00 - 17:00 h on the fringing reef in Bonaire.

Species richness

Nine species of fish from seven families were observed being cleaned at *C. gigantea*, ranging from 0 - 4 species per observation period, and *B. annulata* had ten species of fish from six families – ranging from 0-3 species per anemone. Mean species richness of visiting client fishes was higher for *C. gigantea* than *B. annulata* (Fig. 3) (one-tailed t-test, p = 0.031, df = 17).

Time spent cleaning

P. pedersoni spent a similar amount of time cleaning per client on *C. gigantea* (33.6 ± 34.5 $s \cdot client^{-1}$) and *B. annulata* (27.6 ± 38.6 $s \cdot client^{-1}$) (Fig. 4) (two-tailed t-test, p = 0.491, df = 45).

Discussion

The comparison of cleaner abundance and cleaner interactions between *C. gigantea* and *B. annulata* anemones on Bonaire resulted in more shrimp per anemone, more clients per anemone, and greater species richness on *C. gigantea* than *B. annulata.* However, there was no difference in time spent cleaning between anemone species.

Number of shrimp

The hypothesis (H_1) that there would be similar number of *P. pedersoni* on *C. gigantea* and *B. annulata* was based on studies that reported that there was no difference in abundance of *P. pedersoni* on the two anemones (Silbiger and

Childress 2008) and that abundance of shrimp increased in areas where there was higher fish traffic (Mahnken 1972). In this study there were significantly more shrimp on *C. gigantea* anemones. The difference could be due to the location of anemones - in their position on the reef with regards to relief or their ability to broadcast availability. The size of anemones could also have an effect on the number of *P. pedersoni* that are able to live on the host anemone. It is interesting that although more cleaning occurs at *C gigantea*, the total numbers of anemones hosting *P. pedersoni* are similar (14 *C. gigantea* and 15 *B. annulata*). However, the number of shrimp per anemone is greater and number of clients is greater on *C. gigantea.* Perhaps the greater number of clients is stimulating the greater number of *P. pedersoni* because there are more resources available at *C. gigantea* anemones.

Number of clients

The hypothesis (H_2) that there would be similar number of clients visiting *C. gigantea* and *B. annulata* cleaning stations was based on the lack of research distinguishing any differences in cleaning interactions occurring at the two anemones. This study found there were significantly more clients visiting *C. gigantea* than *B. annulata* to be cleaned. This could be due to the anemone locations on the reef, with *C. gigantea* generally being located more centrally on the reef, as opposed to *B. annulata* often being located on sandy substrates near the reef edge (Mahnken 1972). The different physical characteristics of the two anemones, in color and transparency, may have differing efficiency in broadcasting the availability of *P. pedersoni* for cleaning. The hypothesis by Mahnken (1972) that the transparent color of *B. annulata* would help better broadcast the availability of the shrimp was not supported by this study, as there were less clients visiting the harder to see, more transparent *B. annulata*.

Species richness

The hypothesis (H_3) that *C. gigantea* would have greater client species richness was based on the work of Silbiger and Childress (2008), and Mahnken (1972) that found *C. gigantea* more centrally located on the reef, whereas *B. annulata* found nearer the sand strip, on the edge of the reef. This study did not assess the movement of reef fish in relation to anemone distribution, however there were twice the number of cleaning interactions and twice the mean species richness of clients occurring at *C. gigantea*. In terms of total number of species cleaned, *B. annulata* had ten species, while *C. gigantea* had nine species, yet both species cleaned most commonly Pomacentridae (Fig. 5), which is contradictory to Wicksten (1995) who found that the most common clients were grouper, parrotfish, tang, and goatfish – although grouper, parrotfish and goatfish were common clients for both anemones. This concludes that the main clients of *P. pedersoni* cleaning interactions remain similar regardless of the host anemone; however there are a few species that are found to be cleaned solely at one type of anemone, presumably due to the differing habitat distribution of each.

Time spent cleaning

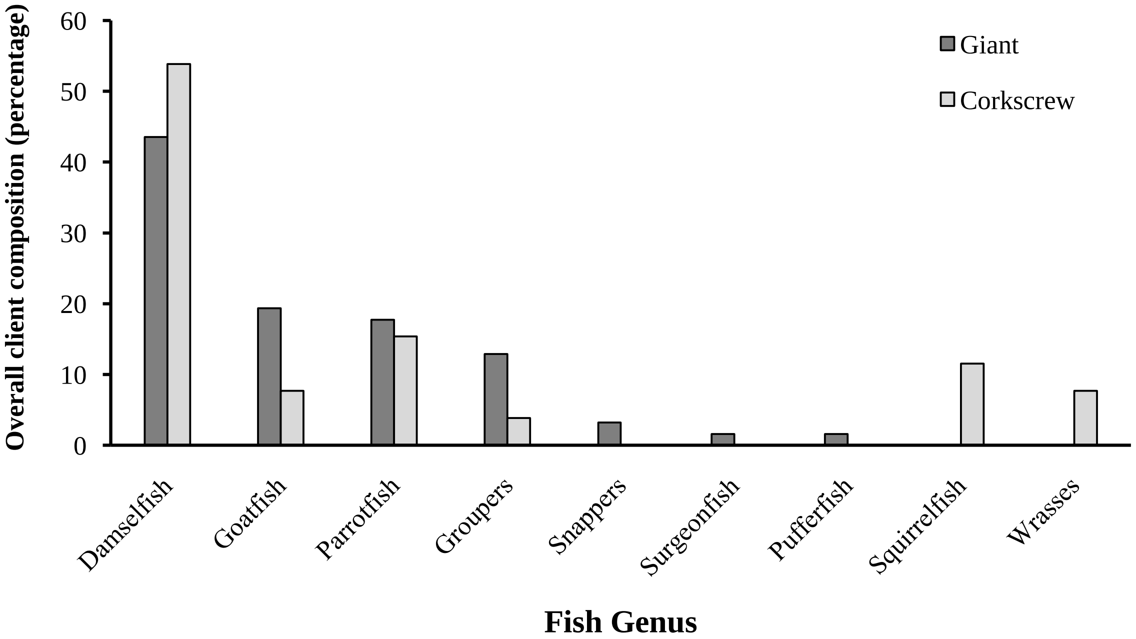

Fig. 5 Comparison of overall percent client composition organized by fish family for *C. gigantea* and *B. annulata* from observations between 13:00 - 17:00 h on the fringing reef in Bonaire.

There was no significant difference in the mean time spent cleaning per client between the two anemones, which supports H_4. Although there are more client fish and more shrimp on *C. gigantea* than *B. annulata*, the actual cleaning interaction is similar. Regardless of the number of clients visiting, the average cleaning time remained the same. This supports the findings by Mahnken (1972) that the cleaning interaction is dictated by the client fish as opposed to the shrimp. Presumably if the interaction were dictated by the cleaner, then the lesser number of cleaning interactions occurring at *B. annulata* may be counterbalanced by longer time spent cleaning, although this was not the case. The findings support the suggestion by Feder (1966) that the cleaning interaction is more likely dependent on client parasite load than the nutritional needs of *P. pedersoni*.

Conclusions

Overall, there are differences in cleaning by *P. pedersoni* between the two most common host anemones that affect the cleaning interactions of *P. pedersoni* and its clients. *C. gigantea* anemones are more efficient hosts for cleaning stations than *B. annulata* anemones, in that a greater number of *P. pedersoni* shrimp were found to live on *C. gigantea* anemones, and the shrimp clean a greater number of client fishes, as well as a greater species richness of client fishes.

The number of cleaning interactions occurring on the reef has been shown to be correlated to overall fitness of the local fish population (Bshary 2007). With the ever growing demand of the aquarium trade for interesting and visually attractive marine organisms such as the colorful *C. gigantea*, it is important to realize that selective removal of said anemone may have ecological effects. There has already been a local extinction of *C. gigantea* on a reef in Brazil (Gasparini 2005), and although not occurring in Bonaire, if similar levels of collection of *C. gigantea* are occurring throughout the rest of the Caribbean this may result in fewer available *C. gigantea* for *P. pedersoni* to live on. A decrease in the number of *C. gigantea* cleaning stations may result in an overall decrease of the number of cleaning interactions occurring on the reef, which may have detrimental effects on the health of the local reef fish population.

Future studies

A comparative study of the distribution of anemones and levels of fish traffic could better explain the distribution of cleaners on the reef. No data were collected on the number of anemones without *P. pedersoni*. These data would be important in a study of the relationship between fish traffic and distribution of *P. pedersoni* on anemones. Lastly, it would be interesting to study the relationship between number of client cleaning interactions at *C. gigantea* and size or longevity of shrimp with shrimp on *B. annulata*. Although there may be a greater number of cleaning interactions occurring at *C. gigantea* cleaning stations, there is the possibility that because there are also more *P. pedersoni* on *C. gigantea* that the shrimp receive less nutrition per individual.

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A comparative study of the feeding ecology of invasive lionfish *(Pterois volitans)* in the Caribbean

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Abstract

Invasive species are often a detriment to the environment due to the lack of parasites, disease and natural predators in the invaded environment, which allows the population to explode. *Pterois volitans* were introduced into the eastern part of the Atlantic in 1980’s, and migrated to the southern Caribbean and in October 2009, lionfish were first documented on the island of Bonaire, Dutch Caribbean. The purpose of this study was to document the feeding ecology of lionfish by identifying and quantifying stomach contents of different size classes of lionfish found on the island using four different metrics- frequency of occurrence, percent by volume, percent by number and Index of Relative Importance (IRI). Of the 70 lionfish stomachs analyzed, there was a positive correlation between lionfish size and amount of fish consumed. Similarly, there was a negative trend seen with size class and the amount of shrimp found in the stomach contents. When IRI was used to compare feeding ecology of Bonaire lionfish to a Bahamas study, the top five ranked families of preyed differed. This study identifies major dietary trends of lionfish on Bonaire, and can be used to better understand the feeding ecology and diet habits.

Introduction

Coral reefs ecosystems around the world are being degraded by a variety of anthropogenic stressors including overfishing, pollution and global warming (Cesar et al. 2003). Coral bleaching, associated with global warming, is an occurrence where the symbiotic zooxanthelle algae that live in the tissue of coral polyps are expelled from the coral (Baker 2008). Outbreaks of coral diseases have become more frequent and diverse over the last 20 to 30 years (Weil et al. 2006) and may cause reefs to be more susceptible to invasions, which could increase the rate of degradation of these already fragile reefs (Weil et al. 2006).

An invasion is defined as the successful survival and reproduction of a species that is not native to the habitat (Lasso-Alcala and Posada 2010). Following the introduction of a non-native species an invasion may occur, which can result in destruction of habitat or a decrease in local biodiversity (Lasso-Alcala and Posada 2010) and decline and extinction of native species due to the invaded species out competing native species for territory and food supply (Clavero and Garcia-Berthou 2005). Invasive species have become a problem following human expansion during the age of exploration up to current time (Whitfield et al. 2002). There are many natural barriers between reefs, but once exploration began, species could travel a much larger distance in the ballast water of a ship, allowing for non-native species to end up in a new environment (Lavole et. al. 1999). More recently, the exotic aquarium trade allows for many different types of fish to be transported all over the world, allowing for a wider range to be established if these animals are released into the native environment (Padilla and Williams 2004).

The invasion of the lionfish into the North Atlantic waters is the first documented case of an invasive marine fish to become established in the North Atlantic and Caribbean Sea (Morris and Whitefield 2009; Schofield 2009). The introduction is thought to have occurred during the 1980's, which was the first documented sighting of lionfish off the coast of Florida (Morris et. al. 2009). The range of the invasive lionfish has been expanding since then and are now found as far north as New York, and as far south as Venezuela (Schofield 2010). The rapid expansion is likely due to lack of natural predators lionfish's venomous spines, ample prey, and a lack of competition from natural apex predators (Morris and Akins 2009). Specifically, lionfish damage Bahamas coral reefs by lowering reef recruitment by as much as 79% (Albins and Hixon 2009), suggesting that lionfish have a direct impact on the overall health of reef ecosystems.

The first documented lionfish capture on Bonaire was in October 2009 by marine park manager Ramon de Leon, and lionfish sightings and captures on the island have since been increasing (personal communication Dr. Rita Peachey 2011). The purpose of this research was to study the feeding ecology of lionfish through stomach content analysis in order to compare the feeding ecology of the *P. volitans* in Bonaire to the lionfish in the Bahamas (Morris & Akins 2009). This study seeks to determine the prey family type, quantity, and volume of prey lionfish consume at different lifecycle stages. If the two populations share similar feeding habits, it might indicate which reef fish lionfish prefer to consume. The specific hypotheses that were tested are:

H_1: As the size of lionfish increase, the amount of fish found in the stomach contents will increase, and the amount of crustaceans found will decrease.

H_2: The reef fish families consumed by lionfish in Bonaire will be ranked the same as the reef fish consumed in Bahamas including: Gobiidae, Labridae, Grammatidae, Apogonidae, Pomancentridae, Serranidae, Blenniidae, Atherinidae, Mullidae and Monacanthidae (Morris and Akins 2009).

Methods

Lionfish collection

Since October 2009, volunteer 'lionfish hunters' brought *P. volitans* into the Center for International Education Exchange (CIEE) research station laboratory in Kralendijk from different locations around the island, supplementing over 1,500 cataloged specimens stored in the CIEE lab. Currently there are over 1550 lionfish cataloged in the CIEE lab book. When live specimens were delivered to the lab, fish were euthanized by placing them on ice. Standard length (cm), total length (cm) and wet weight (g) of each fish were measured and the place, depth, and time of collection were recorded. Each fish was then placed in a freezer bag, the bag was numbered according to the number recorded in the collection book and placed in a freezer for further research. Previous specimens were fixed in 10% formalin and transferred to 70% ethanol for storage.

Stomach content analysis

Total length, measured from the snout of the fish to the tip of the tail, of the lionfish was used to determine the size category of each specimen (Morris and Atkins, 2009) as follows: 6.1-10 cm, 10.1-14 cm, 14.1-18 cm, 18.1-22 cm, 22.1-26 cm, 26.1-30 cm, 30.1 cm and greater. To determine which specimens would be dissected, data from the CIEE logbook were categorized by total size and selected from the size category randomly. Three incisions were made to gain access to the internal organs of the fish. To remove the stomach, the section of alimentary canal from behind the throat to the beginning of the intestine was removed from the specimen and placed into a glass bowl. The stomach was then flushed with water to remove any contents that might have adhered to the stomach wall. The stomach contents were then measured by volume using the displacement method with a graduated cylinder or modified syringes. Volume of each item found within a stomach was recorded. Stomach contents were identified to the lowest possible taxa but comparisons of prey importance are based on family level data. Stomach contents of fresh or frozen specimens were preserved in 10% formalin for a minimum of 24 h, and then transferred to 70% ethanol before examination of the contents occurred.

Data analysis

For each size class, three metrics — frequency of occurrence, percent by volume, and percent by number— were calculated and used to determine the prey importance relative to size class. Frequency of occurrence was calculated by dividing the number of stomachs (in which each identified prey family was found) by the total number of stomachs analyzed for that size class. Percent by volume was calculated by dividing the total volume of each prey family by the total volume of the stomach contents. Percent by number was calculated by dividing the raw number of prey fish within each identified family by the total number of specimens found within the stomachs. To better assess the importance of the differences between fish and crustaceans in the diet of Bonaire *P. volitans*, these metrics were used to calculate the Index of Relative Importance (IRI) for fish and crustaceans using the formula:

$$IRI_a = F_a * (N_a + V_a)$$

In which (F_a) = frequency of occurrence, (V_a) = percent by volume, and (N_a) = percent by number (Morris and Akins 2009). Similarly, the same formula was used to calculate the differences in the importance of different families in the lionfish diet. The resulting values served as the basis by which to compare the lionfish diets.

Results

Of the 70 lionfish dissected, five had empty stomachs and were not included in the analysis. Teleosts were found in 43 stomachs and represented 91% of the lionfish diet by volume, 75 % by frequency and 75 % by occurrence. Of 57 specimens identified five different families were represented: Pomancentridae, Blenniidae, Gobiidae, Serranidae and Mullidae. Crustaceans were found in 21 stomachs and the group was composed entirely by shrimp and represented five percent of the diet consumed by volume, 23 % by frequency, and 25 % by occurrence.

The comparison of percent frequency of occurrence (Fig. 1) between teleosts and crustaceans indicated a notable positive trend between percent occurrence of fish in the stomach contents and size classes. As the size classes increased, the percent occurrence of fish increased. Crustaceans had a reciprocal trend: as size class increased the percent occurrence of shrimp decreased. There was a major increase in percent occurrence of fish between the size classes 14.1 cm and 18.1 cm, and similarly there is a major decrease in percent occurrence of shrimp between the same size classes.

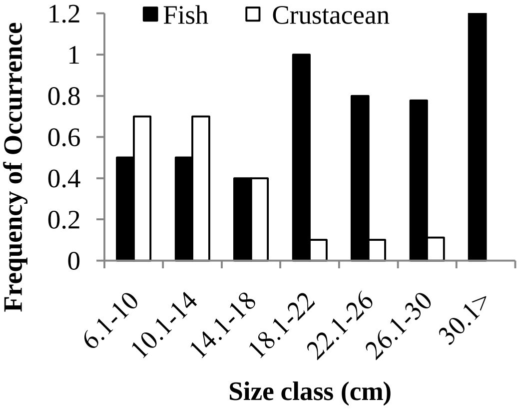

Fig. 1 Data shown is the percent frequency of occurrence of fish and crustaceans found in the stomach contents in 7 different size classes of lionfish.

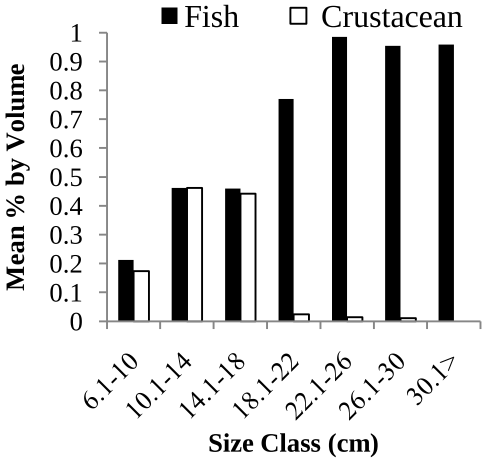

Fig. 2 Data shown indicates the mean percent volume found of fish and crustaceans that were found in the stomach contents of the 7 different lionfish classes.

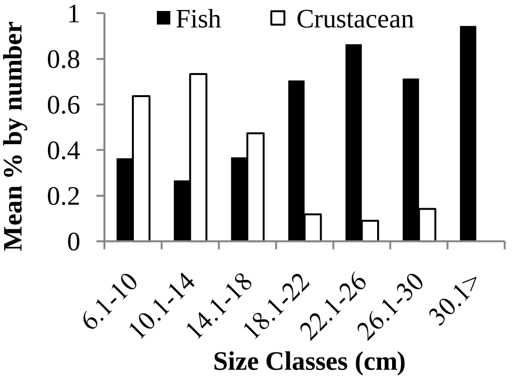

Fig. 3 Indicates mean percent by number of fish and Crustaceans found in the stomach contents of the 7 different size classes of lionfish.

The percent volume of fish and shrimp composition in lionfish diet indicated a positive trend when the size of lionfish and the percent volume of fish were compared (Fig. 2) and the opposite trend was observed in crustacean prey. The percent volume of fish also changed at the 14.1-18.1 cm size class. Similarly, there was a positive trend in percent number of fish in lionfish diet as the percent number of shrimp decreased. Again the changeover occurs at the 10.1 cm and 18.1 cm size class (Fig. 3).

The IRI were calculated and graphed to compare the changes in importance in the diet from crustaceans to fish in size classes. The graph indicates that there is a significant difference in the importance of shrimp in the lionfish diet when comparing size classes (Fig. 4). As the size classes increase, the IRI of crustaceans decreases, and the IRI of fish increase. The largest difference in the crustacean IRI is observed from size class 10.1 cm and 14.1cm, with the IRI dropping to lower than 0.1 at 18.1 cm and to zero after size class 20.1 cm . The highest importance of shrimp in the lionfish diet is found in the three smallest size classes, 6.1-10 cm 10.1-14 cm, and 14.1-18 cm. This study was compared to the study done in the Bahamas (Morris and Akins 2009) (Table 1). The comparisons indicate that the top five teleost families are different between the Bahamas study and this study, though there are similarities in some families in the diet of lionfish.

Table 1 The top 5 teleost prey families found in lionfish stomachs.

IRI	Bonaire	Bahamas
1	Pomancentridae	Gobiidae
2	Blenniidae	Labridae
3	Gobiidae	Grammatidae
4	Serranidae	Apogonidae
5	Mullidae	Pomancentridae

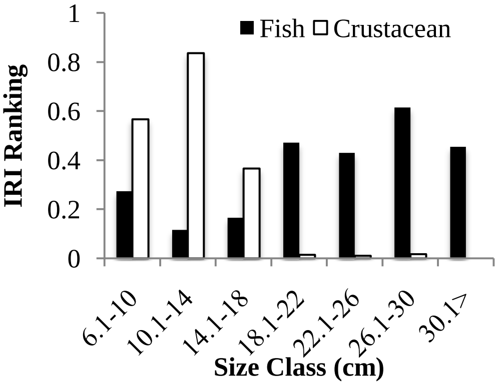

Fig 4 IRI of crustaceans and fish found in the stomachs of lionfish in the 7 different classes of lionfish.

Discussion

Data collected from all four metrics in this study indicate a clear positive trend between the amounts of fish consumed and the size of the lionfish. As lionfish increase in size, the amount of fish that are consumed greatly increases. This gives evidence to support hypothesis 1 and indicates that reef fish are an important dietary factor in larger lionfish, which may be affecting the amount of prey species on the reefs (Albins and Hixon 2009). In addition, when the IRI of fish and crustaceans were compared, the smaller size classes clearly had a higher dependency on crustaceans than larger lionfish. This high importance of crustaceans in the smaller size class has implications on larval reef fish diets, because larval fish also depend heavily on crustaceans as a food source (Scharf et al, 2000). This may indicate another reason why lionfish might be lowering recruitment levels of coral reefs. Since lionfish are eating a major source of prey for younger recruits, the smaller fish may not be obtaining enough nutrients to survive on a reef.

The IRI's of the Bonaire prey families and the Bahamas indicated differences in the rankings and composition of the top five families, therefore hypothesis was not supported. Some similarities were noted and some of the same families of prey fish were found in both diets, Gobiidae and Pomacentridae, but they had different rankings. The difference in the IRI's could be explained by the differences in abundance of certain fish families on the two reefs, and that there is no preference but rather it is more opportunistic to prey on the more abundant species.

The prey species that are being consumed by lionfish are abundant due to overfishing of apex predators on Bonaire (DeBey and Steneck 2009). In environments where predatory fish are not overfished, the presence of lionfish may have a larger affect the population numbers of the reef families. Because of competition by other predators for the prey species this results in depletion of prey species. In places like Bonaire there might not be such a drastic impact on the reef caused by the presence of lionfish because prey species may be overly abundant since there is a lack of natural predators found on these reefs.

This study provides insight on the dietary preferences of different size classes of lionfish and supports the idea that as these fish get bigger their diet switches from crustaceans to a diet of fish. The feeding ecology of lionfish in Bonaire suggest that there is a high predation on small reef fish, yet may not impact populations as much due to the loss of larger predators like groupers and snappers. This is not suggesting that lionfish are not detrimental, but rather the effects on Bonaire reefs might not be as great. Further studies will need to be done to determine whether the families eaten on Bonaire represent a dietary preference. If the abundance of prey in Bonaire differs to the abundant prey found in the Bahamas this may also alter lionfish dietary preferences. Lionfish may have a large impact of reef diversity and numbers in specific family populations and more studies need to be done to understand the feeding ecology.

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The sensitivity of the invasive lionfish, *Pterois volitans*, to parasitism in Bonaire, Dutch Caribbean

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Abstract

Invasive species have a history of damaging their invaded ecosystems and in the case of the *Pterois volitans* invasion to the waters of many Caribbean Island nations, there has been no exception. *Pterois volitans* has caused negative impacts to the coal reef ecosystems such as reduced juvenile coral reef fish recruitment and, consequently damage to the associated fisheries. Management strategies of multiple nations are currently centered upon the reduction of populations via hunting. This strategy requires substantial effort and thus long term management solutions may include biotic controls. Parasitism is an important facet of population dynamics and could be important to the population dynamics for *Pterois volitans* around Bonaire. *Pterois volitans* is rarely a victim of parasitism in its native range and has similarly low rates of parasitism reported in its invasive range. The prevalence or lack of parasitic interactions between *Pterois volitans* and native parasites could be important in planning management strategies and controlling populations in the future. This study examined 200 *Pterois volitans* captured in the coastal waters of Bonaire for parasites in the mouth and gill structure, as well as over the entirety of the skin, to investigate possible interactions occurring between local parasites and *Pterois volitans*. Only one of the 200 investigated specimens was found to have an isopod attached to its gill structure.

Introduction

The introduction of nonnative species has a well established history of negative impacts within their introduced range (Mooney and Cleland 2001; Verlaque et al. 2003; Rauch and Bar-Yam 2006. Invasions are the spread of a nonnative species into a new geographic area or range. From the invasive marine algae *Caulerpa* species, which is able to outcompete local alga and smother the Mediterranean due to its increased thermal stress tolerance, to the Indo-Pacific lionfish, *Pterois volitans* currently encroaching upon the waters of many Caribbean Island nations, they all pose unique threats to the areas they invade (Verlaque et al. 2003; Schofield 2009). The majority of threats to newly invaded areas are often not easily predicted, due to the biotic and abiotic variations during invasions, thus management strategies require adaptability (Ramon de Leon personal communication). There has been a trend that due to lack of natural biotic controls upon invasive species populations, they flourish in their newly established habitat (Clay 2003; Torchin et al. 2003). *Pterois volitans* has been no exception in this trending during its invasion of the eastern seaboard of the United States and the Caribbean Sea (Morris Jr and Whitfield 2009; Schofield 2009). *Pterois volitans* is intriguing in that it is the first documented case in which a Pacific marine fish has successfully invaded the Western Atlantic Ocean (Kimball et al. 2004).

Invasive species success is determined by a number of factors, such as predation ability and fecundity. However among the factors important in determining success of an invasive species is the role of parasites in controlling populations (Clay 2003; Torchin et al. 2003; Prenter et al. 2004). It is well established that invasive species tend to have fewer parasites owing to a lack of coevolution with native parasitic species (Clay 2003; Torchin et al. 2003; Prenter et al. 2004). In many cases, both terrestrial and aquatic, there has been a limitation in the parasitism upon invasive species (Clay 2003; Torchin et al. 2003). This has given rise to the 'enemy release hypothesis' that states that when a species is released from the grasp of a native predator or parasite, the species is able to flourish as a result of the lack of population control (Clay 2003; Torchin et al. 2003). Torchin et al. (2003) showed that in cases in which an invasive species left native parasites behind, they typically had half the number of parasites in their invaded range.

Pterois volitans has successfully invaded the Western Atlantic Ocean; however in their native range they extend from Lord Howe Island, Australia northward to the southern portion of Japan (Schultz 1986). The range also extends east-west into Indonesia, Micronesia and French Polynesia (Schultz 1986) *Pterois volitans* are found upon various bottom compositions with coral and rock being the most prevalent, and have a depth floor of greater than 60 meters within their invaded range (Schultz 1986; Ramon de Leon personal communication). As a consequence of its popularity in the ornamental fish trade, many specimens of *P. volitans* have been exported from their home region (Whitfield et al. 2002; Ruiz-Carus et al. 2006). With such a booming trade comes risk of release, with *P. volitans* becoming an established aspect of many marine ecosystems and currently threatening the biological composition of some invaded reefs (Mooney and Cleland 2001; Whitfield et al. 2002; Ruiz-Carus et al. 2006; Schofield 2009, 2010).

Pterois volitans was initially discovered along the coast of Florida in 1985 and became well established in the mid 1990's (Whitfield et al. 2002; Schofield 2009; Betancur-R et al. 2011). In the following years, *P. volitans* had been reported between Miami, Florida and Cape Hatteras, North Carolina (Schofield 2009). The invasive population of *P. volitans* was established by between 8 and 12 individuals, and thus there is little genetic variation among the populations currently invading the Caribbean Sea and the east coast of South America (Betancur-R et al. 2011). The thermal limitations placed upon *P. volitans* are a key element in preventing its further migration northward (Kimball et al. 2004). As no such limitation is present in the Caribbean and Gulf of Mexico, *P. volitans* has made considerable progress in invading the reef systems and coastal waters of the region. Schofield (2009, 2010) noted invasions into the insular waters of the Caribbean and the coastal areas of the mainland extending south to Venezuela. As *P. volitans* has progressed further into the Caribbean, Bonaire was invaded and the first *P. volitans* was captured in October of 2009 (Ramon de Leon personal communication). Although abiotic factors such as thermal resilience and currents are an important component in determining the spread of nonnative species, the biotic components of population controls should also be considered.

In any invasion the aspects directly attributable to survivability include ability to hunt, ability to mate, and the ability to avoid predation. *Pterois volitans* high fecundity and ability to hunt, effectively coupled with a distinct lack of predators has allowed for a population boom (Whitfield et al. 2002; Schofield 2009). However, there is also another aspect critical to the control of any population that should not be overlooked: parasitism. In the range of successful invasion, there is a distinct lack of widespread parasitism as well as studies regarding parasitism prevalence upon *P. volitans* (Ruiz-Carus et al. 2006; Morris Jr and Whitfield 2009; Morris Jr et al. 2009; Schofield 2009). An important aspect of parasitic relationships is resistance and genetic diversity, and as Betancur-R et al. (2011) found, there is currently a lack of genetic diversity in the *P. volitans* population invading Caribbean Island nations. Parasitism upon *P. volitans* occurs infrequently in its native range, and several instances of parasitism have been reported within the invaded range (Diamant et al. 2004; Ruiz-Carus et al. 2006; Morris Jr et al. 2009). Parasitism upon *P. volitans* has been reported in the invaded areas of the Red Sea and Florida in which both ectoparasites and endoparasites were observed (Diamant et al. 2004; Ruiz-Carus et al. 2006). However, the examples of parasitism upon *P. volitans* are limited to a few cases observed worldwide, with only singular instances of parasitic species having been recorded in Japan and the Red Sea (Morris Jr et al. 2009). Parasitism in the native range has been restricted to the few reported cases of ectoparastism reported in Japan involving leaches (Morris Jr et al. 2009).

The invasion of *Pterois volitans* into the coastal waters of Bonaire, Dutch Caribbean has thus far had many negative effects upon the native flora and fauna. As there are currently very few reported instances of parasitism upon *P. volitans* reported worldwide, presence of parasites could indicate susceptibility of the invasive populations to native parasites. As such, this study seeks to determine if parasitism upon *P. volitans* has begun in the waters surrounding Bonaire in the time since its introduction.

> H_1: Given the presence of parasites on *Pterois volitans* in Florida and the Bahamas, there will also be parasites present on *Pterois volitans* in the waters surrounding Bonaire.

Methods

Study Site

This study has been undertaken on the island of Bonaire, D.C., (12° 9’ 44.74” N, 068° 16’ 53.62” W) (Fig. 1), in the waters around which *Pterois volitans* has been present since October of 2009 (Ramon de Leon personal communication). As many of the specimens have been retained, there has been a unique opportunity to examine them in detail to determine the prevalence of parasitism. Captured specimens were delivered to the CIEE Bonaire Research Station where, upon arrival the total length and depth of capture were recorded and they were cooled to temperatures below their thermal limitations. The specimens were then frozen for later examination. The sample size for this study was composed of 200 *P. volitans* collected by volunteer lionfish hunters and given over for study. The majority of specimens collected are from dive sites along the western coast of Bonaire. Those *P. volitans* selected for analysis were captured between 12 December 2010 and 18 March 2011 and were selected due to the recency of capture. The size of P. volitans examined ranged between 4.7 cm and 29.5 cm, with an average total length (± SD) of 16.9 ± 4.2 cm (Fig. 1). *Pterois volitans* were captured over a depth range between 1 and 65.1 meters, with an average capture depth (± SD) of 24.6 ± 12.4 meters (Fig. 3).

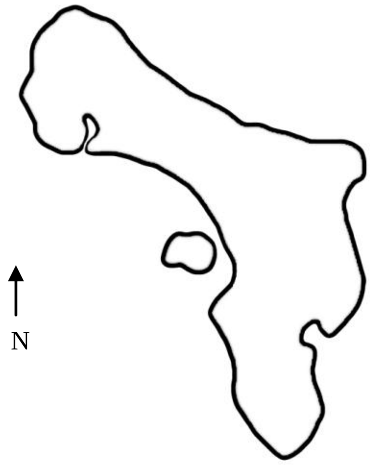

Fig. 1 Map of the island of Bonaire, Dutch Caribbean.

Examination

Pterois volitans were thawed and examined for ectoparasites under high powered fiber optic illuminators to effectively illuminate the areas of interest. The mouth was opened to its maximum aperture and examined, then each set of gills was examined independently. Following this, the skin was scrutinized, and each fin structure was spread open to comprehensively examine the specimen. Special attention was paid to areas of the skin at which parasites could easily attach, such as folds of skin and fin articulation points.

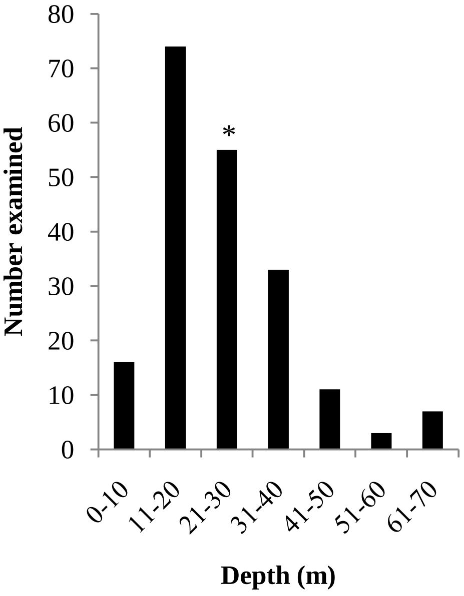

Fig. 2 The distribution of total length among 200 *Pterois volitans* examined. Average size of examined *P. volitans* (± SD) is 16.9 ± 4.2 cm. Asterisks denote parasites found within a given range.

Results

Of the 200 *Pterois volitans* examined, none had parasites, except for one specimen, which had a singular ectoparasite in the gill structure (found by C. McCleery). The parasite in question was an isopod and was identified by Dr. P. Sikkel as a member of the species Exocorolana (Paul Sikkel personal communication). It had a length of 1.0 cm and a width of 0.5 cm. The *P. volitans* on which the isopod was found was captured at a depth of 27 meters at the Cliff dive site. It had an overall length of 26.1 centimeters.

Discussion

Of the 200 *Pterois volitans* specimens examined in this study, only one showed evidence of parasitism. This *P. volitans*, which was 26.1 cm in total length and was collected in mid-February of 2011 was investigated by C. McCleery and found to have an isopod attached to the gill structure. This isopod was 1.0 cm by 0.5 cm. It did not appear to have negatively affected the overall fitness of the *P. volitans* in question, as the specimen did not appear emaciated. However, in past cases negative effects of isopod attachment to gill structure have been reported (Thatcher et al. 2000). In such cases the growth rate of the fish was negatively impacted due to the consumption of the gill filament by the isopod (Thatcher et al. 2000). As such, the isopod may have had a negative impact upon growth rate, however without further examination it is impossible to determine true impact. Another instance of parasitism was reported by STINAPA in which a leech was removed from a *P. volitans*. This specimen of *P. volitans* was collected at a depth of 60 meters and had an overall length of 40 cm (Ramon de Leon personal communication). Additionally, there have been reports of parasitism from Florida and the Bahamas which could indicate large scale developments of parasitism upon *P. volitans* in their invaded range (Ruiz-Carus et al. 2006; Morris Jr et al. 2009; Paul Sikkel personal communication). These limited instances of parasitism are interesting developments given the scarcity of parasites in *P. volitans* native range (Ruiz-Carus et al. 2006; Morris Jr et al. 2009). Within the native range, the instances of parasitism were limited to infrequent discoveries of a few parasitic species. These parasitic species, such as leeches in the Sea of Japan,

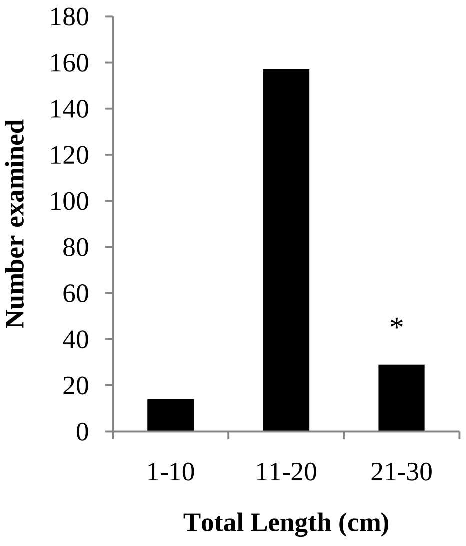

Fig. 3 The distribution of total length among 200 *Pterois volitans* examined. Average size of examined *P. volitans* (± SD) is 16.9 ± 4.2 cm. Asterisks denote parasites found within a given range.

have poorly understood effects upon *P. volitans* (Morris Jr et al. 2009). Such interactions are also poorly understood within the invasive region of *P. volitans*, although further work is ongoing (Paul Sikkel personal communication). The lack of research regarding parasitism upon *P. volitans* within its native range allow for few conclusions to be made regarding its importance to the population dynamics within the region. However, it may be an important factor in controlling *P. volitans* populations in its native range and thus further study should be undertaken. The discovery of this isopod could indicate an evolving parasite-prey relationship present within the reef ecosystems of the Caribbean. As there is a genetic bottleneck present among the members of the invasive *P. volitans* populations, the presence of parasitism may indicate that native parasites in the Caribbean and Florida could specialize and parasite upon *P. volitans* (Betancur-R et al. 2011). However, in order for such relationships to have effects upon the population, these relationships would require large scale developments of parasitism. Furthermore, these interactions would require negative effects to be experienced by *P. volitans* to effect the general population. In addition, susceptibility of *P. volitans* to native parasites in Bonaire could indicate susceptibility of invasive *P. volitans* to parasitism due to the limited genetic variability within the invasive range (Betancur-R et al. 2011).

Parasitic relationships are a contributing factor in the control of populations within their native range, so much so that when released from native predators, invasive species populations tend to explode (Clay 2003; Torchin et al. 2003). This is due to the parasites ability to draw sustenance from the host and force the host to compensate for the loss of nutrients and energy. This has led to the ‘enemy release hypothesis’ that states that when a species is released from predation or parasitism typically found within a native range, the given species tends to excel and become abundant (Clay 2003; Torchin et al. 2003). As such, parasitic relationships between *P. volitans* and parasites within its invasive range could signal the evolution of such relationships and consequently, a possible biological control for populations.

This topic begs further investigation as the development of such relationships could be critical in future population dynamics. Studies regarding the parasites upon *P. volitans* within the native range could prove useful as a comparative baseline. Inquiries regarding the development of parasitism within the invasive range are already underway in the Bahamas and this study may provide useful baseline details regarding instances of parasitism in invaded ranges (Paul Sikkel personal communication). This is an interesting topic for further review and will allow for a greater understanding of parasitic relationships as they develop in invasive tropical marine fishes.

There are several limitations of this study that may affect the conclusions drawn. The small number of examined *P. volitans* prevents drawing conclusions regarding parasite density and similar statistics which may have proved useful. The spatial and temporal limitations imposed may leave confounding variables, such as *P. volitans* population fluctuation with season, unaccounted for. Moreover, *P. volitans* were collected more frequently at popular dive sites around the island which may have influenced the results of this study. However, given the lack of genetic diversity found by Betancur-R et al. (2011) these factors would have negligible effect upon results.

With the discovery of further cases of parasitism upon *P. volitans* within its invasive range come further implications for the invasive populations. Additionally, with the development of such parasitic relationships in the brief time since introduction to Bonaire, there is the prospect of the development of similar relationships in other invaded waters. As there is genetic homogeneity among the invasive populations, the parasitism experienced by this population may well indicate the susceptibility of the population at large. Therefore, it is important that additional research be conducted over a greater spatial and temporal scale, as well as for research to encompass external and internal parasites, in order to establish the nature of parasitism upon *P. volitans*. Ergo, this study may indicate that parasitism could become an important defining characteristic of the invasive population densities of *P. volitans*.

Acknowledgments

To Charlotte McCleery and Ramon de Leon, I am deeply indebted to both for their contributions, without which this study would not have been possible. Dr. Paul Sikkel, your supply of knowledge throughout my research has had an inexorable impact. My greatest gratitudes are extended to my classmates and friends for their support, but most of all to my research partner and dive buddy, Ashton Williams, for her endless patience and flexibility, as well as a relentless sense of humor and daring. STINAPA and the BNMP, thanks for allowing me work within the reserves. I also wish to thank my research advisor, Dr. Eva Toth for her understanding through this project & thanks to Jennifer Blaine and Camerron Crowder for their aid through the entirety of this ordeal. Thanks to CIEE Bonaire for use of resources. And finally, to the volunteer lionfish hunters who provided the specimens to be examined, this study certainly would not have occurred without your tireless efforts and donations.

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Are damselfish detrimental to Bonaire's coral reefs?

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Abstract

Damselfish are small herbivores that cultivate algal gardens on coral heads throughout Caribbean reefs. These gardens are used to grow specific types of algae that they can digest easily. However, algal gardens can affect coral both physically and physiologically. In addition to competing with coral for light and space, algal gardens cause coral to spend valuable metabolic energy to remove the algae. This study aimed to determine whether there is a correlation between damselfish density, percent dead coral cover, and coral diversity. A total of thirty-two 20 x 1m belt transects were laid out randomly at a highly impacted and less impacted site. The damselfish density, the number of species, and their size distribution were recorded along with percent dead coral present and coral composition of the substrate. Data were analyzed and compared to deduce whether or not there was any significant evidence that high damselfish numbers are correlated with an increased percentage of dead coral. Although no connection was found between high densities of damselfish and increases in dead coral, it is possible that factors not quantified in this study, such as human impact, could have had an influential role on both damselfish densities and the health of the reef.

Introduction

Damselfish are herbivorous fish that are widely abundant on Caribbean reefs and are known for their distinct cultivation of algal gardens on scleractinean corals. Due to their lack of masticatory organs and specialized digestive enzymes for breaking down cell walls, damselfish can only digest filamentous algae (Hata and Kato 2010). Therefore, damselfish gardens consist of carefully tended areas of turf algae that exclude calcareous or indigestible algae. It is also shown that they favor delicate algal species from the *Polysiphonia* genus. In their studies, Hata and Kato (2010) found that *Polysiphonia* nearly exclusively inhabited the gardens of *Stegastes nigricans*. Damselfish territories can range in size, but research has suggested that there is a tradeoff between the digestible algae within the territory and the size of the territory. Studies have found that the amount of digestible algae in the territories of damselfish species *Hemiglyphidodon plagiometapon*, *Pomacentrus wardi*, and *Pomacentrus adelus* are higher in smaller gardens compared to larger gardens (Ceccarelli et al. 2006).

In order to create gardens, damselfish actively damage parts of the scleractinian corals by biting the living tissue and then tend to the algal mats that develop on the exposed skeleton (Precht et al. 2010). These lawns of algae are able to grow because damselfish are fiercely protective of them and will attack large herbivores, such as parrotfish, that attempt to graze on or near these algal mats. This territoriality reduces the threat of grazing from other herbivorous fish, and thus, algal mortality (Hinds and Ballantine 1986). Damselfish presence has another beneficial effect on the cultivated algae, as it has been hypothesized that the supply of Nitrogen and Phosphorous from damselfish excrement may enhance algal productivity. This natural fertilizer can cause the territory to possess five times more biomass than areas outside of the garden (Klumpp and McKinnon 1987, Sammarco and Williams 1982).

Damselfish size can contribute to the growth of algae on coral as well. Recent studies observed that larger damselfish attack intruders at a higher and more efficient rate compared to their smaller counterparts. Consequently, the territories of larger damselfish were found to contain higher algal turf biomass (Foster 1985). The size of damselfish may also be attributed to the increase of nutrients in the water. In a study done by Loma et al. (2000), the sizes of *S. nigricans* were compared at a nutrient enriched site and an undisturbed site. They found that the *S. nigricans* species were significantly larger at the nutrient enriched site compared to the undisturbed site Additionally, algae matter was richer in organic Carbon and Nitrogen nutrients at the disturbed site (de Loma et al. 2000).

Increased algal overgrowth is a common threat to coral reefs. Algae are fierce competitors with corals for factors such as light and space (McCook and Jompa 2007). Additionally, when damselfish algal mats cover corals, the corals expen extra energy in an attempt to keep their surfaces clean of algal overgrowth (Potts 1977). When coral colonies rid themselves of the algae, they waste metabolic energy that could have been directed towards recruitment, calcification, or photosynthetic purposes (Potts 1977). Algal mats can also shade the photosynthetic zooxanthellae that provide corals with nutrients and energy (Potts 1977).

The focus of this study is to determine whether or not high numbers of damselfish are a significant stress on coral health. The hypotheses for this study are as follows:

H1: There will be a greater amount of dead corals present on a reef with high damselfish densities.
  a. Increased damselfish numbers will lead to more coral death due to the larger numbers of algal gardens smothering the corals.

H2: Damselfish at Yellow Submarine will be larger in size compared to those at Red Slave.
  a. The influx of nutrients at Yellow Submarine from human waste and other pollutants increases the amount of algae available for damselfish consumption.

The results of this study will provide data on a possible relationship between damselfish abundance and the percentage of dead coral. This study will also provide data on whether or not increased sizes of damselfish are correlated with increased coral death. Identifying damselfish as a potential coral stressor could provide insight into coral reef dynamics.

Materials and methods

Study site

This study was carried out from 5 March to 25 March 2011 in the waters of Bonaire, D.C. One site, Yellow Submarine Dive Shop, (12º 15’ 1” N, 068º 28’ 1” W) on the western coast might be impacted by increased human activities such as waste water coming from hotels and restaurants built close to shore, and the many divers that frequent the reefs. The other dive site, Red Slave, (12º 01’ 52.59” N, 068º 15’ 24. 93” W) on the southern coast of the island served as a control site due to its fairly clean waters coming from the open ocean and infrequent diving activity.

Data collection

At each site, sixteen 20 x 1 m transect surveys were conducted randomly between the depths of 6m - 18 m using SCUBA. The number of damselfish present within 50 cm on both sides of the transect were counted, recorded by species, and grouped into three size categories of 0.0 - 3.0 cm, 3.1 - 5.0 cm, and greater than 5.0 cm. Damselfish species counted and identified were *Stegastes partitus, Chrysiptera parasema, Stegastes planifrons, Stegastes adestus,* and *Stegastes diencaeus.*

The percent dead coral of all coral colonies within the same belt transects were recorded. Dead coral was defined as any head of coral in which the skeleton was white from being recently eaten or killed, or completely overgrown with algae or other benthic organisms. Four additional 20 m transects were laid out at Yellow Submarine in order to record the number of coral species present. These transects were compared to eight randomly selected AGRRA benthic 10 m belt transects conducted at Red Slave.

Data analysis

Damselfish densities were normally

distributed (Shapiro-Wilk normality test for Yellow Submarine: N = 16, W = 0.94, P = 0.33; Shapiro-Wilk normality test for Red Slave: N = 16, W = 0.97, P = 0.82). An unpaired t-test assuming equal variances was then used to determine whether damselfish densities at the two sites differed significantly. Percent dead coral at Yellow Submarine was normally distributed (Shapiro-Wilk test, N = 16, W = 0.94, p = 0.34), but not at Red Slave (Shapiro-Wilk test, N = 16, W = 0.89, p = 0.465). Therefore, a Mann-Whitney U test was used to compare percent dead coral at the two sites. To determine if the abundance of damselfish size categories differed at Red Slave and Yellow Submarine, only the data for *Stegastes partitus* was used. *S. partitus* at Yellow Submarine were normally distributed (Table 1), but were not normally distributed at Red Slave (Table 2). A Kriskal-Wallis test, the non-parametric equivalent of ANOVA, was used. In all tests executed, the significance level was at $\alpha = 0.05$.

Table 1 Results from the Shapiro-Wilk test to determine if damselfish sizes at Red Slave were normally distributed. The α-value significance level was 0.05. Data was not normally distributed.

	0-3 cm	3-5 cm	>5 cm
W-value	0.9	0.8	0.8
p-value	0.33	0.1	0.05

Table 2 Results from the Shapiro-Wilk test to determine if damselfish sizes at Yellow Submarine were normally distributed. The α-value significance level was 0.05. Data was normally distributed.

	0-3 cm	3-5 cm	> 5 cm
W-value	0.9	0.9	0.9
p-value	0.13	0.2	0.13

Table 3 Results run from the Kruskal-Wallis test (non-parametic equivalent of the ANOVA). Data from the 0-3 cm category was statistically significant, data from the 3-5 cm category represented a trend, and date from >5 category was not statistically significant. The α-value significance level was 0.05.

	0-3 cm	3-5 cm	>5 cm
$\chi 2$-value	6.9	3.4	0.17
Degrees of Freedom	1	1	1
p-value	0.009	0.065	0.67

Results

Damselfish abundance and densities

The dive site Yellow Submarine contained more damselfish species than Red Slave. *Stegastes partitus* was the most abundant species at both sides, making up 95.4% and 83.4% of the total damselfish at Red Slave and Yellow Submarine respectively. *Stegastes planifrons* comprised 3.9% and 11.7%, and *Chrysiptera parasema* comprised 0.67% and 1.2%. *Stegastes nigricans* and *Stegastes diencaeus* were found only at Yellow Submarine and were 0.46% and 3.0% of the total damselfish recorded (Fig. 1).

Red Slave, the site farther away from direct human impact, had an average density (± SD) of 2.3 ± 0.709 damselfish/m^2, while the heavily impacted Yellow Submarine site had an average density (± SD) of 2.025 ± 0.450 damselfish/m$^{2.}$. These differences were not significant (two tailed t-test, p=0.14), indicating that both sites were equally suitable habitats (Fig. 2). However, Red Slave had a statistically greater amount of bicolor damselfish (*S. partitus*) in the small category 0.0 - 3.0 cm than Yellow Submarine (Fig.3, Table 3). There was no significant difference in 3.0-5.0 cm bicolor damselfish numbers (Table 3) or bicolor damselfish greater than 5.0 cm (Table 3) between the two sites (Fig. 3). The other species, *Chrysiptera parasema, Stegastes planifrons, Stegastes adestus, and Stegastes diencaeus* that were present at Yellow Submarine in higher numbers than at Red Slave are larger than bicolor damselfish that overly dominated Red Slave.

Percent dead coral cover and coral diversity

Percent dead coral averaged 48.0% (± 31.5% SD) at Red Slave and 60.0% (± 27.6% SD) at Yellow Submarine. This difference proved to be significant (Mann-Whitney U test, N = 32, U = 32.5, p< 0.0001, Fig. 4).

The number of corals belonging to different species was similar at both sites: eleven species were found at the experimental site Yellow Submarine and the control site Red Slave. Both sites contained: *Colpophylia natans, Montastraea annularis, Montastraea cavernosa, Siderastrea, Agaricia, Diploria labyrinthiformis, Eusmilia,* and *Meandrina*

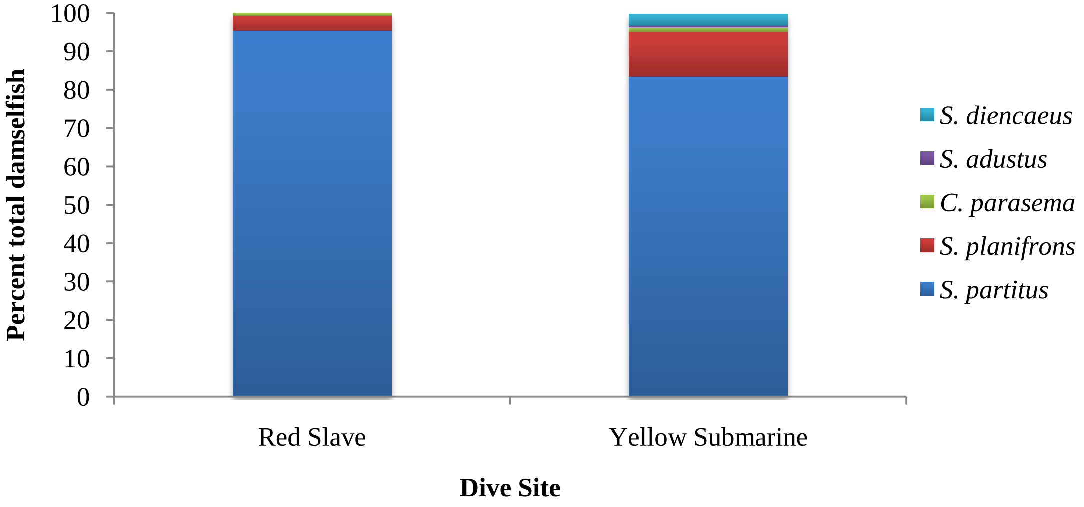

Fig. 1 Total number of damselfish separated by percent species found at sixteen 20 x 1 m transects at Red Slave and Yellow Submarine dive sites.

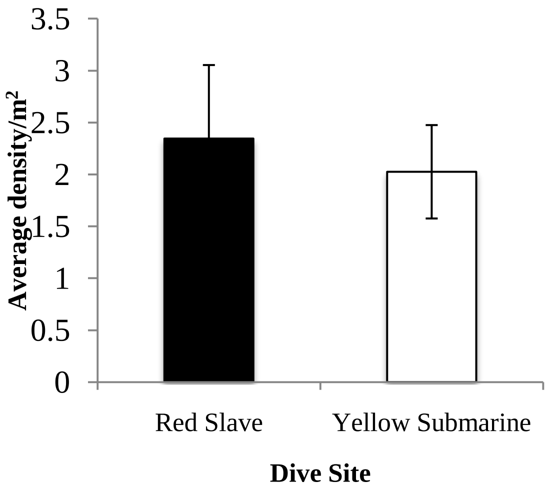

Fig. 2 Average damselfish density (±SD) per transect at Red Slave and Yellow Submarine dive sites.

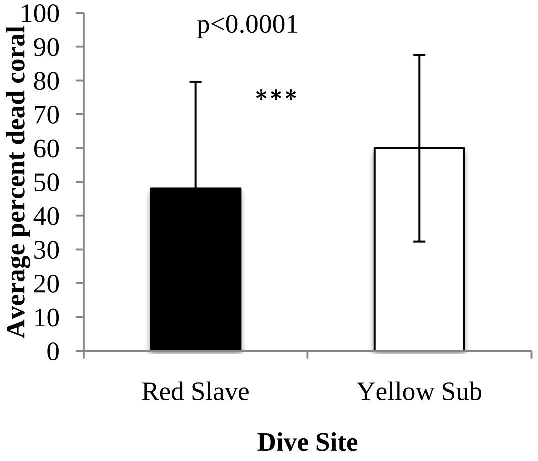

Fig. 4 Average percent dead coral (±SD) at Red Slave Yellow Submarine. The asterisks indicate that the difference between the two averages is highly significant (p=0.0001).

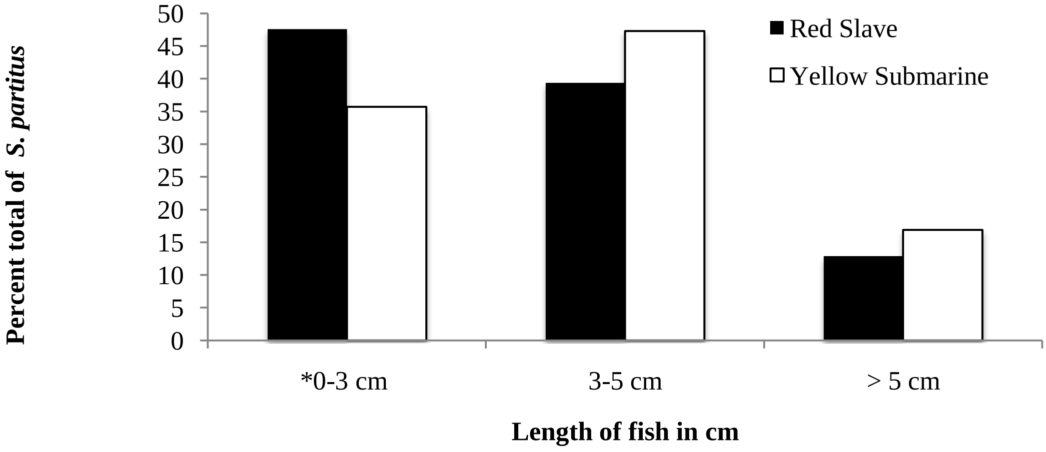

Fig. 3 Number of bicolor damselfish categorized by sizes of 0-3cm, 3-5cm, and >5 cm at Red Slave and Yellow Submarine dive sites. The asterisk indicates a significant difference on the 0.05 level.

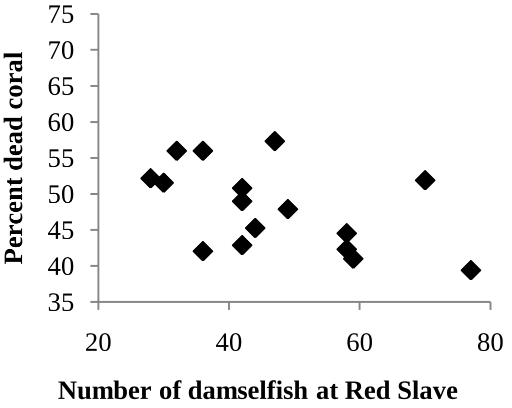

Fig. 5 Number of damselfish and percent dead coral per transect at Red Slave dive site. Data points have an R^2-value of 0.25208.

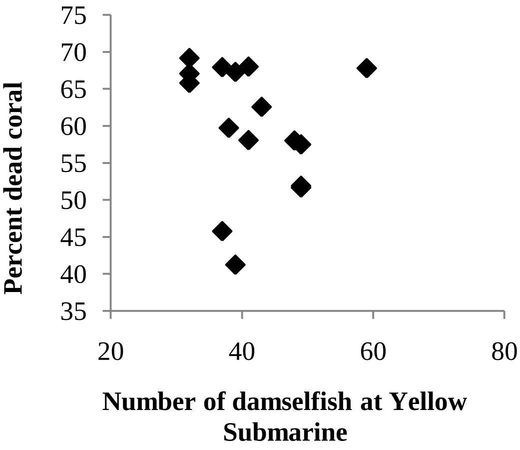

Fig. 6 Number of damselfish present and the percent dead coral per transect at Yellow Submarine dive site. Data points have an R^2 -value of 0.0298.

coral species. *Dendrogyra cylindrus*, *Stephanocoenia*, and *Porites asteroides* were found only at Yellow Submarine, while *Diploria strigosa*, *Madrascis mirabilis,* and *Montastrea faveolata* were found solely at Red Slave.

At both Red Slave and Yellow Submarine, there was no correlation between damselfish numbers and dead coral cover (Red Slave: N = 16, R^2 = 0.25208, Yellow Submarine: N = 16, R^2 = 0.02984, Fig. 5 and Fig. 6).

Discussion

Although the percentage of dead coral was higher at Yellow Submarine, against expectations the results suggest that there is no correlation between densities of damselfish and the percentage of dead coral present at both sites. In addition, both Red Slave and Yellow Submarine had a similar number of coral species present. Therefore, it is highly probable that factors not quantified in this study played an influential role in determining damselfish densities and the percentage of dead coral cover. One likely factor is human impact. In a study focusing on the effects of sewage pollution on coral reef communities, Pastorok and Bilyard (1985) noted that moderate levels of nutrient enrichment cause increased production and biomass of benthic algal levels. High levels of nutrients can cause increased levels of sedimentation and toxicity, further damaging the corals. In another study focusing on the effects of sewage pollution from tourist hotels in Jamaica, research showed that increased nutrient levels from sewage gave rise to algal growths. This can obstruct the passage of light to the corals and may lead to the expulsion of the zooxanthellae (Barnes 1973). Because of its close proximity to human impact such as hotels, restaurants, and dive shops, the Yellow Submarine site most likely has similar nutrient levels in its waters that the experimental Jamaica sites had. Therefore, Yellow Submarine is probably experiencing comparable effects such as increased algal growth. In addition, the influx of dive shops in the area could be a possible factor adding to increased coral death. The high number of divers that frequent the Yellow Submarine site can cause significant damage compared to the relatively reclusive Red Slave site. These effects are most likely much more detrimental to the reef than damselfish gardens.

Another possible explanation for the lack of a correlation between high damselfish densities and increased coral death could be due to the pre-existing conditions of the reefs. Even though Yellow Submarine had a significantly greater percent of dead coral compared to Red Slave (Fig. 4), both sites contained very high levels of dead coral (60% dead coral at Yellow Submarine and 48% dead coral at Red Slave). However, both sites also had relatively similar densities of damselfish (Fig. 2). Therefore, it can be hypothesized that perhaps the damselfish did not cause any significant damage on the reefs due to the fact that there was already ample space to cultivate their algal gardens.

The increased amount of nutrients and dead coral also support the hypothesis that there are larger damselfish at Yellow Submarine compared to Red Slave. The study showed that there was a significantly greater number of smaller bicolor damselfish at the control site compared to the high impact site. These results regarding damselfish size are consistent with those found by de Loma et al. (2000), in that damselfish were larger at sites with increased levels of nutrients. Red Slave had definite higher numbers of *S. partitus* in the 0 - 3 cm category, but had lower numbers in the categories of 3 - 5 cm and > 5 cm than Yellow Submarine (Fig. 2).

Another possible reason that *S. partitus* is smaller at Red Slave than Yellow Submarine could be that the Red Slave *S. partitus* population is comprised mostly of juveniles. Like most tropical fish, *S. partitus* larvae are pelagic before transitioning into the benthic juvenile stage (Nemeth 2005). Since Red Slave receives continuous circulating currents from the open ocean, it is possible that Red Slave is a close and convenient site for the larval recruits to establish their primary territories. Studies have also shown that certain species of juvenile damselfish prefer to settle near conspecifics compared to adults (Cheney and Côté 2002). This may also explain the higher densities of damselfish at Red Slave compared to Yellow Submarine.

The results of this study suggest that there is no correlation between elevated damselfish densities and decreased coral health. Instead, other factors due to human impact such as increased nutrients play a larger part in the decline of coral reefs. Yet, the possible detrimental effects of damselfish on coral reefs cannot be discounted and further studies should be conducted on the subject. These studies should include water samples to determine the contents of the ocean water as well as an increased number of impacted and non-impacted study sites. Even though this study did not find any serious detrimental effects on corals from damselfish gardens, it is a possibility that high damselfish densities may become a biological stress on coral reefs in the future.

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The environmental impact of the reverse osmosis desalination plant on the immediately surrounding water and coral reef ecosystem in Bonaire, Dutch Caribbean

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Abstract

One of the most promising solutions to the growing shortage of potable water is the tapping of the oceans through desalination. Until recently the high energy costs of this technology has kept it restricted to oil rich countries in the Middle East. As the need for fresh water has grown and the technology has improved, municipal desalination has slowly expanded out of this region and several plants have been built in the Caribbean and Australia, with more planned. These two regions also house some of the highest concentrations of reefs in the world, and exceedingly little research has been done on the impact of effluent from desalination plants on coral reef ecosystems. Desalination plants release hypersaline water with heightened thermal energy, lowered O_2 levels, and a variety of chemical additives. This study used a modified AGRRA benthic survey method to compare coral mortality and reef composition outside the desalination plant to a control site in Bonaire, Dutch Caribbean. The LaMotte Salt Water AquaCulture Test Kit and Hanna Instruments HI 713 Phosphate Low Range kit were used to compare water samples within and between sites. The reef in close proximity to the desalination plant was found to have significantly lower live hard coral, gorgonian, and fire coral cover, and higher sand cover by percent than the control site. The reef also showed significantly higher rates of coral mortality than the control site. The water around the plant was also found to have higher salinity, but lower levels of CO_2 and nitrates than the control site. These results suggest that the desalination plant has a negative effect on the surrounding coral reef ecosystem's health and complexity. These results should give pause to governments planning the development of desalination plants near coral reef environments until more research can be done into this technology's environmental impact.

Introduction

It is estimated that the world population will grow by 47% to a total of 8.9 billion humans by 2050 (United Nations Department of Economic and Social Affairs Population Division, 2004). While our population continues to grow, however, our resources do not. Of the many concerns this situation raises, one of the most pressing is a lack of potable water (Naim and Monir 2002). One of the most promising solutions to this problem is the desalination of ocean water (Drioli et al. 2002). Only 2.5% of the world's water is freshwater, leaving the vast majority of this vital resource in previously inaccessible saline ocean environments (United Nations Water 2008).

Until recently the energy costs of the desalination process have restricted it almost exclusively to oil rich countries, such as those in the Middle East (Roberts et al 2010). In 2001 it was estimated that only 2.43% of the water obtained for domestic use originated from desalination plants (Tsiourtis 2001). However, as the need for potable water becomes more pressing and the technology improves, desalination plants are expanding out of this region and rapidly growing in number (Bremere et al. 2001). Bremere et al. (2001) estimated that the municipal desalination market will increase by 200% over the next 25 years in water poor regions, such as the Caribbean.

The sudden explosion in the popularity of desalination has one major problem; the environmental impact of this technology has not been sufficiently studied. Desalination plants uptake large amounts of seawater and return hypersaline water with heightened thermal energy (Dweiri and Badran 2002). In addition, most plants release all or some combination of corrosive metals (e.g. copper, nickel, and zinc), antifouling additives (e.g. chlorine and hypochlorite), antiscaling additives (e.g. orthophosphates), antifoaming additives (e.g. fatty acids, fatty acid esters, and acylated polyglycols), corrosion inhibitor additives, and Oxygen removing additives (e.g. sodium sulfate) (Dweiri and Badran 2002).

Little research has been done on the effects of this hypersaline effluent on the marine environment. Past research has been largely restricted to the effects of Multi-Stage Flash technology plants on the marine environments in the Mediterranean and Red Sea. Multi-Stage Flash plants desalinate water by repeatedly evaporating the seawater in different stages until the result is freshwater. This process requires a high input of thermal energy (water-technology.net 2011). Reverse osmosis plants, on the other hand, work by separating fresh and saltwater with a semipermeable membrane and using a hydraulic pump to force water against its concentration gradient, resulting in freshwater (Malki 2008). This process tends to be more energy efficient, but requires more pretreatment of the water with chemicals (Malki 2008). Regardless of the type of plant, studies have shown that brine discharge from desalination plants has a negative impact on the surrounding environment. The range and the severity of the effect, however, is hotly contested, ranging anywhere from minor damage to only a 10 meter area, to extensive damage up to 100 m (Roberts et al. 2010). In addition, Sánchez-Lizaso et al. (2007) showed that hypersalinity and heat pollution alone significantly increased the mortality rate and decreased the vitality of the marine organisms *Posidonia oceanica*, a Mediterranean species of sea grass, and *Paracentrotus lividus*, a species of sea urchin.

When evaluating the potential effects of the effluent from desalination plants on coral reef environments, Hopner et al. (1996) projected that coral reefs will be the third most sensitive ecosystem, being only more resilient than mangrove forests and saltmarshes. One study examining the effects of desalination plant discharge on a coral reef environment in Saudi Arabia found increased temperature and salinity in surface waters as well as lower levels of dissolved oxygen (Osman and Al-Gadaani 1984). Several factors can affect the extent of environmental impact, however, including site to site differences such as current and water flow at the site (Lattemann and Hopner 2008).

Bonaire, Dutch Caribbean offers a unique opportunity to study the effects of a reverse osmosis desalination plant on a Caribbean coral reef ecosystem. Bonaire constructed a reverse osmosis desalination plant to provide the island with fresh water. Yet, to date, very little research has been done on the environmental effects of this plant's effluent. The importance of the degree of impact to the coral reef ecosystem is heightened in an economy like Bonaire's which largely relies on tourism to its diverse coral reef ecosystem.

The current study evaluated the size and severity of the desalination plant's effect on the surrounding coral reef environment and water quality. I tested two hypotheses.

H1: Water samples from the area surrounding the plant will show significantly heightened salinity and reduced oxygen levels compared to water samples from control sites taken at equal depths.

H2: There will be statistically significant impacts to coral reef health and substrate composition in the nearby vicinity of the plants discharge site compared to control sites.

The importance of this study is threefold. First, this was one of the first studies to examine the effects of desalination plants in the Caribbean, whose marine ecosystem is distinct from that of the Mediterranean and Red Sea. Second, this study investigated the effects of a reverse osmosis plant, which have been studied less than the Multi Stage Flash plants. Thirdly, it examined the effects of a desalination plant on a coral reef environment, one of the more fragile marine ecosystems, but an ecosystem that exists in Australia and the Caribbean, two areas planning the construction of desalination plants (Roberts et al. 2010).

Methods

Study Site

All research was conducted at two sites in Bonaire, DC, within the limits of the Bonaire National Marine Park (BNMP) between February 9 and March 26, 2011. Two sites, one experimental site and one control site were chosen. The experimental site was located in the vicinity of the desalination plant's discharge (012° 10' 32.8" N, 068° 17' 30.43" W). The control site was the dive site called Red Slave (012º01'52. 59" N, 068º15'24. 93"W) on the southern tip of the island (Fig. 1). This site was selected as a control since the current comes in from the open ocean and so island activities have little effect on the site.

Fig. 1 Map of Bonaire, Dutch Caribbean. The X denotes the location of the desalination plant, while the star marks the location of the dive site Red Slave.

Benthic survey

Data collection was carried out using SCUBA. Both sites were surveyed with twenty 10m transect lines, following the AGRRA Benthic methods version 4 (as described by AGRRA's website: www.agrra.org). Three alterations to the methods were made. First, on the first pass all species of urchins were recorded. This was done because of Sánchez-Lizaso et al.'s (2007) findings that the temperature and salinity pollution of desalination plants can increase mortality in at least one species of Mediterranean sea urchin. Second, the fourth pass was not made. Third, substrate types observed within the category "other" were recorded.

The first transect was laid out parallel to shore at a depth of 18 m. Transects were then laid out at 3 m intervals moving closer to shore until the end of the reef substrate was reached or ten transects had been done. The remaining transects were then laid out perpendicular to shore starting at the reef substrate and extending 10 m away from shore. An equal number were done to the left and right of the discharge site or its proxy at the control site.

This procedure resulted in 10 perpendicular transects at the experimental site and 14 at the control site. The proxy at the control site was represented by going straight out from the marked entry point until a depth of 5 m was reached (Fig. 2).

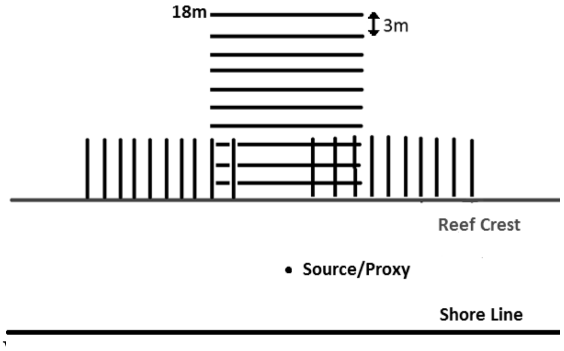

Fig. 2 The pattern of the sampled transects as laid out at each site. The ones parallel to the reef substrate were done first, followed by the perpendicular ones. "Source" represents the approximate location of the site of effluent release at the desalination plant or its proxy at the control site.

Four water samples were taken at each site to assess the overall water quality. One was taken at the site of discharge, or its proxy, and three more were taken at the start of the reef substrate at a depth 1m above the bottom. Water samples were taken using the procedure outlined in the LaMotte Salt Water AquaCulture Test Kit (Chestertown, MD) instruction Manual (Model AQ-4/AQ-5).

These samples were analyzed for Ammonia Nitrogen, CO2, dissolved O2, nitrates, pH, and salinity using the LaMotte Salt Water AquaCulture kit (Chestertown, MD) and procedures. The samples were also analyzed for phosphates using Hanna Instruments HI 713 Phosphate Low Range kit (Woonsocket, RI) and procedures. These measures were taken because the resources to test for specific chemicals in the effluent were unavailable, yet these measures give a good overall picture of general water quality.

Data analysis

The average percentage for each benthic component was calculated per transect. The sites were then compared using two-tailed t-tests, $\alpha = 0.05$ for the categories percent hard coral cover, percent sand cover, percent fleshy macro algae cover, percent calcareous macro algae cover, percent crustose coralline algae, percent coral recently deceased, percent coral old deceased, total percent coral dead, mean coral head size. A trend was defined as $p < 0.1$. Mann-Whitney tests were used to compare the subcategories of “other” due to the non normality of these data.

The low number of water samples from each site prevented any statistical tests to be run on the data. In addition, no analysis was done on differences in sea urchin density between sites as none were observed on any transects at either site.

Results

Analysis of the AGRRA benthic data revealed significant differences in substrate makeup between sites (Fig. 3). Red Slave had significantly higher coverage of percent live hard coral cover (paired t-test, $t = 2.958$, $df = 32$, $p < 0.01$), soft coral cover (Mann-Whitney test, $N = 38$, $U = 40$, $p < 0.0001$), and fire coral cover than the desalination plant (Fig. 3). A trend towards higher percent fleshy macro algae cover at Red Slave was also found (paired t-test, $t = 1.925$, $df = 28$, $p = 0.064$). In contrast, the desalination plant site had significantly higher percent sand (paired t-test, $t = -4.528$, $df = 33$, $p < 0.001$) and rubble coverage (Mann-Whitney test, $N = 38$, $U = 100$, $p = 0.011$).

Also, evaluation of coral mortality between sites revealed significantly higher percents of old and total coral death at the desalination plant compared to Red Slave

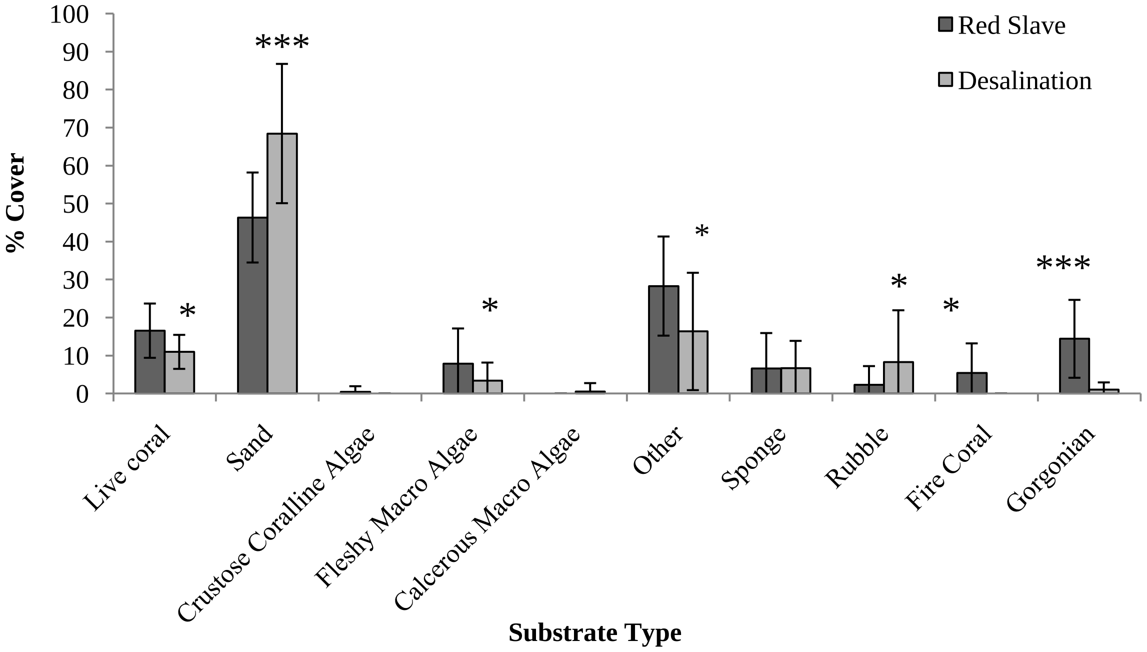

Fig. 3 Comparisons between the substrate composition (mean ± SD) at Red Slave (dark bars) and desalination (light bars) study sites. *, **, *** indicate significant differences at $\alpha = 0.05$, 0.01, and 0.001 respectively.

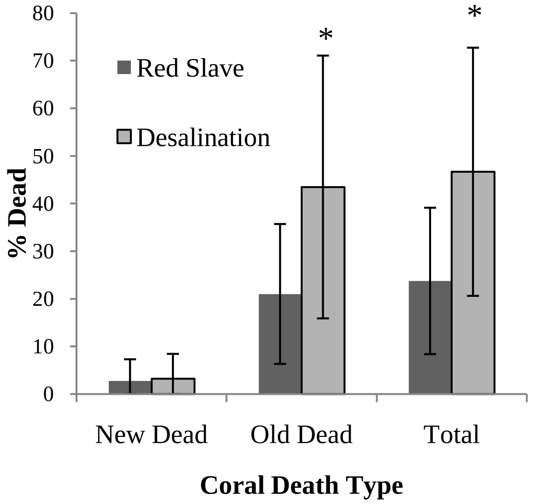

Fig. 4 Mean percent death of coral found (± SD) at the sites Red Slave and Desalination. Type of coral death was sub categorized into percent new dead and percent old dead. * indicates a significant difference at $\alpha = 0.05$.

(paired t-test, $t = -3.195$, $df = 29$, $p = 0.003$; paired t-test, $t = -3.366$, $df = 31$, $p = 0.002$). The desalination plant had a mean old coral death percent (± SD) of 43.469 ± 27.592% and a mean total coral death percent (± SD) of 46.667 ± 26.051%, whereas Red Slave had a mean old coral death percent (± SD) of 21.006 ± 14.690% and a mean total coral death percent (± SD) of 23.747 ± 15.370% (Fig 4).

A comparison between mean coral head size between sites found no statistically significant difference, but there was a trend towards the desalination plant having larger coral heads than Red slave (paired t-test, $t = -1.848$, $df = 23$, $p = 0.077$). Coral heads at the desalination plant had a mean area (± SD) of $0.49 \pm 0.78m^3$ compared to the mean coral head size of $0.15 \pm 0.26m^3$ found at Red Slave.

While no statistical analysis of the water samples could be conducted, there appeared to be several differences between the sites. It appears that samples from the desalination plant had higher salinity and O_2 levels and lover levels of CO_2 and Nitrates than the control site (Table 1).

Table 1. Salinity, O_2, CO_2, and Nitrate levels of the water samples from the sites Red Slave and the desalination plant (Desal.). Coral indicates the mean of the three samples taken over the coral substrate. Source indicates the sample taken at the site of discharge or its proxy.

Site	Salinity ppm	O_2 mg/L	CO_2 ppm	Nitrates ppm
Red Slave Coral	32.33	35.77	5	0.1
Red Slave Source	34	44.2	5	0.25
Desal. Coral	37.07	52.5	1.67	0.03
Desal. Source	36.87	95.2	3	0.02

Discussion

The results of this experiment support the initial hypothesis that there is a statistically significant impact on coral reef health and substrate composition in the vicinity of the desalination plant's effluent compared to control sites. The reef near the plant showed significantly higher percentages of coral death, a lower percent of live coral and gorgonian cover, and a higher percentage of sand cover. All of these results suggest a less healthy and less complex, more sand dominated, reef near the effluent release site of the desalination plant compared to a control site unaffected by the desalination plant.

The results of the water samples, however, largely disprove the initial hypothesis that water samples from the area surrounding the plant will show significantly heightened salinity and reduced Oxygen levels compared to water samples from control sites taken at equal depths. While, as predicted, the water near the desalination plant was more saline than the control site, there was actually a trend towards higher dissolved O_2, rather than lower. In addition, the water samples from the desalination plant actually had lower levels of nitrates and CO_2 compared to the control site Red Slave. While one must be cautious in evaluating these results given the low sample size and lack of statistical analysis, these findings actually suggest a superior water quality close to the desalination plant compared to Red Slave.

The water sample comparisons suggest that the effluent from the desalination plant has not had a negative impact on the surrounding water quality, apart from elevated salinity. This must be reconciled, however, with the data from the AGRRA Benthic survey which showed a significantly less healthy reef substrate than the Red Slave control site. Four possibilities can account for this seeming inconsistency. One is that the heightened salinity alone has a detrimental effect significant enough to account for the increased coral mortality and decreased substrate complexity. This seems possible given the findings of Sánchez-Lizaso et al. (2007) showing that heightened salinity and temperature alone can cause greater mortality in a Mediterranean species of sea grass and urchin. Alternatively, there could be detrimental nutrients or chemicals in the effluent that were not tested for in the scope of this experiment.

Another possibility is that the observations between water samples are not actually statistically significant and are only the result of standard deviation. The last possible explanation I propose is that another variable; either unrelated to the desalination plant or not tested for at all, can explain the heightened coral mortality, reduced coral cover, and water sample results.

The results of this study, while not conclusive evidence that desalination plants degrade reefs and water quality, suggest that the effluent from reverse osmosis desalination plants have a detrimental effect on Caribbean coral reef health and composition. This experiment should give pause to future developers about the potential environmental impacts of desalination plants until more research can be done. This future research should be targeted towards other desalination plants located on a Caribbean coral reef ecosystem and especially towards more extensive and sensitive water sample testing, as well as testing whether the AGRRA benthic results can be generalized to other sites. In addition, baseline studies which can evaluate the temporal impact of the plants by studying the environment pre and post plant construction would increase our understanding of the environmental effects of desalination plants. This research would help eliminate the possibility that a third variable is responsible for the differences observed in coral morality and cover and more firmly establish the dangers of desalination plants to Caribbean reef ecosystems.

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Assessment of local lionfish (*Pterois volitans*) densities and management efforts in Bonaire, Dutch Caribbean

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Abstract

The Indo-Pacific lionfish *Pterois volitans* is an invasive species that was released along the U.S. Atlantic coast and spread to the Caribbean. Lionfish can inhabit a wide range of depths and they lack predation in their invasive range, leading to a successful invasion into their introduced range. Their large appetite combined with having no predators may pose an ecological threat to the Caribbean coral-reef ecosystem. *Pterois volitans* invaded Bonaire, Dutch Caribbean in 2009, and the extent of their presence and the impact they have upon the reefs surrounding Bonaire has yet to be investigated. Basic components to managing an invasive species are monitoring their occurrence and controlling their abundance. Around Bonaire, managers and diver volunteers catch and kill *P. volitans* to control their numbers in an attempt to reduce their negative impacts on the ecosystem. This study investigated the effectiveness of the current management strategy, *P. volitans* removal, by comparing their densities at frequented hunting sites with no-hunting sites. To achieve this, the densities of *P. volitans* at eight hunted and less frequented sites were calculated by searching for their presence in 50 x 4 m belt transects at five different depths (6 m, 12 m, 18 m, 24 m, 30 m) at selected sites along the west coast of the island. All *P. volitans* recorded were categorized into three size classes (small: 0-10 cm, medium: 11-30 cm, large: ≥ 30 cm). The results of this study show no significant differences between the densities of lionfish found at hunted sites (10/ha) compared to less frequented sites (16/ha). This study is the only known effort that attempts to determine the effectiveness of *P. volitans* removal from invaded areas in Bonaire.

Introduction

In recent years the Indo-Pacific Lionfish *Pterois volitans* has invaded the Northeast U.S. and Caribbean waters, the extent of the invasion has been recorded as far north as New York in the U.S. and as far south as Venezuela; the southern extent of the invasion is still continuing. This species has covered a vast territory and become the first successful non-native marine species to be established in these waters (Schofield 2009). To be considered invasive a species must be present outside of their natural distribution area and pose a threat to or have harmful effects in the invasive area. The genus *Pterois* includes seven species of venomous fish native to the Indo-Pacific. Within the seven species, two have been thought to be invasive outside their home range; *Pterois volitans* and *Pterois miles* (Hamner et al. 2007). Through genetic testing it was determined that *Pterois volitans* are the species of lionfish found in the Caribbean. This ornamental fish was likely introduced through multiple aquarium releases of either the adult fish or their eggs, off the coast of Florida (Betansur-R et al. 2011).

In the Caribbean, lionfish have surpassed the size and depth limitations present in their native range. In the Indo-Pacific, *Pterois volitans* have been recorded up to a maximum size of 38 cm and in depths up to 50 m; in their invasive range, sizes range from 5 to 45 cm and depths range between 48 m and 84 m depth (Whitfield et al. 2006). The success of lionfish is due to their biological characteristics; they are equipped to survive in much colder and harsher environments than they face in the Caribbean (Kimball et al. 2004). The ability to survive in harsh environments is merely one characteristic lionfish exhibit that contributes to their success. Lionfish are growing to increased sizes in their invaded range (45 cm) compared to in their native range (38 cm). Size of the fish is important as larger fish have the ability to produce increased quantities of eggs. *Pterois volitans* can become sexually mature within two years of age or when reaching 17-18 cm total body length (J Potts, NOAA, personal communication). Once individuals reach sexual maturity, *P. volitans* exhibit high rates of fecundity; producing approximately two million eggs a year (Morris and Whitfield 2009). Abundance of *P. volitans* in their invasive range has been reported to be approximately five times higher compared to in their native range (80/ha) (Green 2008). With such an elevated reproductive success rate, lionfish are already overpopulated in some areas and could become overpopulated in others as they continue to invade (Albins and Hixon 2008).

Pterois volitans are predatory carnivorous fish and very few carnivorous species prey upon them. The lack of predators of *P. volitans* is likely accredited to their long pectoral and dorsal spines that are highly venomous; their venom is their main defense mechanism and is lethal to several species of fish (Bernadsky and Goulet 1991). *Pterois volitans* are not selective feeders, preying on a variety of species, over 50 different fish species in the Caribbean (Morris and Akins 2009). Fishelson (1997) reported that the stomach of an adult *P. volitans* can be expanded 30 times its normal size by volume when feeding and can consume 2.5 - 6% of its body weight per day. During a five-week study, it was shown that *P. volitans* can reduce native fish recruitment on a Caribbean reef by an average of 79% (Albins and Hixon 2008).

Higher densities of lionfish have been recorded in their invasive range compared to in their home range. The average density of *P. volitans* in a 1-km long transect in the Red Sea was estimated to be 80 individuals per hectare (Fishelson 1997). Whitfield et al. (2007) recorded densities (± SD) of *P. volitans* off the coast of North Carolina, USA (21.2 ± 5.1/ha); these densities are lower than those observed in the home-range (~80/ha) of *P. volitans*. However, a study done in New Providence, Bahamas, recorded densities of *P. volitans* (393.3 ± 144.4/ha); that were five times higher than that of the home range and significantly higher than the densities recorded off the coast of North Carolina (Green 2008). The higher densities of lionfish found in the Caribbean suggests that the impact of lionfish may be more extreme in the southern extent of the invasion compared to in the northeast United States.

In 2009 the first *P. volitans* was found for the first time in Bonaire, (R Peachey personal communication). Since the invasion, various catching and/or hunting methods were used by SCUBA divers to keep their numbers at bay in an effort to maintain minimal abundance of lionfish. Their early reproductive age, frequent production of many eggs and the fact that Caribbean predators do not recognize them as prey combined with a lower fishing pressure on this invasive species compared to native fish species; result in an expected increase of abundance and distribution of *P. volitans*. Their increased population will amplify their negative impacts upon the invaded ecosystem (Whitfield et al. 2006). As with most invasive species, the ecological impact of *P. volitans* is still not fully understood and should be researched in more detail. The current management method (hunting) will not completely eradicate lionfish; divers are limited in number and by depth.

In Bonaire, so far no efforts to quantify *P. volitans* abundance have been undertaken. In order to analyze the current management methods used to control the invasive species, this study aimed to: (1) determine approximate *P. volitans* abundance and average densities on the western and south-west region of Bonaire, (2) analyze the effectiveness of the local management of the catch and removal of *P. volitans* by comparing sites where hunting effort is absent with sites with substantial hunting effort, (3) establish a baseline of *P. volitans* densities and average size.

The following hypotheses were tested:

H1: Higher densities of *Pterois volitans* will be recorded in hunted sites compared to less frequented sites.

H2: Because there is lack of hunting effort at depth, *Pterois volitans* will be more abundant in deeper depths (24 m, 30 m) than at shallower depths (6 m, 12 m, 18 m)

H3: Because they grow larger in their introduced range average size of *P. volitans* found in Bonaire will be in the large size class (small: 0-10cm, medium: 11-30 cm, large: ≥ 30 cm)

The importance of this study is to verify whether the current management effort (hunting) of lionfish is effective. Moreover this study will provide a baseline of *P.volitans* abundance in hunted and less frequented sites around Bonaire to determine if current management is having an impact on species abundance. The baseline densities recorded in this study will provide a possible gateway for long term monitoring of lionfish.

Methods

Site selection

The study sites were distributed approximately at even distances along the west side of the island. To ensure full coverage of the coastline, two sites were selected in each geographical area (Fig. 1). In total, each area surveyed along the coastline included two dive sites where *P. volitans* were frequently hunted and two less frequently hunted dive sites. Sites where hunting efforts were low or absent, were established 300m north or south of a particular dive site; to calculate this distance an odometer in the dive vehicle was used. In geographical location from north to south along the coast, the hunted (marked) dive sites were: Karpata, Oil Slick Leap, Small Wall, Ebo's Reef, 18th Palm, Angel City, The Invisibles Reef and Atlantis (Fig. 1).

Fig. 1 Map of hunted sites versus less frequented sites (300 m N/S of hunted sites) visited in Bonaire, Dutch Caribbean.

Data collection

At each dive site, a survey was conducted using 50 m transect tapes where *P. volitans* abundance was investigated within a 4m belt at five depths (6 m, 12 m, 18 m, 24 m, 30 m). Using SCUBA, divers were located on each side of the transect tape as it was laid along the specified depth contour parallel to shore. The dimensions of the belt transect were 4 x 50 m. Five transects were conducted at each site totaling 0.8 ha surveyed per site. A total of sixteen sites (eight hunted & eight less frequented) were surveyed, equaling a total of 1.6 ha surveyed. Each *P. volitans* discovered was measured with a half-meter stick and sorted into three size classes (small: 0-10 cm, medium: 11-30 cm, large: ≥ 30 cm). While observing the reef structure, divers looked under large structures or contours enabling them to discover not easily visible *P. volitans*. *Pterois volitans* were recorded by depth and categorized by size.

Calculations and statistical analysis

Size categories were approximated using length calculations of *P. volitans* observed in their native range (Whitfield et al. 2006) and their densities were determined per hectare. A General Linear Model was used to statistically analyze the location densities (hunted and less frequented sites) and size class (small, medium, large) in which *P. volitans* were recorded. A paired t-test assuming unequal variance was conducted in excel to analyze the difference in density of lionfish at deeper depths (24m, 30m) compared to at shallower depths (6m, 12m, 18m). For both statistical analyses a p-value of ($p \leq 0.05$) was used to determine statistical significance.

Results

Location and Distribution

The observed total abundance of *P. volitans* was lower at hunted sites (126.2/ha) when compared to frequented sites (83.7/ha). Using a General Linear Model test, the recorded mean density of *P. volitans* found at less frequented sites (16/ha) compared to hunted sites (10/ha) were not found to be statistically different (Table 1, Fig. 2). The overall density of *Pterois volitans* (13/ha) recorded, including abundances from hunted and less frequented sites.

Table 1: Density of Pterois volitans categorized by location, size and interaction over 16 sites in Bonaire, D.C.

Source	Type III sum of squares	df	F	Significance level
Model	407.25	5	4.24	0.003
Location	24.083	1	1.254	0.269
Size	377.375	2	9.823	0.0001
Interaction location & size	5.792	2	0.151	0.861

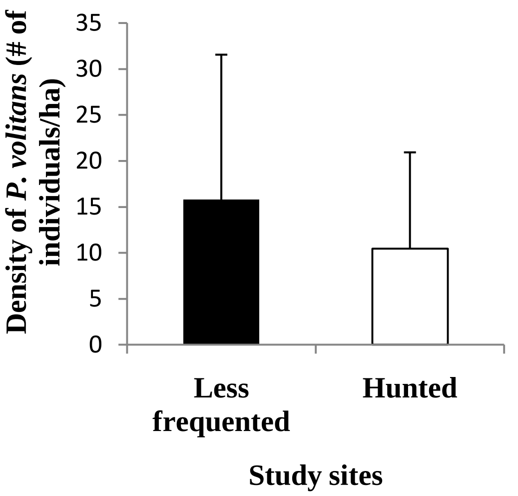

Fig. 2 Density (# of individuals'/ha) of total number of P. volitans recorded at less frequented (16/ha) and hunted sites (10/ha).

Depth of occurrence

Densities of *P. volitans* (# of individuals per depth/ha) recorded at shallow depths (6 m = 9.3/ha, 12 m = 31.2/ha, 16 m = 40.6/ha) and deeper depths (24 m = 228.1/ha, 32 m =215.6/ha) were found to be significantly different (p = 0.0002, Fig. 3).

Lionfish size distributions

Pterois volitans were most frequently recorded being within the medium size range (10-30 cm, Fig. 3); it was determined that size is statistically significant (Table 1). Total number of *P. volitans* recorded in each category (Fig. 4): small (≤ 10 cm; 12/ha), medium (11-30 cm; 43.12/ ha) and large (≥ 31 cm; 7.5/ ha).

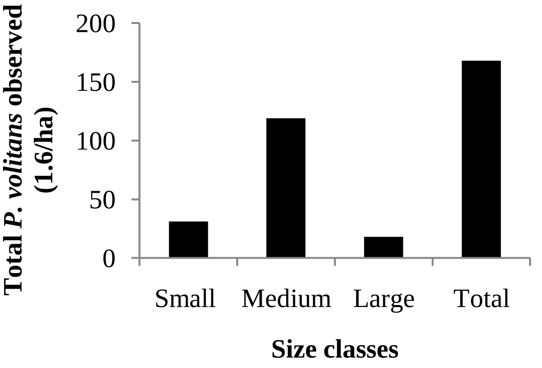

Fig. 4 Total P. volitans observed in each size class; (small ≤ 10 cm; 20/ha), (medium 11 - 30 cm; 69/ha) and (large ≥ 31 cm; 12/ha; overall total of P. volitans observed during study (168/1.6 ha).

Discussion

This study showed that the average lionfish densities between more and less frequently hunted sites did not differ significantly (t-test, p = 0.269) even though the average density (± SD) of *P. volitans* found at less frequented sites was higher (16 ± 3.75/ha) compared to the average density of *P. volitans* at hunted sites (10 ± 3.75/ha). The hypothesis stating a higher density of lionfish would be found at less frequented sites was thus, not supported.

Data collected in this study can be used as a current abundance baseline in specific

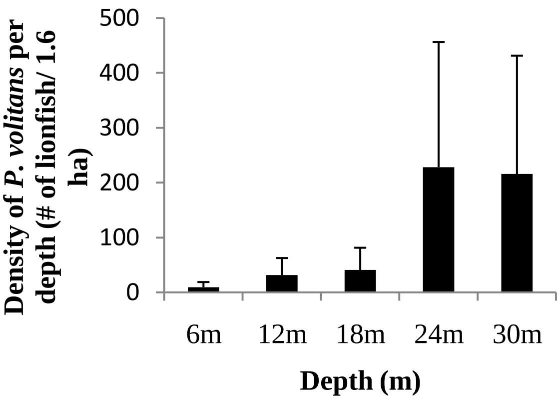

Fig. 3 Average density (# of individuals per depth/1.6 ha) for total number of P. volitans (168 /1.6 ha) collected at less frequented and hunted sites combined, per depth (m).

areas; the current baseline suggests an average amount of *P. volitans* (13/ha) recorded along the west coast of the island. Compared to lionfish density (21.2 ± 5.1/ha) recorded by Whitfield et al. (2007) along the coast of North Carolina, USA and those recorded by Green (2008) in New Providence, Bahamas (393.3 ± 144.4/ha); the average density of lionfish recorded in this study is much lower. In a study done in the U.S. North Atlantic, *P. volitans* were the second most abundant species present in the ecosystem compared to other native species surveyed in the area, (Whitfield et al. 2006) in Bonaire, they may be the most abundant predators (personal observation).

Some characteristics that contribute to the success of invasive species are an increased growth rate and higher maximum size in their invaded habitat. One example is *Lutjanus kasmira*, the blue-striped snapper that was purposefully introduced into Hawaii. *Lutjamus kasmira* had a higher growth rate compared to the snappers normal growth rate in their home range in the Atlantic (Morales-Nin and Ralston 1990). Lionfish are also exhibiting higher growth rates and reach larger maximum sizes in their invasive range (≤ 45 cm) compared to in their native range (≤ 38 cm), suggesting a successful invasion (Whitfield et al. 2006). The average size of *P. volitans* recorded in this study were in the medium size range (11 - 30 cm); suggesting that most *P. volitans* observed in this study are sexually mature (≥ 17 cm) and capable of reproducing and possibly contributing to an increase in population size.

Pterois volitans were most abundant in the deeper depths (24 m, 30 m) possibly suggesting that these depths are where the least amount of hunting occurs as divers are rarely reaching these depths. Based off of the size range of lionfish recorded in this study and the depths at which they were observed; it is likely that the population of *P. volitans* will continue expanding, further causing an impact on native fish species and the coral reef ecosystem (Whitfield et al. 2007). The negative impact is likely to continue; leading to reduced recruitment of native juvenile fish species as lionfish can consume large quantities of prey.

The importance of this study was to analyze current management methods used to control the population of *P. volitans*. Plausible suggestions as to why current management methods are shown to not have a significant impact could be due to the limited sample size of this study (n = 16); only eight hunted sites and eight less frequented sites were visited. It is thus recommended that abundances of *P. volitans* should be recorded in more areas around the island. An uncertainty in data is that smaller fish might have been overlooked, leading to an underestimation of densities. Another uncertainty is the timing at which dive sites were visited by lionfish hunters before data collection happened. Future studies should focus on more dive sites and the extent of hunting effort for every site would need to be better known.

The implications of the results of this study to manage lionfish densities around Bonaire are threefold. First, even though there were less lionfish found at hunted sites, the difference between less frequented sites was not prevalent. For this reason it is recommended that hunting effort be increased at all sites. Second, when possible, divers should also focus on hunting at deeper depths, because a higher density of lionfish was present there. Third, because lionfish reproduce in the medium size range, when possible, hunters should attempt to remove those and larger specimens. This should be done to take sexually active individuals out of the population thus preventing further increase in the local and wider population. This study is the first of its kind, as it analyzed current management methods used to control the population of lionfish by evaluating local hunting efforts. It is suggested that future studies similar to this one are conducted in order to monitor and evaluate the management of the invasive species *P. volitans.*

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Is the introduced cup coral *Tubastraea coccinea* an invasive species in Bonaire, Dutch Caribbean?

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Abstract

The orange cup coral *Tubastraea coccinea* has expanded its range from the Indo-Pacific into the western Atlantic region. It grows on a wide variety of natural and man-made substrates, including rock ledges, docks, and shipwrecks. Its early reproductive age, fast growth rate, and ability to thrive where other species cannot could potentially make *T. coccinea* a valid threat to native species. The goal of this study is to provide baseline data on the size, depth, and range of *T. coccinea* on the island of Bonaire in the Dutch Caribbean. Substrate and light intensity preferences were also investigated by estimating percent cover on a variety of substrates and light conditions. A clear preference was observed for concrete substrates and low light conditions. Interactions between *T. coccinea* and a variety of other coral and sponge species were also investigated for potential harmful effects, but despite documentation of harmful coral-coral interactions in Brazil, no evidence of *T. coccinea* exhibiting harmful effects on native coral species was found in the study area of Bonaire. While *T. coccinea* does not currently appear to exhibit negative effects on native species in Bonaire, it may simply be in the early stages of expansion and this expansion needs to be monitored for future development of harmful effects on native species.

Introduction

The orange cup coral *Tubastraea coccinea* is an alien to the western Atlantic region (de Paula and Creed 2004). Despite its common name, *T. coccinea* is found in shades of red, orange, and yellow, with its tentacles usually colored bright yellow and orange (personal observation). This fouling azooxanthellate coral species has been found at depths ranging from less than one meter to over thirty meters, and possibly deeper, but in general it is a shallow-water species (Fenner and Banks 2004; de Paula and Creed 2004). Colony sizes recorded in Brazil ranged from 45-105mm, making it a relatively small coral species (de Paula and Creed 2004). It was first recorded in the Caribbean in1943 in Puerto Rico and Curacao, and in 1951 the first date-documented specimens were taken, some from the hulls of ships (Humann and Deloach 2003; de Paula and Creed 2004). Since then it has spread across the Caribbean to Florida and the Gulf of Mexico, as well as down to the coast of Brazil, and it is now the most widespread azooxanthellate species in the tropics (Fenner and Banks 2004; de Paula and Creed 2004).

A key factor in the success of *T. coccinea*'s spread is its ability to thrive on a variety of substrates. While many coral recruits require specific surface conditions and substrates to survive, *T. coccinea* appears to grow on almost any hard-made objects; colonies have been found growing on rock ledges, caves, ship hulls, buoys, docks, jetties, shipwrecks, airplane wreckage, and other man-made objects (Cairns 2000; de Paula and Creed 2004; Fenner and Banks 2004). Some studies have shown that the species grows best on concrete, perhaps because concrete has relatively high levels of calcium carbonate, the same chemical which forms the skeleton of all stony corals (Creed and de Paula 2007). In the Galápagos *T. coccinea* grows preferentially in caves, suggesting a species preference for low light intensities (de Paula and Creed 2004). *Tubastraea coccinea* has also been found growing on several oil rigs in the Gulf of Mexico, which are treated with anti-fouling agents and are generally unsuitable for life (Fenner 2001). Although *T. coccinea* is generally absent from areas of dense coral growth, in habitats not dominated by other corals *T. coccinea* can cover almost 100% of a substrate (de Paula and Creed 2004; Mantelatto et al. 2011). It reproduces by brooding and fragmenting, and has a relatively early reproductive age of 1.5 years (Fenner and Banks 2004). It grows at a rate of 3-5 cm^2 per year, a rapid rate compared to other stony corals, and can reach a diameter of $5cm^2$ in just one year (Fenner and Banks 2004; Vermeij 2005). Furthermore, *T. coccinea* appears to be unaffected by air exposure during low tides (de Paula and Creed 2004; personal observation).

Tubastraea coccinea is certainly an introduced species, but debate remains over the classification as 'invasive,' which implies that the exotic species is harmful to native species. Much of the scientific literature on *T. coccinea* identifies the species as invasive; on the coast of Brazil, especially, *T. coccinea* was clearly demonstrated to have negative effects on native shallow corals, especially a scleractinian endemic coral, *Mussismilia hispida* (Creed 2006). Creed (2006) studied the effects of cup corals on *M. hispida* and found that in 100% of *T. coccinea–M. hispida* contacts, *M. hispida* showed damage when at distances of ≤ 5 cm from the *T. coccinea* colonies. *Tubastraea coccinea* generally caused necrosis to the nearby *Mussismilia* tissues, and dead areas were often overgrown by sponges, crustose coralline algae, and even cup coral recruits (Creed 2006).

Cup corals are known to grow in the water of Bonaire, Dutch Caribbean (Pacheco 2008). However, no literature has suggested that *T. coccinea* is damaging to or competing with other coral species in Bonaire. On the contrary, a short study by Pacheco suggested that *T. coccinea* has a positive effect on native reef species by providing a habitat to a variety of reef creatures (2008). Juveniles of several fish species, including the yellowtail damselfish (*Microspathodon chrysurus*), longfin damselfish (*Stegastes diencaeus*), redlip blenny (*Ophioblennius macclurei*), bearded fireworms (*Hermodice carunculata*), sharpnose puffers (*Canthigaster rostrata*), bicolor damselfish (*S. partitus*), trumpetfish (*Aulostomus maculatus*), saddled blenny (*M. triangulatus*), ocean surgeonfish (*Acanthurus bahianus*), and snails of the Sorbeoconcha order have all been observed living on or in cup coral colonies (Pacheco 2008; personal observation).

Little research has been conducted on *T. coccinea* and its effects on neighboring species in the southern Caribbean. Even fewer studies have been conducted to determine the introduced or invasive status of this species. The aim of this study is to provide baseline data investigating the size, depth, distance from source, substrate, and light preference of *T. coccinea*, as well as its possible negative effects on other organisms on the west coast of Bonaire. I hypothesize that:

H_1: *T. coccinea* will not be found in water deeper than 10m, will spread from the site of introduction, and will be of comparable size to colonies measured in Brazil.

H_2: *T. coccinea* will grow preferentially on concrete substrates;

H_3: *T. coccinea* will prefer to grow in shady, low-light areas rather than areas exposed to high intensities of sunlight;

H_4: *T. coccinea* will exhibit damaging effects on neighboring coral colonies located ≤5cm from the cup coral colony.

Results arising from this study will provide information on the current state of species spread from a single study site in Bonaire. Due to their lack of zooxanthellae, *T. coccinea* may be negatively affected by high levels of direct sunlight. Establishing a preference for low light conditions would assist in predicting areas vulnerable to cup coral introduction and would confirm findings in Brazil. Information about preferred substrates and conditions can help identify vulnerable areas and objects. This information will be useful in classifying *T. coccinea* as either introduced or invasive in Bonaire, which will indicate whether this species currently needs to be kept under observation or actively managed.

Methods

Site determination

This research was conducted on the island of Bonaire (Fig. 1), in the Dutch Caribbean in the months of March and April of 2011. As cup corals are thought to have originally arrived in the Caribbean on the hulls of ships, it was

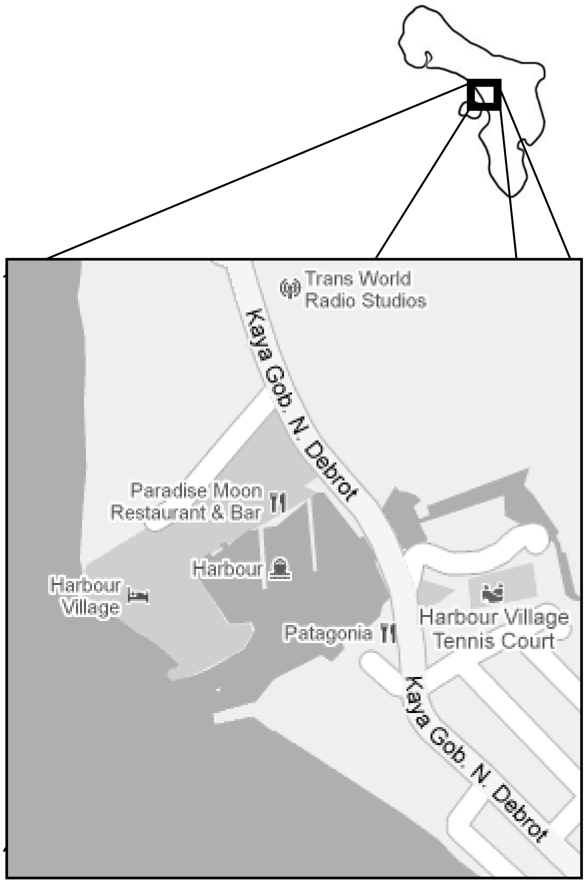

Fig. 1 The Harbor Village Marina and surrounding area. Courtesy of Google Maps.

assumed that a similar pattern occurred in Bonaire and that a marina was a likely point of introduction. Therefore, the dive site Something Special (12°09'39" N, 068°16'59" W) on the west side of Bonaire was chosen as a research site because it is the closest dive site to the Harbor Village Marina (12°09'50" N, 068°17'05" W). The Harbor Village Marina was chosen due to the known occurrence of cup corals and the variety of substrates in the area.

Size, depth and spread of T. coccinea from source site

Cup coral colonies were measured for a size range comparison against measurements taken in other known areas of introduction. The diameter of each colony was measured to the nearest millimeter at the widest point using a ruler. Several substrates were surveyed with a minimum of 40 randomly chosen colonies each. A size range (minimum to maximum) was established, and mean size was determined for each substrate and across all substrates. Size data were analyzed using an ANOVA test.

In addition, the depths inhabited by *T. coccinea* were determined by 100 x 2 m belt transects laid parallel to shore at depths of 1 m, 7 m, 14 m, and 20 m. Transects were laid in the area of the Something Special dive site. Transects were conducted by SCUBA and all encountered *T. coccinea* colonies were recorded for size and depth. All depth measurements were taken using a Suunto Zoop dive computer.

Finally, the range of occurrence from the marina was determined by a snorkel survey. Starting at the marina, the survey was conducted by swimming either north or south, and distances between individual colonies were observed until the maximum allowed distance was passed without encountering a colony. The maximum allowed distance between the most distant individual cup coral colonies was 100 m; if a length of more than 100 m was surveyed without encountering a colony, the range was considered to end at the last encountered colony. *Tubastraea coccinea* reproduces by brooding and fragmenting, so the larvae have a short competency period and cannot travel far, and fragmenting pieces typically do not land far from the original site. Therefore, 100 m was considered the maximum distance at which colonies could reasonably be expected to have originated at the marina (Creed and de Paula 2007).

Substrate preference

Substrate preferences for *T. coccinea* were evaluated by determining average percent cup coral cover on several natural and artificial substrate types (Table 1). Natural substrates surveyed were shallow shoreline rock ledges, sand, and coral reefs, and artificial substrates were submerged concrete and metal. Concrete substrates surveyed in this study were concrete dock supports and the marina's concrete jetty. Metal substrates surveyed in this study were metal dock supports and sunken metal drums and bars. Metal and concrete substrates were surveyed by laying a 0.5 x 0.5 m^2 PVC quadrat divided into a 5 x 5cm grid over the largest possible surface area of the substrate and photographing the quadrat (Fig. 2). Photographs were analyzed on a computer for number of colonies in each quadrat and percent coral cover to the nearest one-tenth of a percent. At least 40 photographs of each substrate were taken, and a minimum of 15 photographs were randomly selected for analysis using a random number generator. Quadrat photographs were

Table 1. A list of substrates surveyed in this study, their depths, and whether *T. coccinea* colonies were observed growing on that substrate.

Substrate	Depth	Cup Corals Observed?
Natural Substrates		
Shallow Rock Ledges	>1 m	Yes
Sand	0-6 m	No
Coral Reefs	5-20 m	No
Artificial Substrates		
Concrete (Marina Jetty)	0-5 m	Yes
Dock Supports (Concrete)	0-2 m	Yes
Dock Supports (Metal)	0-2 m	Yes
Metal Drums (Metal)	0-2 m	Yes
Misc. Metal Bars (Metal)	0-2 m	Yes

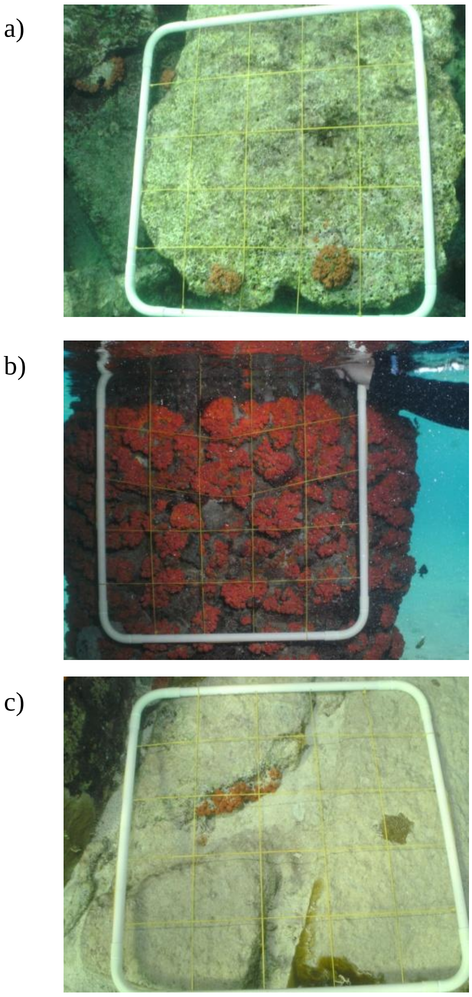

Fig. 2 0.5x0.5m^2 PVC quadrats were laid over the maximum available surface area of the substrate. Quadrats were used to estimate percent T. coccinea cover on a) rock, b) cement, and c) metal.

taken over the largest surface area of substrate possible in order to obtain the most accurate determination of percent cover. In order to investigate *T. coccinea*'s growth patterns in optimal conditions, only percent cover in low light conditions was evaluated for this part of the study.

Percent cover estimates for rock were conducted using the line-intercept method. A 30 meter transect was laid along the rock ledge and percent cover was visually determined on the underside of the ledge. It was assumed that the results of the line-intercept method were comparable to the results of the quadrat method. Again, percent cover was estimated to the nearest one-tenth of a percent. Number of colonies could not be determined for rock ledges due to the dangerous conditions.

In order to analyze the difference between the substrates, a Mann-Whitney U test was used. Rock percent cover was excluded from the analysis due to sample size limitations (N = 1).

Light preference of T. coccinea

Light preferences for *T. coccinea* were evaluated by determining average percent cover and average colony size of cup coral inhabiting several natural and artificial substrate types. Light preference was evaluated in a qualitative manner: high light areas were considered the areas receiving direct sunlight, while low light areas were considered the areas receiving little or no direct sunlight (Fig. 3). Metal samples (not including metal dock supports) were assigned a position based on their physical position on the substrate and the amount of light determined to reach them. In the case of rock ledges, the colonies growing on the top or side of the ledge were considered high light, and those growing on the underside of the rock ledge were considered low light; the shallow rock ledges could not be photographed due to space and safety issues.

The average colony size in each light condition was determined by dividing the percent cover of each individual quadrat by the number of colonies in that quadrat. The purpose of this calculation was to determine whether colonies growing in low light conditions were on average larger than colonies growing in high light conditions. This calculation is completely separate from the average colony size measurements found in the previous section, as those measurements did not distinguish between

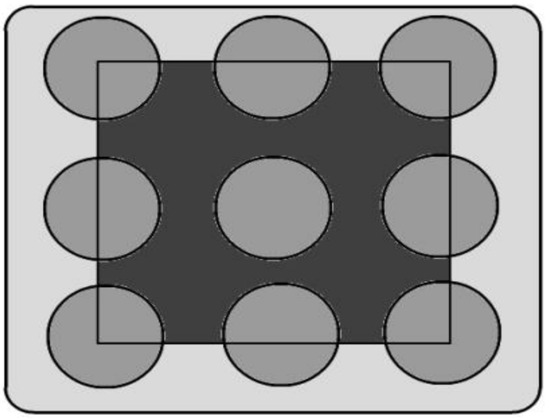

Fig. 3 Light intensity of the dock supports was determined to be high or low intensity. Grey circles are dock supports, light grey area is high intensity dark grey area is low intensity.

light conditions. These calculations were analyzed with a Mann-Whitney U-test.

Interactions between T. coccinea and neighboring corals and sponges

T. coccinea colonies growing in close proximity (≤ 3 cm) to another species of coral or sponge were surveyed for damage to either organism. Interactions were encountered by snorkel survey along the shallow rock ledge, on the dock supports, and on the marina jetty. Distances between the colonies were measured using a ruler. Deleterious effects such as necrosis, bleaching, disease, algal overgrowth, and coral overgrowth on either cup coral or neighbor were observed and recorded. Data were analyzed with an ANOVA test.

Results

Size, depth and spread of T. coccinea from source site

Colonies of *Tubastraea coccinea* were found to vary in size from 17 - 137 mm, with an average size (± standard deviation) of 69 ± 25 mm across all substrates (Fig. 4). Mean colony size (± SD) was largest on concrete substrates at 74 ± 28 mm, followed by metal with 67 ± 23 mm. Rock was found to have the smallest mean colony size at 64 ± 19mm. An ANOVA test did not yield significant results for average colony size between substrates.

In the 50 x 2m belt transects conducted, several colonies were encountered on the 1m transect. However, no orange cup corals were encountered on or below the 7 m transect.

On the southern side of the marina cup corals were found only within 230 m of the marina jetty. On the northern side of the marina cup corals were observed at least as far as 3400 m away (dive site name: Andrea II).

Substrate and light intensity preference

No *T. coccinea* colonies were observed growing on coral reefs or sand. Several small colonies of *T. coccinea* were observed in the sand beneath rocks and dock supports, but closer inspection revealed that these colonies were fallen fragments from colonies growing on rocks and dock supports, and were not actually growing in the sand.

Concrete dock supports were observed to have the overall highest average cup coral percent cover (± SD) of any substrate (56.0 ± 20.4%), as well as the maximum percent cover of 80.0% (Table 2). Rock had the next highest percent cover (44.3 ± 4.5%) with a maximum percent cover of 47.4% (Fig. 5). Metal followed with an average percent cover of 32.5 ± 25.6% and a maximum percent cover of 75.0%. Average percent cover estimations were analyzed with an ANOVA test and were found to be significant (p < 0.001).

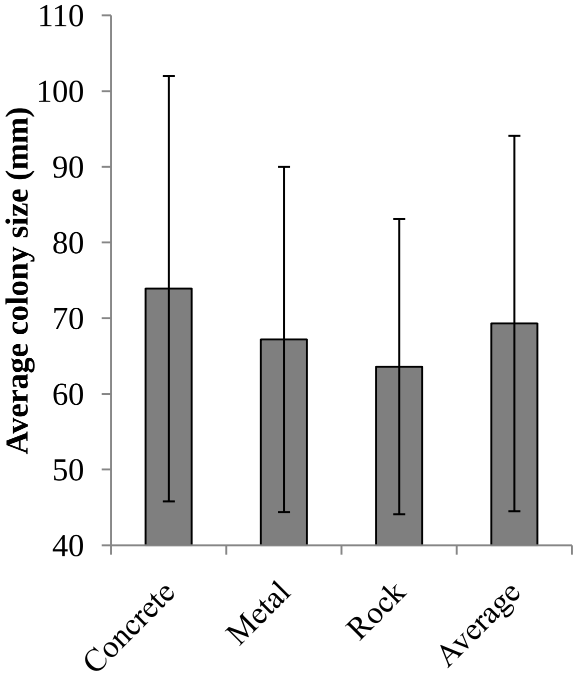

Fig. 4 T. coccinea colonies were measured for diameter at their widest point. Cement colonies were the largest on average.

Table 2 Maximum percent cover and average percent cover of *T. coccinea* on a variety of substrates in high and low light intensity conditions.

	Low Light		**High Light**	
Substrate	Maximum Percent Cover	Average Percent Cover (± SD)	Maximum Percent Cover	Average Percent Cover (± SD)
Metal	75.0	32.5 ± 25.6	40.0	8.0 ± 13.4
Concrete	80.0	56.0 ± 20.4	9.0	3.5 ± 2.6
Rock	47.4	44.3 ± 4.5	3.5	2.0 ± 2.1
Sand	0.0	0.0	0.0	0.0
Coral Reef	0.0	0.0	0.0	0.0

Light condition was observed to have a large effect on cup coral percent cover of all substrates. As shown in Fig. 5, concrete percent cover was significantly higher under low light conditions than under high light conditions (N = 45, U = 0, p < 0.0001). Metal percent cover was significantly higher under low light conditions than high light conditions (Mann-Whitney U-test: N = 33, U = 47, p < 0.0001). Rock percent cover was also observed to be much higher under low light conditions, but the data were not able to be statistically analyzed due to the small sample size (N = 1).

The number of colonies per unit area was significantly larger under low light conditions than under high light conditions for both metal (N = 35, U = 56, p = 0.01) and concrete (N = 45, U = 3.5, p < 0.0001). Average cover of individual cup coral colonies (Fig. 6) was found to be significantly larger in low light conditions than high light conditions (N = 35, U = 63, p = 0.03), but no significant difference was found between substrates.

Interactions between T. coccinea and neighboring corals and sponges

Of the 124 *T. coccinea* and a coral/sponge species interactions surveyed, only 3 (< 3%) of neighboring coral colonies of other species were observed to be damaged (Table 3). Necrosis was observed on one *Meandrina* colony and bleaching was observed on two corals both belonging to the genus *Diploria*. In all cases healthy colonies of the same genus were also observed. In *T. coccinea*-sponge interactions the sponges never exhibited signs of damage; however, in 17% of such interactions *T. coccinea* exhibited necrosis at the contact site.

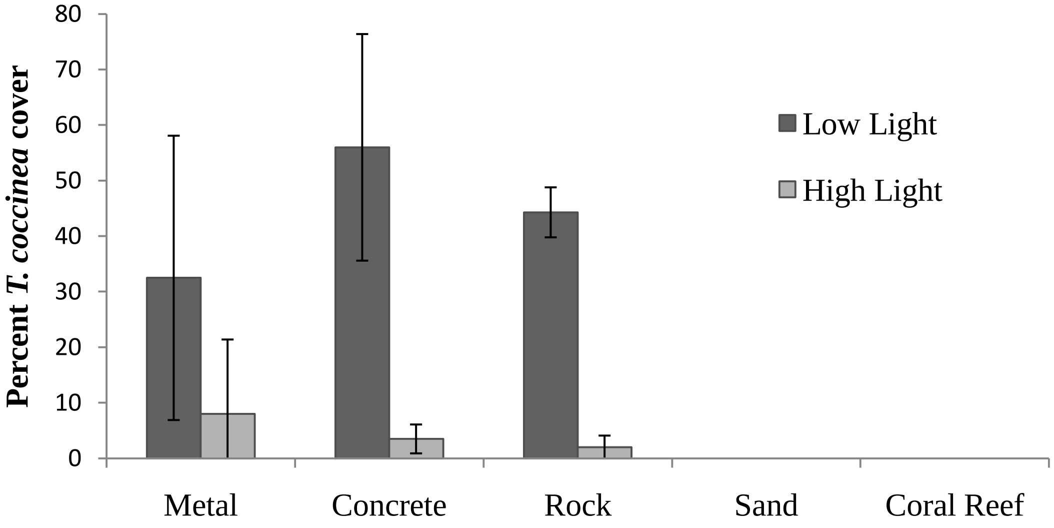

Fig. 5 Percent *T. coccinea* cover on a variety of substrates at high and low light intensities.

Table 3 A list of corals and sponges growing within 3cm of a *T. coccinea* colony, and damage observed on either party. Three neighboring corals of non-*Tubastraea* species were damaged but in all cases healthy colonies of the same genus were observed. In 17% of sponge-*T. coccinea* interactions, *T. coccinea* was damaged; sponges appear to be a threat to *T. coccinea*, but not the other way around.

Coral Genus	Total Interactions	Neighbor Damaged	*T. coccinea* Damaged
Porites	11	0	0
Millepora	48	0	0
Diploria	14	2	0
Siderastraea	7	0	0
Meandrina	2	1	0
Other Coral	6	0	0
Sponges (misc.)	36	0	6
Total Observed:	124	3	6

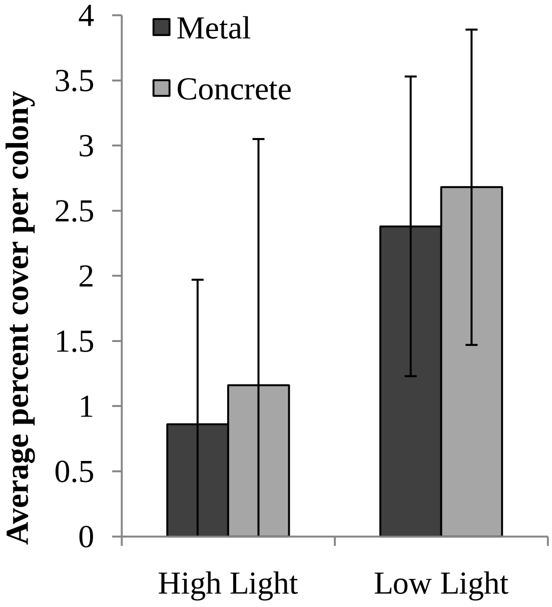

Fig. 6 Average percent cover per *T. coccinea* colony was found by dividing the percent cover of each quadrat by the number of colonies in that quadrat. In low light conditions, individual colonies tend to be larger and thus cover a larger area of the quadrat.

Discussion

Size, depth and spread of T. coccinea from source site

Tubastraea coccinea was found to have a somewhat larger size range in Bonaire than previous studies had found in Brazil, where the size range was reported as 45 - 105mm (de Paula and Creed 2004). Coral recruits were not identified for this study, and because *T. coccinea* colonies tend to grow to about 5 cm^2 in diameter within one year I assume that all colonies measured were several years old. This difference in minimum size could be explained by date of introduction; *T. coccinea* was recorded in Brazil long before Bonaire, so it is quite possible that cup corals have been growing in Brazil for a longer period of time and are therefore larger. On the larger end of the size range, it appears that cup coral colonies grow to be somewhat larger in Bonaire than in Brazil. This could potentially conflict with the previous assumption that cup corals are larger in Brazil due to earlier introduction, but introduction date is not the only factor in coral growth. In Brazil, *T. coccinea* is competing with native species of coral, but in Bonaire *T. coccinea* does not appear to have much competition and could perhaps grow more quickly as a result. A large variety of other factors, including water quality and free substrate abundance, are likely to have contributed to the difference in maximum size, but this study does not address those factors and more research would need to be conducted to more fully explain the difference in size range.

In the area surveyed, *T. coccinea* colonies were not found in water 7 m deep or deeper. However, *T. coccinea* is known to grow quite deep on a shipwreck called the Hilma Hooker, located in 30 meters of water on the southwestern side of Bonaire; however, this area could not be accessed for this study. This ship sunk in the 1980's so it is entirely possible that the cup corals had already been growing on the hull of the ship prior to sinking; genetic testing would be necessary to identify the source of the cup corals. If the coral was not already present on the hull of the Hilma Hooker, it is also possible that coral recruits landed on the Hilma

Hooker and, without a dense cover of native corals, began to proliferate.

Cup corals were found to have a much larger range on the northern side of the marina, probably due to the northward-flowing current. Colonies were found as far north from the marina as the Andrea II dive site, but because this was the maximum range of the snorkel survey, it is likely that cup corals can be found even farther north of this site. *Tubastraea coccinea* is a brooding coral species, so larvae tend to have a short competency period and do not travel as far from the parent as the larvae of broadcast spawners (Nishikawa et al. 2003). Interestingly, while no density data was collected from the north side of the marina for this study, it was observed that *T. coccinea* was found at a much higher density on the southern side of the marina than on the northern side. The small corner-cove formed by the jetty and rocky shoreline could provide a low-current area and allow for more larvae to settle near the parent; the strong current on the north side of the jetty is more likely to quickly spread the larvae thinly across a larger area.

Substrate and light intensity preference

A clear preference for concrete substrates has been shown by the cup coral populations in this study site, supporting Creed and de Paula's findings in Brazil that *T. coccinea* grows preferentially on concrete substrates (2007). Creed and de Paula suggested that this may be due to the chemical composition of concrete; concrete contains high levels of calcium carbonate, the same material that forms the skeletons of stony corals (2007). The complete lack of cup coral growth on the nearby coral reef is somewhat surprising, especially with the amount of literature describing cup corals as 'invasive' and harmful. This is perhaps due to the sandy stretch between the coral reefs and the shallow rock ledges and dock supports. The jetty is located beside a dredged harbor, and so the lack of surrounding reef may explain the absence of spread.

The differences between average colony size measurements for each substrate were not found to be statistically significant, although a trend of larger colonies on concrete was observed. The average cover of individual cup coral colonies was found to be slightly larger on concrete substrates than on metal substrates under both light conditions, again suggesting a trend of larger colonies on concrete substrates. However, the difference was less than 0.3% in both high and low light conditions and was also not statistically significant. No data was collected on the size or number of colonies on rock substrates, so no conclusions regarding the size of colonies can be made about rock substrates.

A clear preference for low light conditions was also observed, supporting the hypothesis that *T. coccinea* prefers low light conditions. Average percent cover and average colony size were both much higher in low light conditions than in high light conditions, indicating a strong preference for low light conditions. This is most likely because *T. coccinea* is an azooxanthellate species, and therefore does not require sunlight to survive. In corals living symbiotically with zooxanthellae, the zooxanthellae can produce mycosporine-like amino acids (MAAs) that act as a sunscreen for the coral host, protecting it from damaging ultraviolet radiation. Corals without zooxanthellae cannot produce these MAAs on their own, and so they may be damaged by high levels of sunlight exposure, explaining their preference for shady areas (Yakovleva and Hidaka 2004). Additionally, *T. coccinea* may have evolved as a shade-loving specialist species, allowing it to thrive in conditions that would starve zooxanthellate corals.

Interactions between T. coccinea and neighboring corals and sponges

Of the 124 surveyed interactions within 3cm of a *T. coccinea* colony, the greatest damage was actually observed on the *T. coccinea* colonies growing near sponges. In these *T. coccinea*-sponge interactions, the sponges appeared to be killing cup coral polyps that were in direct contact with the sponge. In *T. coccinea*-other coral interactions, less than 3% of the neighboring corals were observed to be damaged in any way, and in all cases other colonies of the same genus were observed without damage within 3 cm of *T. coccinea*. There is no evidence or reason to believe that the damage to these corals was due to the neighboring colonies of *T. coccinea*, and so the hypothesis that *T. coccinea* damages other organisms was not supported. Despite Creed's (2006) findings that cup corals were damaging the endemic *Mussismilia* corals of Brazil, there is no evidence that *T. coccinea* is currently exhibiting any negative effects on the corals of Bonaire. It is possible that *Mussismilia* is simply more susceptible than other corals to damage by *Tubastraea*; however, there is currently no research or evidence to support this theory.

Based on the results of this study, it can be concluded that in the studied area of Bonaire *T. coccinea* colonies are not harming native coral and sponge species. No colonies were encountered on the coral reef in the study area, and no negative effects were observed on neighboring coral colonies. This suggests that *T. coccinea* is currently not an 'invasive' species in this area, but merely introduced. However, it is likely that *T. coccinea* may begin to compete with and exhibit negative effects on native species in the future, and so continued observation of *T. coccinea* is necessary to determine its introduced or invasive status in the future. *T. coccinea* may simply be in the early stages of expansion, and this expansion must be monitored. If *T. coccinea* does in fact become invasive, it may become necessary to implement management practices for this species to prevent it from outcompeting native species in Bonaire.

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"A lot of people attack the sea, I make love to it."

-Jacques Cousteau