Physis

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Physis

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CIEE Research Station

Tropical Marine Ecology & Conservation Program

Volume 14 Fall 2013

PHYSIS φύσις

Imagine the world as a piece of clockwork. The most intricate set of cogs, moving parts, motors. The movement of every piece moves another, and then another. In this near infinite clock, every piece is a living being, a rock, a river. A part of an ecosystem. The inner workings of this clock are so delicate, and so vast. We, as scientists, look at one of these tiny wheels and try to see how it fits onto the ones that surround it.

Our generation has inherited a world in the brink of crisis. Small ailments have accumulated in virtually every ecosystem in the world over time, and we're nearing a breaking point. It is extremely disturbing to think that a single species has artificially disrupted the natural processes of the entire planet when that same species has the means and intellect to prevent the disruptions in the first place. We've invested billions of dollars into trying to create our image of what nature "should be". We idealize it as we see it in a painting, still and unchanging, and become frustrated by its insistence on fluctuating. We do not like the idea of the unknown, so we insist on restricting nature to what we think is safe.

Ecosystems are in a perpetual state of change. Storms, floods and disease are not new to this earth, and the destruction they may reap is also a part of the system. A healthy ecosystem is one that can self-heal; one with unhindered cogs that can move freely to adjust to a new turn in the clockwork, changing with time at its own pace. This state is called *Physis*, and it is what we should strive to achieve.

Nature's resiliency is a thing of wonder, but it must be given the time and space to repair itself. We as humans often convince ourselves that we have the capacity to create and direct nature to comply with our whim. The damage we repeatedly inflict upon Mother Nature prevents its healing processes. Only if we humans manage to cease the course of our vicious cycle, can nature truly take hold of *Physis*, for it is impossible for a wound to heal without a moment of rest. When we keep trying to ply nature to fit our image, we are denying it this moment. We cannot run the world, but the world can run itself. We need to reassume our place as a tiny part of a dynamic ecosystem, for its sake and ours.

We are marine biologists and we want to know the why and the how of everything we see around us. Our heart is in the ocean, that most incredible and delicate realm, foreign to us land animals and largely unexplored. We want to learn from our oceans, but we find ourselves chasing questions that the system is not capable of answering anymore. We can't ask about the shape of a system that has lost half of its pieces, and is losing more and more every day.

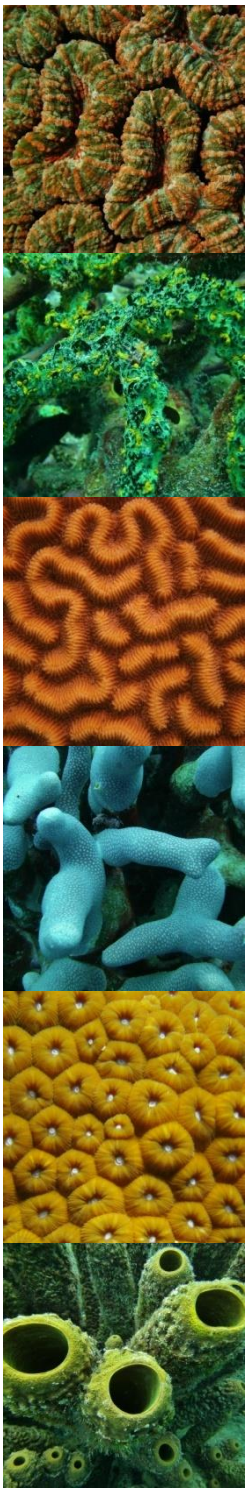
Instead of trying to create new technologies to engineer our way out of the problems we have created, we should try to reduce our impact and restore natural processes to allow nature to heal itself. If we take down the dams we have put up, rivers begin to flow as they once did; if we do not continue to pave over it, lush grass will break through the sidewalks; a fallen tree that is left alone will soon be brimming with new life. Harsh storms may crush magnificent branching corals but eventually the wreckage is replaced by new structures and life begins to return. Released from external forces, life flourishes, becomes stabilized, and is replenished.

Megan Beazley, Pam Denish, Elizabeth Groover, Austin Lin, Lucia Rodriguez, Jennifer Shaffer

FOREWORD

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

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



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

The student research reported herein was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Dutch Caribbean. Projects this semester were conducted on the leeward side of Bonaire where most of the population of Bonaire is concentrated. Students presented their findings in a public forum on the 20th and 21st of November, 2013 at the research station.

The proceedings of this journal are the result of each student's research project, which is the focus of the course that was co-taught this semester by Rita B.J. Peachey, PhD; Patrick Lyons, PhD; and Enrique Arboleda, PhD. In addition to faculty advisors, each student had an intern that was directly involved in logistics, weekly meetings and editing student papers. The interns this semester were Yannick Mulders, Fadilah Ali, Estelle Davies, McCrea Sims, and Gabrielle Lout. Astrid de Jager was the Dive Safety Officer and provided scientific dive training and oversight of the research diving program.

Thank you to the students and staff that participated in the program this semester! My hope is that we succeeded in our program goals and CIEE's mission and that the students' all succeeded in their goals.

Dr. Rita Peachey

FACULTY

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

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

Dr. Patrick Lyons is the Tropical Marine Conservation Biology faculty for CIEE and co-teaches Independent Research and Marine Ecology Field Methods. Patrick received his B.S. in Marine Biology from the University of Rhode Island and his Ph.D. in Ecology and Evolution from Stony Brook University. His research broadly focuses on the behaviors that coral reef animals employ while interacting with competitors, predators, prey, and mutualist partners. His goal has been to describe these behaviors and clarify their evolutionary basis. Patrick's main line of research has been on the fascinating mutualism between alpheid shrimp and gobiid fishes in which blind shrimp provide shelters for goby partners and gobies warn their blind shrimp partners when predators are present. Patrick's research has clarified the benefits and costs of gobies that use this strategy versus those that don't.

STAFF AND INTERNS

Amy Wilde is the Program Coordinator 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 and she is the student resident hall manager.

Amber Anna Jasperse is the administrative assistant at CIEE Bonaire. She graduated from HAVO in 2012 on Bonaire, and studied graphic design in Holland. In total she's lived on the island for more than 17 years. She is hard working and loves to help around where help is needed. In her free time she loves to go windsurfing at Sorobon beach.

Astrid de Jager is the Dive Safety Officer. She came to Bonaire in 2009 and has been working in the dive industry ever since. She developed from Dive Master all the way to SDI Instructor Trainer, Padi Staff Instructor and IAHD instructor. Currently she is the owner of a small dive training center, from which she teaches beginning divers as well as professional level classes.

Sjoukje Hiemstra is the lab technician at CIEE Bonaire. Sjoukje has extensive experience in working with zoo animals throughout the Netherlands. In the last couple of years Sjoukje was involved in post-mortem research with harbor porpoises. Beside the harbor porpoises Sjoukje was involved in many necropsies on seal and dolphin species, large baleen whales and oystercatchers. Sjoukje has co-authored several scientific publications about harbor porpoises and reports for the Dutch Government. On Bonaire Sjoukje is starting different pathology projects besides her work in the lab.

Fadilah Ali has worked at CIEE on and off since 2010 as an intern and is currently a final year PhD student at the University of Southampton. Her research focuses on the lionfish invasion in the Caribbean and to date, she has dissected more than 10,000 lionfish. Fadilah is the Intern Coordinator at CIEE but also acts as the Director of all Arts and Crafts.

INTERNS

Estelle Davies is a Tropical Marine Conservation Biology Intern at CIEE Bonaire for the fall semester 2013. Estelle received her B.Sc. Honours in Marine and Environmental Biology from St Andrews University in Scotland spending her 3rd year on exchange at the University of California Santa Cruz. She received her Masters of Applied Science in Tropical Marine Ecology from James Cook University in Australia where she studied the sex ratio of sponge-inhabiting brittle stars on the Great Barrier Reef. Before moving to Bonaire she worked for Jean-Michel Cousteau's Ocean Futures Society, teaching Marine Education Programs in French Polynesia, Fiji and the Cook Islands. Estelle is involved in diving, research and teaching at CIEE Bonaire.

Yannick Mulders is the Coral Reef Ecology intern at CIEE. After growing up on Curacao, he moved to the Netherlands and obtained a Bachelors degree in Biology, and a Masters degree in Environmental Biology at the University of Utrecht. During his Masters he focused mostly on tropical reef ecology and his degree was complemented with the "Marine Scientist of the Netherlands" annotation. He first came to CIEE in 2012, when the fieldwork of the research he was involved in brought him to Bonaire for a month.

McCrea Sims is the Marine Ecology Field Research Methods intern at CIEE. She holds a B.S. in Biology from Wofford University. She was a student at the research station in the spring semester of 2013, with her research focusing on algae and its self-healing properties. Currently she is gaining experience to further her education at the graduate level. She is an active PADI Diver and AAUS Scientific Diver.

Gabrielle Lout is a volunteer intern at CIEE. She is currently working on a B.S. in Marine and Conservation Biology at Seattle University. After graduation, she plans to continue her education at a Master's graduate program in Marine/Ocean Sciences. She was a student at the research station in the spring semester of 2013, with research focusing on exotic corals in the Caribbean. She is an active PADI Open Water Instructor, AAUS Science Diver, and assists with the scuba dive program at CIEE.

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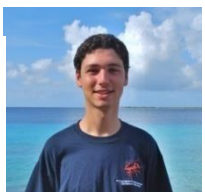

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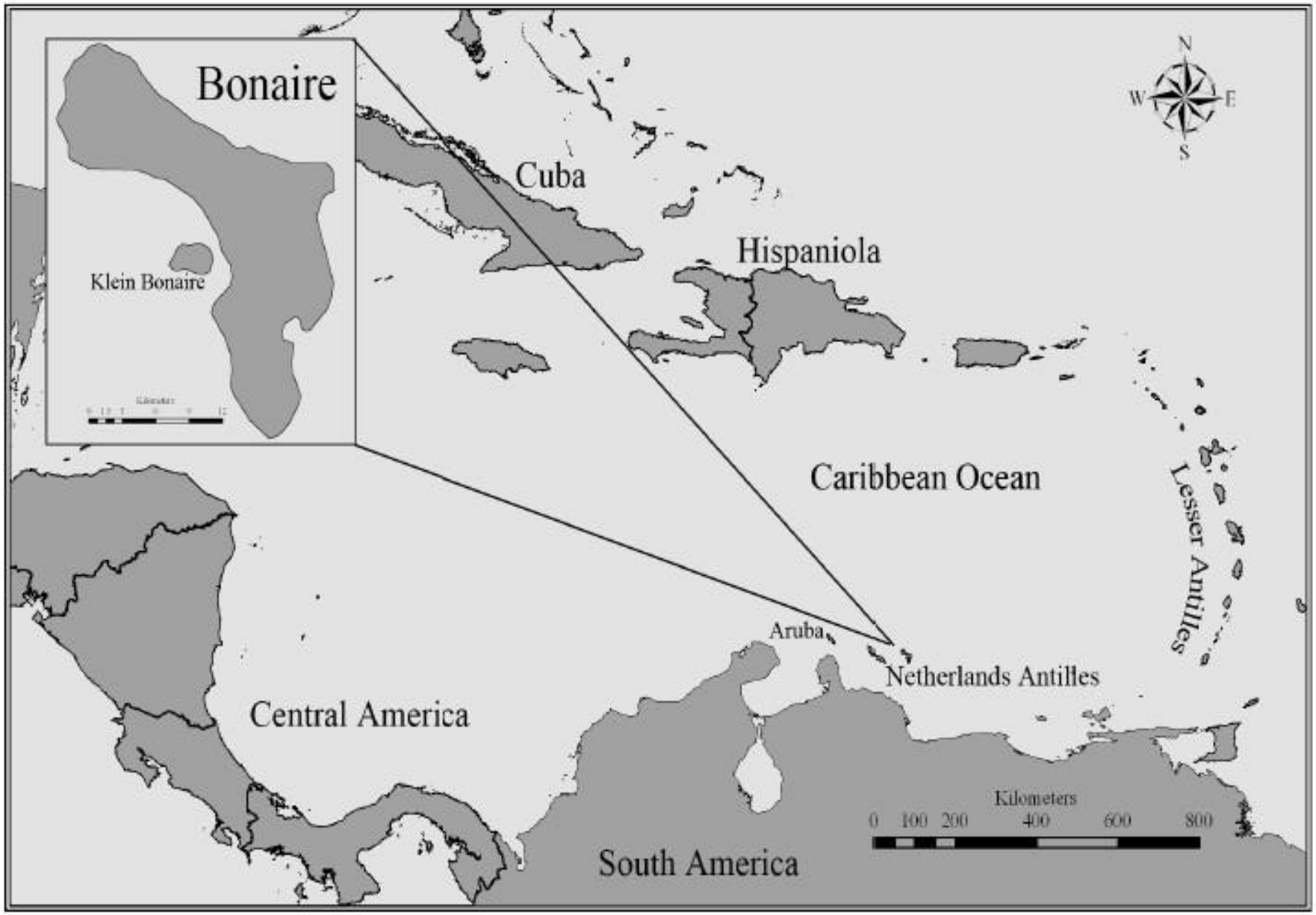
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REPORT

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Influence of habitat on defecation behavior of queen (*Scarus vetula*) and princess (*Scarus taeniopterus*) parrotfish

Abstract Herbivores are important structuring agents for ecosystems worldwide. While effects of grazing by herbivorous fish are well studied, their roles in organismal dispersal have only recently become a topic of interest. Location preference and range of defecation may indicate the importance of their contribution to organism spreading. This study therefore examined the distribution and frequency of defecation of the princess parrotfish (*Scarus taeniopterus*) and queen parrotfish (*Scarus vetula*) between coral reef and sand flat habitats. Observations were performed using SCUBA in Bonaire, Dutch Caribbean. Target species were observed for 20-minute trials in each habitat. Defecation frequency, bite frequency, maximum distance between defecations, and location of defecation were recorded and averaged for each species in each habitat, and compared between species and habitats through two-way analysis of variance (ANOVA). Additionally, unique food sources observed during trials were sampled and examined in the lab. *S. taeniopterus* individuals were found to defecate significantly less and have smaller maximum distance between defecations within the reef habitat than the sand habitat, while *S. vetula* did not show significant behavioral changes for any of the variables between the two habitats. Lab results also suggest that *S. taeniopterus* may be opportunistic omnivores. This study offers insight to behavioral plasticity and specificity to habitat type, and provides a broader understanding of dietary plasticity and ecological roles for *S. taeniopterus* and *S. vetula.*

Keywords Parrotfish • Defecation behavior • Habitat differences

Introduction

Herbivores serve as important structuring agents for ecosystems worldwide by controlling plant biomass, assisting in plant dispersal, and serving as a mode of energy transfer from primary producers up trophic levels (McNaughton 1985; Collins et al. 1998). Loss of herbivore abundance in marine ecosystems threatens the persistence of coral reefs, and can induce an ecological phase shift from coral dominant reefs to macroalgae dominated reefs (Cyr and Pace 1993; Hughes 1994; Aronson and Precht 2001; Gardner et al. 2003; Carpenter et al. 2008).

Reef fish are particularly important herbivores and bioeroders of marine ecosystems because they maintain coral reefs and assist in recruitment of corals by selectively feeding on macroalgae that would normally outcompete coral recruits (Lewis 1986; Hughes 1994; Bellwood and Choat 1990; Bruggemann et al. 1994; Bruggemann et. al. 1996; Bellwood et al. 2004; Vermeij 2006; Vermeij and Sandin 2008). Additionally, herbivory benefits coral reefs by removing organisms that would compete with coral through shading and abrasion (McCook et al. 2001), and controlling biochemicals that influence the

growth, survival, and reproduction of corals (Rasher and Hay 2010).

While roles of grazing by herbivores are well studied, roles in organismal dispersal by herbivorous fish have only recently become a topic of interest (e.g. Castro-Sanguino and Sanchez 2012; Vermeij et al. 2012). Within marine and aquatic ecosystems, herbivorous fish may participate in seed, plant, and fruit distribution through defecation (Vermeij et al. 2012). Studies done by Vermeij et al. (2012) found that algae fragments are not always fully digested when eaten. Survival of fragments suggests that algae may therefore tolerate or even benefit from grazing by herbivorous fish through release by defecation into a potentially more favorable environment. Viable macroalgae assemblages were found in the feces of blue tang (*Acanthurus coeruleus*), ocean surgeonfish (*Acanthurus bahianus*), princess parrotfish (*Scarus taeniopterus*), and stoplight parrotfish (*Sparisoma viride*) (Vermeij et al. 2012). Of the algae fragments that survived digestion, 43% of algal fragments from all four species were capable of regrowth (Vermeij et al. 2012).

In addition to distributing plant biomass within the reef system, other studies have shown that zooxanthellae (*Symbiodinium*) within the fecal matter of fish are still photosynthetically active and capable of re-establishing symbiosis after ingestion by herbivores (Muller-Parker 1984; Castro-Sanguino and Sanchez 2011). Castro-Sanguino and Sanchez (2011) documented viable *Symbiodinium* surviving *S. viride* ingestion, where 93% of all samples of *S. viride* fecal matter contained viable zooxanthellae. Castro-Sanguino and Sanchez (2011) concluded that *S. viride* are contributing to the distribution of *Symbiodinium* in the coral reef ecosystems. Though these findings reveal important ecological relationships, little else has been documented on zooxanthellae dispersal through herbivore defecation.

If parrotfishes act as vectors for organism dispersal, selectivity and location preference for defecation may indicate the importance of their contribution to organism spreading. It has been observed that heavybeak parrotfish (*Chlorurus gibbus*) make a distinct movement between reef zones before defecation, targeting sand gullies or moving completely off the reef and out of their feeding area (Bellwood 1995). Furthermore, other herbivorous marine fish such as the whitespotted devil damselfish *Plectroglyphidodon lacrymatus* (Polunin and Koike 1987), surgeonfish species *Acanthurus glaucopareius, Acanthurus lineatus* (Robertson 1982), and *Ctenochaetus striatus* (Krone et al. 2008) move outside of their feeding territory before defecating. In addition to defecating off of grazing zones, it has been documented that other reef fish species defecate non-randomly on sand (Vermeij et al. 2012). It is possible that these behaviors are an evolutionary adaptation to separate excess sedimentation from their food sources and reduce the risk of infection from microorganisms that may be present in their feces (Krone et al. 2008).

It is important to consider the environment in which defecation distribution occurs. While differences of foraging techniques of parrotfish species have been well studied (Bellwood and Choat 1990; Bonaldo and Bellwood 2009), previous studies have not described differences in defecation location when comparing the same species between two habitats. However, preliminary surveys of this study revealed that behavioral differences in parrotfish species between habitats might occur.

This study examined the distribution and frequency of defecation of the *S. taeniopterus* and *Scarus vetula* between the coral reef and sand flat habitats, where sand flats were directly alongside the reef. Based on preliminary observations, the

objectives were tested with the following hypotheses:

H_1: Defecation frequency and defecation range will be significantly smaller within reef habitats than sand habitats

H_2: Defecation behavior between habitats will not be significantly different between *S. vetula* and *S. taeniopterus*

Studying defection behavior of *S. vetula* and *S. taeniopterus* may give insight into behavioral plasticity and specificity between environments. Studying behavior may also give insight to behavioral adaptations for coexistence within and between species, and provide a better understanding of ecological relationships on coral reef habitats.

Materials and methods

Study organism

S. taeniopterus and *S. vetula* are common species of the Western Tropical Atlantic. Previous studies have noted that several species, including *S. taeniopterus*, defecate non-randomly on sand (Vermeij et al. 2012). The mode of foraging for *S. vetula* and *S. taeniopterus* has been defined as scrapers because both species simply scrape algae off of the substrate without excavating a large portion of substrate (Cardoso et al. 2009). If *S. taeniopterus* and *S. vetula* display the same foraging techniques, it is possible they might also have similar preferences or patterns for defecating. Additionally, *S. taeniopterus* has been documented to contain viable macroalgae assemblages within its feces (Vermeij et al. 2012). Only terminal phase (TP) parrotfish species were studied, as preliminary studies indicated behavior may vary between life stages, and therefore observations within species could not be pooled. Both species also appear to be undisturbed by diver presence.

Study site

Studies were conducted from 28 September to 3 November 2013 on the leeward fringing reef on Bonaire, Dutch Caribbean between the dive sites Yellow Submarine (N 12° 9’ 36.648”, W 068° 16’ 55.578”) and Something Special (N 12° 9’ 40.9062”, W 068° 17’ 0.7362”). Reef and sand habitats within these sites were observed. These dive sites span ~250 m along shore, and consist of the same reef structure and have a sand flat extending ~100 m from shore before reaching the reef crest. Many species of parrotfish have been found to have territorial ranges varying between 41 to 1400 m^2 (Mumby and Wabnitz 2002). However, there are a lack of studies explaining the spatial range of *S. taeniopterus* and *S. vetula* and their tendency to stay in the same territory over a matter of days or weeks. Therefore all surveys were done in different areas at both dive sites to avoid surveying the same parrotfish more than once. This location is easily accessible from shore and frequently visited by divers. Visibility is generally >15 m within the water column which allows easy observation at a distance to avoid potential alterations in parrotfish behavior and also provides an unobstructed view for accurately recording fish activity.

Upon arrival to the study site during observational surveys, the observers would immediately start scanning for target individual along the specified habitat. When targeting species on the reef habitat, observers would scan along the reef slope from the reef crest to a depth of ~18 m (AAUS research dive limits). Along sand habitat, observers would scan from the surface (~5 m above substrate) until the target species was located, and then descend to the target species.

Field techniques

Subject characteristics and behaviors

Each parrotfish in the study was observed for a 20-minute trial. Once a target species was found, a 1-2 minute period allowed for the parrotfish to acclimatize to the presence of observers. Measurements of parrotfish were also taken during this time to avoid disturbing the parrotfish during the timed trial. To measure parrotfish behavior, one consistent surveyor recorded species, size, start depth, substrate grazed, bite frequency, defecation frequency, location of defecation (i.e. water column, on sand, or on dead coral), and end depth. Size was estimated with a 1-m stick marked with ten-centimeter increments.

During several of the timed trials, a sample of the parrotfish feces was also taken for observation. Fecal samples were only taken when parrotfish released matter directly over substrate, and not within the water column to avoid collecting microbial organisms that were already suspended in the water column. Samples were gathered using a spoon, and placed into a glass vial with a secure screw cap. Samples were not taken if fecal matter could not be separated from the sediment it landed on to avoid gathering sand or microbes within the benthos. Samples were also not taken if all defecations were made outside of dive limits, or gathering samples posed potential risk to damaging surrounding organisms (e.g. coral). Additionally, if the parrotfish were observed to eat an unknown food source, a sample of the food source was collected and stored in glass vial with a secure screw cap to analyze in the lab directly following the survey.

Each surveyor practiced the same procedures for every parrotfish surveyed for the duration of the study to reduce surveyor bias. The same waterproof datasheets were used during trials, and data was recorded into a field notebook after each trial. Fecal samples were observed in the lab after each trial. If equipment was already in use and samples could not be analyzed directly after a trial, the sample was placed in ethanol until it was possible for them to be analyzed. Trials were all taken between 7:30-17:30, with the majority of trials starting between 10:30-16:30.

Foraging and defecating range

To measure habitat range and distances between defecation locations, defecation and grazing activities were marked using numbered, weighted, orange tape markers. Numbers on tags were recorded next to the foraging and defecating activity on the surveyor's datasheet. Preliminary tests showed that the presence of the markers did not visibly change parrotfish behavior, or prevent them from defecating there again. After each timed trial, a 100-m tape was used to measure the distances between defecation locations, and distances between grazing boundaries.

Lab techniques

Fecal samples and unique food sources were observed under a microscope. Observations were made on the content of the feces using an AMScope microscope camera, which allowed visual display and image capture on the lab desktop.

Data analysis

To measure behavioral differences between sand and reef habitats for both species, a two-way analysis of variance (ANOVA) was used to analyze the maximum distances between defecation locations, defecation frequencies, bite frequencies, and frequency of defecations made above each substrate. Where significant p-values were found, separate t-tests were done for a specific species for both habitats. Mean defecation frequency and mean maximum defecation distances were each compared between each species

in each habitat. Confidence intervals with an alpha value of α=0.05 were also calculated and used for comparative bar graphs between species and habitats.

Results

Field observations

A total of 20 fish were surveyed, with 5 samples for each species within each habitat (e.g. queen, reef habitat).

Additional behaviors that were recognized (but were not part of this study) were noted. One such behavior displayed by *S. taeniopterus* was defined in this study as "streaming," where individuals being surveyed would stream a fine layer of feces while displaying territorial behavior against another *S. taeniopterus* individual. If another *S. taeniopterus* streamed within the territory of the individual being surveyed, the *S. taeniopterus* individual would stream directly over the invading stream. However, *S. taeniopterus* being surveyed were observed to stream while chasing another *S. taeniopterus*, even when the other individual did not stream. Territorial defecations were not included in analysis of defecation behaviors or distances, as the purpose behind defecation appeared different and therefore could not be pooled with the behavior and preference of other defecations made when the individuals were not displaying aggressive territorial behavior.

Additionally, this study aimed to survey *S. taeniopterus* and *S. vetula* in the reef habitat between the reef crest and down the reef slope to a depth of 18 m. However, *S. vetula* were always found on the reef crest specifically, and did not venture deep into the reef slope. While *S. vetula* were seen on the reef slope below the reef crest, *S. vetula* individuals roaming below the reef crest for periods longer than a minute or two were not observed during this study. Therefore, all reef habitat observations for *S. vetula* in this study were made on the reef crest specifically, as subjects were not found deeper in the reef slope during trial times or otherwise.

Behavioral frequencies

To measure defecation frequency differences for both species between habitats, a two-way ANOVA was used to examine the effect of species (*S. taeniopterus* or *S. vetula*) and habitat type (sand or reef) on defecation frequency (Table 1). Analysis showed that when all data from both habitats and species were pooled, species had no effect on defecation frequencies, but habitat did (Table 1). However, habitat was found to be a significant factor explaining defecation frequency primarily because of *S. taeniopterus*. Two sample t-tests showed *S. taeniopterus* had a significantly higher mean defecation frequency in the sand habitat than the reef habitat (t= 2.8, p=0.02), where *S. vetula* did not have a significant difference in defecation frequency in the sand habitat compared

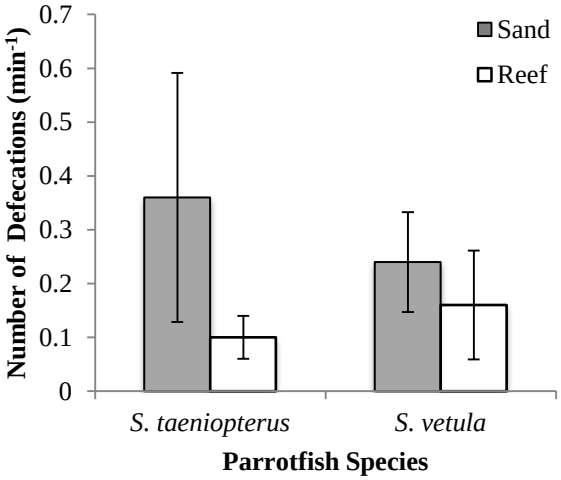

Fig. 1 Comparison of mean defecation frequency between sand and reef habitats for *Scarus taeniopterus* and *Scarus vetula* (n=5 for each species in each habitat). Error bars indicate 95% confidence intervals. *S. taeniopterus* had a significantly higher defecation frequency in sand than reef habitat, whereas *S. vetula* did not have a significant difference in defecation frequency between the sand to the reef habitats

with the reef habitat (t=1.5, p=0.2; Fig. 1).

A two-way ANOVA was used to examine effect of species (*S. taeniopterus* or *S. vetula*) and habitat type (sand or reef) on bite frequency (Table 2). Analysis showed that species did not have an effect on bite frequencies (Table 2). Neither *S. taeniopterus* nor *S. vetula* displayed significant mean differences in bite frequency between habitats (Fig. 2).

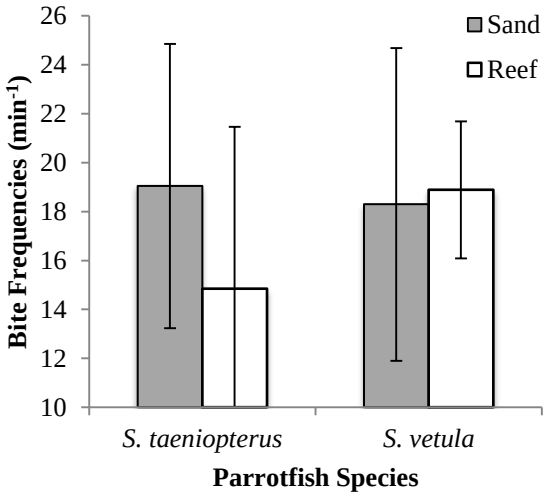

Fig. 2 Comparison of mean bite frequency between sand and reef habitats for *Scarus taeniopterus* and *Scarus vetula* per minute (n=5 for each species in each habitat). Error bars indicate 95% confidence intervals. *S. taeniopterus* did not have a significant difference between sand and reef habitats. Similarly, *S. vetula* did not have a significant difference in bite frequency between sand and reef habitats

A two-way ANOVA was used to examine the effect of species (*S. taeniopterus* or *S. vetula*) and substrate type (sand or dead coral) on location that defecations were made above in the coral reef habitat (Table 3). Analysis showed that when all data for both species within the coral reef habitat were pooled, species had no effect on defecation frequencies, but substrate type did (Table 3). However, substrate type was only found to be a significant factor explaining location of defecations primarily for *S. taeniopterus.* Two-sample t-tests showed *S. taeniopterus* had a significantly higher frequency of defecations over sand substrate than dead coral substrate (t= 2.6, p=0.03), where *S. vetula* did not have a significant difference of frequency of defecations made over sand substrate than dead coral substrate (t=1.6, p=0.2; Fig. 3). While there were no individuals that defecated on live coral heads, small coral recruits were sometimes present on dead coral substrates. However, dead coral was the dominant substrate that was defecated on.

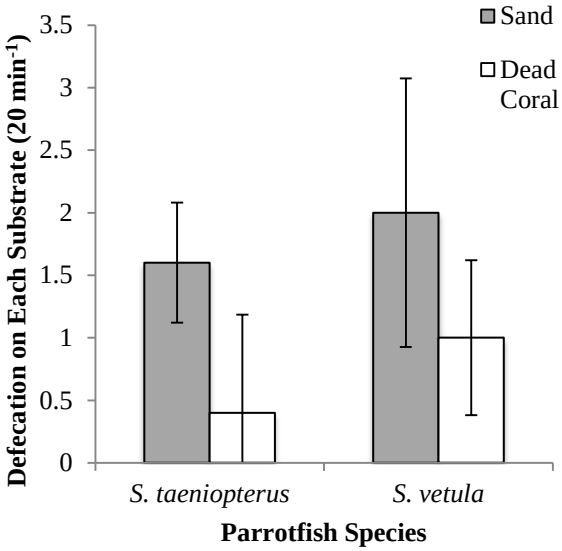

Fig. 3 Comparison of mean number of times defecations were made on sand and dead coral substrates within the reef habitat during 20 min surveys for *Scarus taeniopterus* (n=5) and *Scarus vetula* (n=5). Error bars indicate 95% confidence intervals. *S. taeniopterus* defecated significantly more on sand than dead coral during 20 minute trials, but there was no significant difference in the average number of times *S. vetuala* made on

Maximum defecation range

A two-way ANOVA was used to examine the effect of species (*S. taeniopterus* or *S. vetula*) and habitat type (sand or reef) on maximum defecation range. Analysis showed that when all data from both habitats and species is pooled, species has no significant effect on defecation range, but habitat does (Table 4). However, two sample t-tests showed that habitat was only found to be a significant factor explaining mean maximum defecation range for *S. taeniopterus* (t=4.9, p=0.001), where *S. vetula* did not have a significant

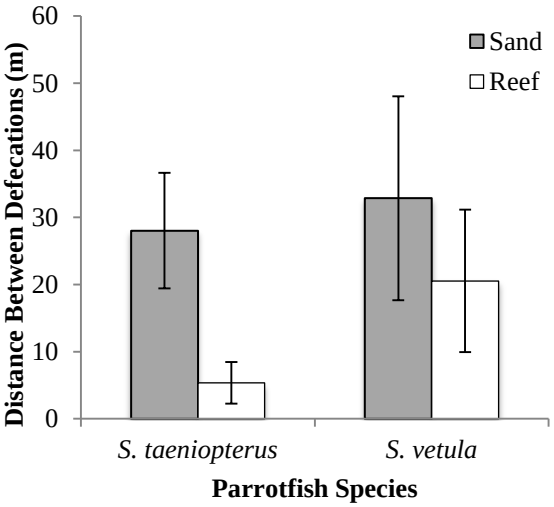

Fig. 4 Comparison of mean maximum defecation distance between sand and reef habitats for *Scarus taeniopterus* and *Scarus vetula* (n=5 for each species in each habitat). Error bars indicate 95% confidence intervals. *S. taeniopterus* had a significant difference between sand and reef habitats, whereas *S. vetula* did not have a significant difference in mean maximum defecation distance between sand and reef habitats

difference in mean maximum defecation distance between sand reef habitats (t=1.3, p=0.2; Fig. 4).

Lab observations

Of the fecal samples (n=5) taken for both *S. taeniopterus* and *S. vetula*, all were found to have live algal fragments and sponge spicules (Fig. 5). Zooxanthellae were also found in at least one sample of both species.

During data collection, all *S. taeniopterus* and one *S. vetula* individuals in the reef habitat were found to forage in the water column on a unique food source. Once this activity was recognized, a sample of the food source was taken to the lab and identified as a polychaete in the family Maldanidae (Fig. 6). *S. taeniopterus* individuals frequently entered the water column to forage, and consumed between 1-6 Maldanids during each trial.

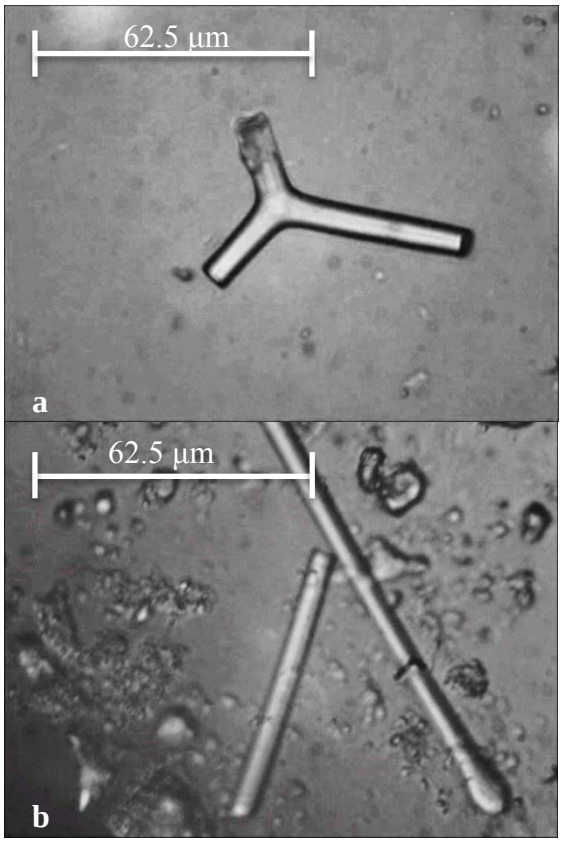

Fig. 5 Examples of sponge spicules found in feces under 1000x magnification of **a** *Scarus vetula* **b** *Scarus taeniopterus*

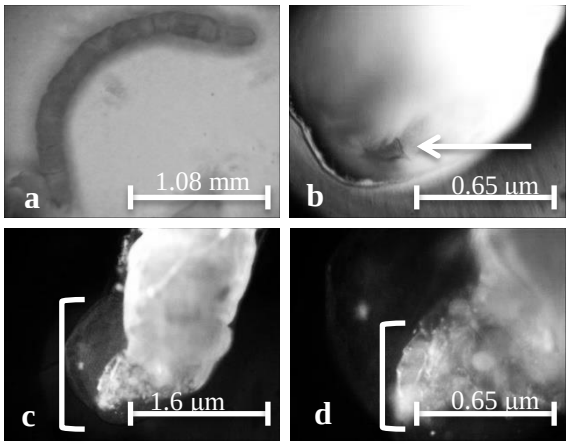

Fig. 6 Pictures samples showing **a** Maldanids under a dissecting microscope with 7x magnification, bottom left end of Maldanid appeared to be torn, as did all Maldanid samples **b** An unknown structure of a Maldanid, seen in with a *white arrow* under a microscope with 100x magnification. **c** One end of a Maldanid with an unknown structure highlighted with *white brackets*, where part of the structure appeared translucent under 40x magnification **d** A closer view at the unknown structure in c under 100x magnification, where a jagged, plated structure can be seen with *white brackets*

Table 1 Two-way analysis of variance (ANOVA) table for defecation frequencies of *Scarus taeniopterus* and *Scarus vetula* between reef and sand habitats

Source of Variation	SS	DF	MS	F	P-value
Species	0.005	1	0.005	0.312	0.584
Habitat	0.145	1	0.145	10.009	0.006
Interaction	0.040	1	0.040	2.805	0.113
Within	0.231	16	0.014		

Table 2 Two-way analysis of variance (ANOVA) table for bite frequencies of *Scarus taeniopterus* and *Scarus vetula* between reef and sand habitats

Source of Variation	SS	DF	MS	F	P-value
Species	13.530	1	13.530	0.472	0.617
Habitat	16.290	1	16.290	0.568	0.589
Interaction	28.680	1	28.680	1.000	0.500
Within	657.859	16	41.116		

Table 3 Two-way analysis of variance (ANOVA) table for substrates defecations were made on for *Scarus taeniopterus* and *Scarus vetula* within the reef habitat

Source of Variation	SS	DF	MS	F	P-value
Species	1.250	1	1.250	1.613	0.222
Location	6.050	1	6.050	7.806	0.013
Interaction	0.050	1	0.050	0.065	0.803
Within	12.400	16	0.775		

Table 4 Two-way analysis of variance (ANOVA) table for maximum defecation distance of *Scarus taeniopterus* and *Scarus vetula* between reef and sand habitats

Source of Variation	SS	DF	MS	F	P-value
Species	497.503	1	497.503	3.559	0.077
Habitat	1525.131	1	1525.131	10.912	0.004
Interaction	135.981	1	135.981	0.973	0.339
Within	2236.349	16	139.772		

Discussion

While some findings supported original hypotheses, other findings during this study were unexpected. When fecal content was observed under a microscope, live algal samples and zooxanthellae could be seen, which supports studies that described both algae and zooxanthellae surviving Scarid ingestion (Castro-Sanguino and Sanchez 2011; Vermeij et al. 2012). Additionally, sponge spicules were observed in all fecal samples. While sponges are not generally described as a food source for Scarids, several individuals observed in the present study took bites of turf algae from sponge.

In addition to finding sponge spicules in feces, it was also found that *S. taeniopterus* were frequently observed to forage in the water column. While individuals occasionally ate a free-floating alga, *S. taeniopterus* seemed to specifically target dead polychaetes floating in the water column. Polychaete samples were taken during surveys, and lab observations determined that they belonged to the family Maldanidae. Although *S. taeniopterus* frequently participated in this behavior, *S. vetula* were only observed to forage on Maldanids in the water column once. Additionally, *S. taeniopterus* individuals were observed consuming half of a ~4-cm dead or dying silver fish in the water column. The fish remains could not be taken back to the lab to analyze because the individual resided below program depth limits. These observations may suggest that *S. taeniopterus* are

opportunistic omnivores and have a broader dietary plasticity outside of macroalgae preference.

Analysis of defecation behavior for *S. taeniopterus* and *S. vetula* showed that habitat was a significant factor explaining the mean defecation frequency and range for *S. taeniopterus*, but not *S. vetula*. This supports the initial hypothesis that species would display a significantly smaller defecation frequency and range in the reef habitat than the sand habitat, but only for *S. taeniopterus.* It was also found that neither *S. taeniopterus* nor *S. vetula* defecated on live coral during this study. All defecations were made on sand and dead coral, but *S. taeniopterus* was the only species found to defecate significantly more on sand than dead coral. These observations support the theory that herbivorous fish species such as *S. taeniopterus* may have an evolutionary adaptation to separate excess sedimentation and microorganisms that may be present in their feces away from their food sources (Krone et al. 2008; Vermeij et al. 2012). Although it may be advantageous to specify certain areas for defecation for fish species, a minimized defecation range in the coral reef habitat may not support the idea that Scarids are vectors for organismal dispersal, especially when defecations are concentrated on sand substrates where reestablishment of algal fragments may be difficult.

S. vetula did not show the same behavioral changes between habitats. Habitat was not a significant factor explaining the mean defecation frequency and range of *S. vetula*, which rejects the first hypotheses that defecation frequency and defecation range would be significantly smaller on reef habitats than sand habitats. Different S. *vetula* individuals varied greatly in their maximum defecation distances and defecation frequencies in both habitats. While some seemed to defecate in a specific spot within the sand habitat, others defecated sporadically over their entire foraging territory. Similarly, where some may have only defecated twice on the coral reef crest within a few centimeters of each other, others defecated many times over their entire territory. Although these results are not consistent with the hypothesis, these results may show that *S. vetula* play a larger role as vectors for organismal dispersal in coral reef habitats than *S. taeniopterus.* Vector roles may be more relevant if organisms are spread over a greater distance, as seen in terrestrial plant species that use herbivores to increase the dispersal range of their offspring (Howe and Smallwood 1982; Tiffney 2004). Additionally, perhaps vector roles would be more relevant if defecations were made on more favorable substrates than sand for algal growth, as seen in the frequency of defecations made on dead coral by *S. vetula*.

A possible explanation for behavioral differences between *S. taeniopterus* and *S. vetula* may be that they were found in different microhabitats within the reef habitat. While *S. taeniopterus* individuals were found from the reef crest and all the way down the reef slope, *S. vetula* individuals were consistently found within the reef crest specifically. Even when *S. vetula* swam deeper within the reef slope, the time spent within the reef slope lasted roughly a minute before the individual would return to the reef crest. If *S. vetula* only inhabit a small portion of the reef, there is less available space among the reef for *S. vetula* individuals to define their territories. Therefore, the rest of the *S. vetula* population might be pressured to define their territory within the sand flat habitat where more space is available. *S. taeniopterus* may not face this problem because individuals will define their territories anywhere within the reef habitat. If most of the population is found within the reef habitat, it may be that *S. taeniopterus* is more of a reef specialist and consequently, there is more selective pressure for them to have specific

defecation behavior. Whereas *S. vetula* may be more of a sand flat specialist, and there is no selective pressure for specific defecation behavior. If *S. taeniopterus* and *S. vetula* are habitat specialists, behavioral differences between habitats would be expected. In the sand flat habitat, dead *Porites* spp. rubble and conspicuous objects (e.g. sunken boats, concrete slabs, mooring blocks) are present, but sand dominates the habitat. Spending time to swim to a specific area within their territory before defecating is energetically costly. Because sand is so readily available for individuals to defecate on at any time in the sand flat habitat, it is reasonable that both *S. vetula* and *S. taeniopterus* individuals with territories defined in the sand flat habitat would avoid spending time and energy to specify an area away from where they forage, because the chances of defecations landing on sand are very likely. In the reef habitat, however, the benefits of specifying an area to defecate away from foraging areas within the reef habitat may outweigh the energetic costs of travelling to a specific area before defecating for *S. taeniopterus.* If *S. taeniopterus* are reef specialists, it is likely that there was a selective pressure for *S. taeniopterus* to develop a behavioral change to separate their feces from the live and dead coral heads that they forage on. Decreasing the amount of energetically costly trips to their defecation area may counteract the energetic costs of traveling to a specific area within their territory, which may additionally explain why defecation frequency decreases significantly for *S. taeniopterus* in the reef habitat.

Another behavior that was observed (but not part of this study) was the minimum distance between a feeding area and the area of defecation. If species are non-randomly defecating in certain areas to avoid the spread of disease and microbes around algae assemblages (Krone et al. 2008; Vermeij et al. 2012), quantifying the separation between foraging area and defecation area may reveal more insight to evolutionary advantages to separating areas. While this variable was not measured, it was observed that both species of parrotfish defecated while eating on multiple occasions. Parrotfish individuals of both species also defecated between locations they ate, indicating that defecation occurred within their feeding territory. This observation may therefore imply that *S. taeniopterus* and *S. vetula* do not leave their foraging territory to defecate, as described in other herbivorous fish species (Vermeij et al. 2012).

Other explanations for the trends seen in this study may be the small sample size of each species surveyed. It is possible that if there were more than five replicates of each species in each habitat, there may have been a different outcome for the insignificant results found for *S. vetula.* Perhaps the *S. vetula* individuals that had frequent and wide defecations within their territories were outliers from the rest of the population. Similarly, perhaps the *S. taeniopterus* individuals that were observed in this study defecated in the same area within their territory by chance. Longer trial times and more observations of *S. taeniopterus* individuals may reveal a greater variation in their defecation behavior. However, previous studies with more replicates (n>100) have similarly shown differences in defecation behavior between two species on the Great Barrier Reef (Bellwood 1995). Bellwood (1995) observed that *C. gibbus* appeared to have a significant preference in defecation location, but *Chlorurus sordidis* did not display the same preference within the same habitat range (Bellwood 1995). While Bellwood (1995) speculated that the differences in *C. gibbus* and *C. sordidis* were a result of differences in territoriality, it is unclear if the same assumption can be made between *S. taeniopterus* and *S. vetula* as territorial behavior was not measured in this study.

It also appeared that *S. taeniopterus* might seek cover in the reef habitat before defecating. In addition to defecating significantly more on sand than dead coral, it was occasionally observed that the *S. taeniopterus* individual would swim beneath a coral shelf before defecating on sand, and stayed there for several seconds before and after defecation. Scarid species have been observed in previous studies to settle on the bottom under at least partial cover where they remain quiet throughout the night (Hobson 1965), including *S. taeniopterus* but not *S. vetula* (DeLoach and Humann 2003). Seeking cover during the night is a known behavior for *S. taeniopterus*, but the process in which they choose these territories is not well understood. It is possible that the behavior observed in the present study is a strategy displayed by *S. taeniopterus* to test areas necessary for survival while sleeping. Perhaps defecating in these regions is a strategy for designating these areas with partial cover to return to at night.

Unique defecation behavior was also observed when *S. taeniopterus* displayed what appeared to be territorial behavior, defined in this study as streaming. Streaming only occurred when *S. taeniopterus* individuals chased conspecifics out of their foraging range. Studies have explored a variety of territorial behavior displayed by Scarids (Buckman and Ogden 1973; Mumby and Wabnitz 2002), although none described streaming as one of these behaviors. However, marking territory through excretion is not a novel idea. Marking territory through defecation and urination has been well documented in terrestrial animals (Rosell and Sundsdal 2001; Zub et al. 2003). Yet, there are a lack of studies examining territory marking through this behavior in marine environments. Examination of feces content of streams and non-stream defecations may reveal chemical differences in which *S. taeniopterus* use to define their territories.

This study demonstrates that different species, even those displaying similar lifestyles and foraging types, cannot be pooled together and generalized for similar roles within the coral reef ecosystem. While herbivorous fish play significant roles in maintaining coral reefs by selectively foraging on macroalgae, their behaviors, distribution, and ecological roles may vary greatly. Even within scraping Scarid species, *S. taeniopterus* and *S. vetula* display significantly different defecation behavior and their ecological roles among coral reefs should be considered separately.

It appears that while *S. taeniopterus* and *S. vetula* are frequently found in the Western Tropical Atlantic, documentation of their ecological relationships are lacking. Time, space, and resources limited the present study, and may be the reason for insignificant results. In order to more definitely determine these ecological relationships, future studies should take place over a longer period of time, survey a wider range of territory, and include time of day to further understanding of behavioral changes by environment. Through studying the defecation range and specificity to substrate and habitat type, continued inferences can be made of the ecological roles of Scarid species perform in marine habitats. Such roles are fundamental for ecological success of cohabiting species within coral reef ecosystems, and overall health of coral reefs.

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REPORT

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Factors affecting the distribution and abundance of *Tripneustes ventricosus* on Kralendijk's waterfront

Abstract Coral reefs are being threatened by both anthropogenic and environmental factors. One prominent factor is the loss of herbivores on coral reefs due to overfishing and disease. Herbivores play a key role in coral reef ecosystems by grazing away competing algae that threaten coral survivorship and recruitment. The loss of *Diadema antillarum* in the Caribbean due to an unknown pathogen has been detrimental; however, there is another species of sea urchin emerging in the reef environment: *Tripneustes ventricosus*.

Little is known about the environmental factors that influence the abundance and distribution of *T. ventricosus* in the reef environment. This study seeks to quantify the distribution and abundance of a particular population of *T. ventricosus* on the back reef found along the waterfront of Kralendijk, Bonaire, Dutch Caribbean. It also examines the effects of benthic composition and competition on the distribution of *T. ventricosus*. In order to examine these factors, 30-m transects were laid at three different distances from the waterfront wall at five sites. Quadrats were placed at 5-m intervals along each transect. Within each quadrat, algal, benthic, and sea urchin composition was observed and recorded. Water samples were taken from each site to test nitrogen concentration. The diversity and abundance of algae and substrate type appear to be factors influencing *T. ventricosus'* distribution and abundance; competition did not appear to be a factor. The area of the abundance of *T. ventricosus* seems to have ideal conditions for *T. ventricosus*, and many other urchins, including *D. antillarum*, that were observed at this site indicating that it may be an ideal place for sea urchins in general.

Keywords *Tripneustes ventricosus* • West Indian sea egg • Coral reef • Distribution

Introduction

Coral reefs are declining at an alarming rate around the world due to both human impacts and changing environmental conditions (Hughes 1994; Bellwood et al. 2004; Burkepile and Hay 2008; Thyresson et al. 2011). Coral reefs are complex ecosystems with many different trophic levels playing key roles in maintaining health and stability. Herbivores are a key part of the trophic system because they support coral survivorship and recruitment by removing competing algae (Burkepile and Hay 2008). These herbivores are also threatened because of overfishing practices (Thyresson et al. 2011; Hughes 1994). Although protection of herbivores cannot prevent degradation, it can delay coral loss in stressful environments (Edwards et al. 2010).

Sea urchins (Echinodermata: Echinoidea) are important herbivores in coral reef systems (Ogden and Lobel 1978; Moses and Bonem 2001). Historically, *Diadema antillarum* was one of the most prominent and abundant sea urchins in the Caribbean (Williams 1981; Hughes 1994;

Moses and Bonem 2001). Since their decline in 1983 due to an unknown pathogen, they have been slow to recover, and their continued absence has threatened the health of coral reefs (Miller at al. 2007; Lacey et al. 2013; Moses and Bonem 2001). According to Moses and Bonem (2001), *Tripneustes ventricosus* used to distinguish its territory from the abundant *D. antillarum* by residing in the *Thallasia* sea grass beds while *D. antillarum* preferred patch reefs. Since the mass mortality of *D. antillarum* in the Caribbean, there has been a widespread change in *T. ventricosus'* population and distribution to the reef habitat (Woodley et al 1999; Woodley 1999). This could suggest competition between these two herbivores. *T. ventricosus* is ecologically important because sea urchins are key herbivores on the reef (Moses and Bonem 2001). They are also economically important food source in the Caribbean (Maciá and Robinson 2008).

The expansion of *T. ventricosus* may be beneficial to both their population and the reef environment. It has been shown that macroalgae found on the reef is more nutritionally beneficial than the sea grass *Thalassia* and contribute to faster growth rates (Maciá and Robinson 2008). In return, increasing herbivore species richness in the coral reef environment can play a major role in maintaining structure and function (Burkepile and Hay 2008). The loss of *D. antillarum* along with important herbivorous fish on the reef has contributed to the current phase shift from a coral-dominated ecosystem to an algae-dominated system (Hughes 1994). This species of sea urchin has the potential to become the major herbivore in the coral reef environment and aid in the natural preservation and recovery of corals. Understanding the habitat variables on a coral reef that affect *T. ventricosus'* abundance and distribution is important for preserving and conserving their population for the future.The expansion of *T. ventricosus'* population to the reef habitat is evident in a back reef environment on Kralendijk, Bonaire's waterfront. There is one area of the waterfront, approximately 300 meters in length, with a substantial population of *T. ventricosus*.

This study looks at that population's abundance and distribution as well as factors affecting these variables. Algal, benthic, and nitrogen composition and abundance were looked at within the main distribution of *T. ventricosus* and compared to the compositions outside of this distribution. Algae is the primary food source of *T. ventricosus* on the reef (Maciá and Robinson 2008), and substrate has been shown to influence algae distribution and abundance (Sabater et al. 2002, Potapova and Charles 2005). There must be ideal substrate for algae to grow on to support a large population of herbivores. It has been shown that *T. ventricosus* prefers macroalgae such as *Dictyota cervicornis* and *Galaxaura oblongata* as opposed to the seagrass *Thalassia testudinum*, however there was no difference in *T. ventricosus'* preference between the two macroalgae (Maciá and Robinson 2008). Though there was no preference between these two macroalgae, there is the possibility that *T. ventricosus* does have macroalgal preferences for different species or composition of species if algal diversity and abundance is higher. Nitrogen concentration is a limiting factor for algae, and the productivity of the ecosystem within the main *T. ventricosus* distribution may play a role in supporting algal abundance and therefore sea urchin abundance (Hatcher and Frith 1985). Presence and abundance of *T. ventricosus* and *D. antillarum* were also recorded at each site to determine if there was any competition between the two.

The following hypotheses were tested:

H_1: Macroalgal and benthic composition found within the distribution of *T. ventricosus* is different than the composition

found outside of the population's distribution

H_2: There is a higher concentration of ammonium, nitrate, and nitrite within the main distribution of *T. ventricosus* compared to the outside sites.

H_3: There will be a different abundance of *D. antillarum* found within the distribution of the *T. ventricosus* population than that found outside the distribution.

Materials and methods

Study site

This study was conducted on the waterfront of Bonaire's capital, Kralendijk. This area is approximately two kilometers long and is characterized by a road and a boardwalk that run parallel to the coastline. There is a wall that separates the land from the water; this was used as a reference point for the transects that were laid. Two sites inside the main distribution and three sites outside the main distribution of *T. ventricosus* were selected using a random number generator in Microsoft Excel. These numbers corresponded to a certain kilometer distance down the waterfront using Google maps (From north to south: Site 1: 12.16133 N, 68.283103 W, Site 2: 12.159463 N, 68.28125 W, Site 3: 12.155976 N, 68.279209 W, Site 4: 12.15402 N, 68.278437 W, Site 5: 12.152588 N, 68.277927 W).

Population abundance and distribution

To determine the main distribution and abundance of *T. ventricosus* along the waterfront, a preliminary walking survey was conducted. The waterfront was observed from its beginning until the end of the commercial shipping dock. There was a noticeably higher concentration of *T. ventricosus* observed between the recreational pier and the restaurant Karel's (12.154151 N, 68.278501 W, 12. 151817

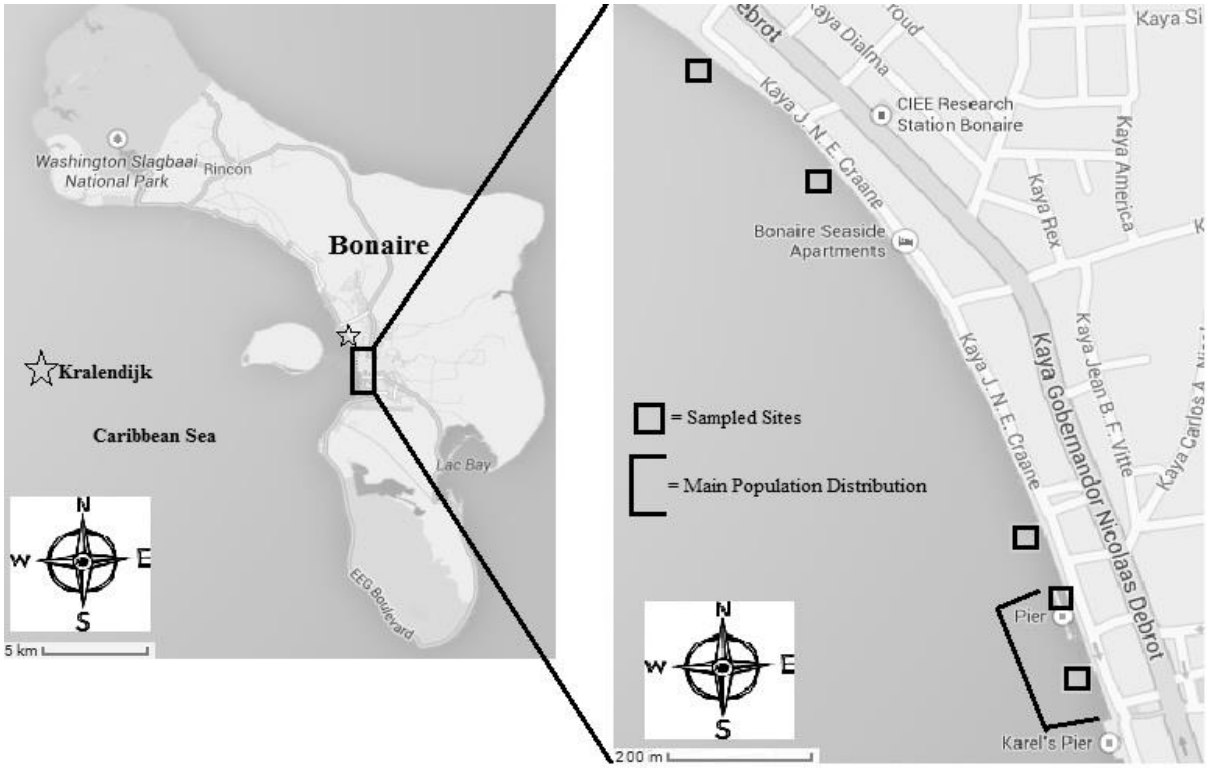

Fig. 1. Map of Kralendijk's waterfront and the study sites on the western side of Bonaire, Dutch Caribbean. "Main Population Distribution" refers to the main local population of *Tripneustes ventricosus* and is defined by the *black bracket* (modified from Google Maps)

N, 68. 277723 W; approximately 300 m) than anywhere else in the surveyed area. These observations were used to determine where the main population of urchins resided. Areas falling outside this site area were classified as outside the main distribution.

Algal, benthic, and sea urchin surveys

Possible factors affecting the abundance and distribution of the *T. ventricosus* were examined using the transect/quadrat method. Factors measured included algal and benthic composition and *D. antillarum* abundance. At each site, three 30-m transects were laid parallel to the waterfront wall at 1-m, 5-m, and 10-m distance from the wall (Fig. 2). A 1-m^2 quadrat was placed at the 5 m, 10 m, 15 m, 20 m, 25 m, and 30 m marks along each transect line. The algal composition, benthic composition (e.g. sand, rock, coral rubble, pavement), and presence and abundance of *D. antillarum* and *T. ventricosus* were recorded in each quadrat. For algal and benthic composition, point intercepts in 10-cm intervals within each quadrat were used to record the algae and substrate directly underneath. The total number of *T. ventricosus* and *D. antillarum* present in each 1-m^2 area were recorded regardless of position. All other sea urchin species in the quadrat were noted but not quantified.

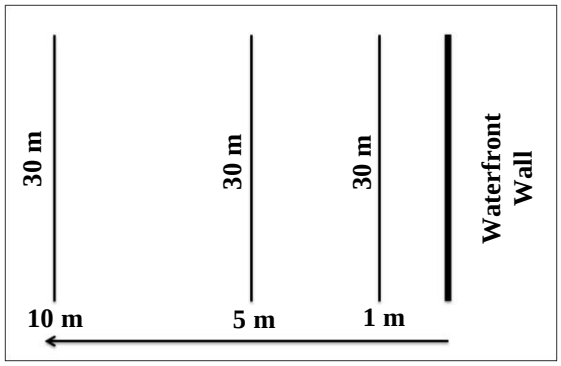

Fig. 2 Model of how transects were laid in respect to the waterfront wall at each site. The transects were all 30m in length. 1m, 5m, and 10m indicate the distances from the wall that each transect was laid

Water quality

To determine if the productivity of each site had an effect on *D. antillarum* and *T. ventricosus* presence, water was collected from the 15-m mark on the transect laid 5-m from the waterfront wall. LaMotte water quality tests for ammonia, nitrate, and nitrite were performed in the laboratory.

Data analysis

Comparative statistical analyses were done for the algal and benthic composition and sea urchin abundances. These analyses used averages of algal and benthic composition and *D. antillarum* and *T. ventricosus* abundance within the quadrats for each 1-m, 5-m, and 10-m transect at each site. The variables of algal and benthic composition and *D. antillarum* abundance were then compared to *T. ventricosus* abundance at each site using bar graphs to examine any trends (See Appendix). Linear regressions were performed comparing the average density of *T. ventricosus* to average relative percent sand cover, average algae cover, total number of algal species present, and average *D. antillarum* density per m^2 for each transect. A linear regression was also performed to compare the average percent of hard substrate to the average algae cover for each transect. The water quality tests were compared graphically (See Appendix) and with a linear regression comparing ammonia concentration at each site to the average percent algae cover at each site.

Results

Substrate

As the average percent of hard substrate increases, the average algae cover significantly increases on each transect (Fig. 3, F= 7.34, p= 0.02).

Though linear regression shows that there is not a significant negative correlation between average relative sand density and the average *T. ventricosus* density per m^2 (R^2= 0.124, F=1.83, p= 0.20), a clear trend can be seen from the graph: all the *T. ventricosus* found were in areas with less than 10% average sand density (Fig. 4).

Algae abundance

Though there was not a significant correlation between average percent algae cover and the average density of *T. ventricosus* per m^2, there is a trend showing that as the average percent algae cover increases, so does the average density of *T. ventricosus* per m^2 (Fig. 5, F=1.68, p=0.23).

Algal species diversity

The total number of algal species present is positively correlated with the average *T. ventricosus* density per m^2 (R^2= 0.567). A linear regression demonstrated that the total number of algal species present had a significant effect on *T. ventricosus* density (Fig. 6, F=17.03, p= 0.001).

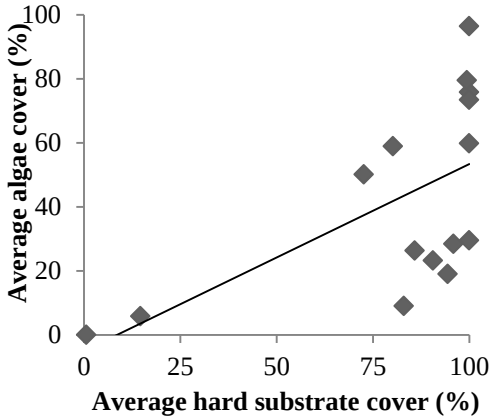

Fig. 3 Average hard substrate compared to the average algae cover for each transect at each sampled site on the western side of Bonaire, DC (n=15) R^2=0.3607

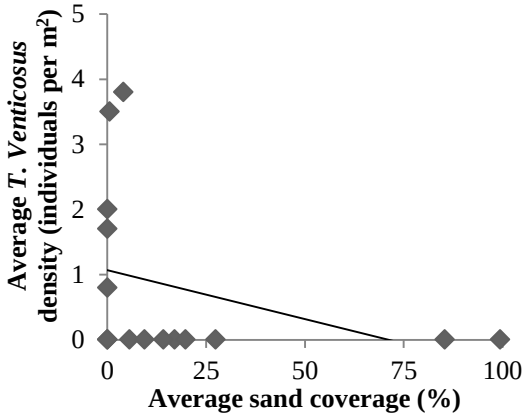

Fig. 4 Average sand coverage compared to average *Tripneustes ventricosus* density (individuals per m^2) on each transect for each site sampled on the western side on Bonaire, Dutch Caribbean (n=15) R^2=0.1236

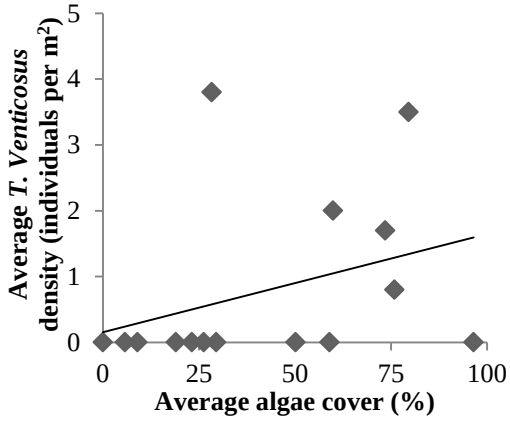

Fig. 5 Average algae cover compared to the average *Tripneustes vetricosus* density (individuals per m^2) on each transect at each sampled site on the western side of Bonaire, DC (n=15) R^2=0.1149

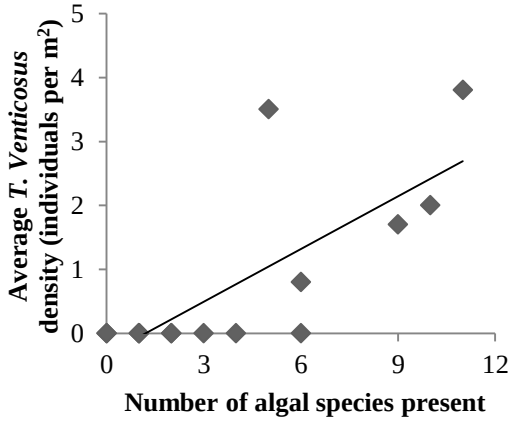

Fig. 6 Total number of algal species observed compared to the average *Tripneustes ventricosus* density (individuals per m2) present along each 1-m, 5-m, and 10-m transect for all sites (n=15) R^2=0.5670

Nitrogen concentration

The amount of ammonia was not significantly correlated with the average percent algae cover at each sampled site, however there appears to be a positive trend between the two (Fig. 7, F= 2.95, p= 0.18).

D. antillarum abundance

The densities of *D. antillarum* and *T. ventricosus* do not appear to be correlated; however, there is a positive trend between the two (R^2= 0.289) A linear regression demonstrated that the average *D. antillarum* density per m^2 had a significant effect on average *T. ventricosus* density per m^2 (Fig. 8, F=5.27, p=0.04).

Discussion

Substrate, algae abundance and diversity, nitrogen concentration, and the presence of *D. antillarum* all appear to be positively correlated with *T. ventricosus* abundance. Compared to the outside sites, these variables combined suggest that the sites inside the main population distribution of *T. ventricosus* seem to have the most favorable conditions to allow a large population to thrive on the back reef of Kralendijk's waterfront (See Appendix).

Macroalgae is a beneficial food source of *T. ventricosus* in the reef environment (Maciá and Robinson 2008). There was a strong relationship between the presence of hard substrate and increased algae coverage found on each transect (Fig. 3, F=7.34, p= 0.02). It has been shown that different species and abundances of algae can occur on varying substrate types (Sabater et al. 2002, Potapova and Charles 2005). Pavement, rock, and coral rubble are hard substrates capable of supporting algae in the back reef environment (Potapova and Charles 2005), and each were observed with varying algal species and abundances (See Appendix). There was a higher diversity and abundance of hard substrate and algae observed within the main distribution sites compared to outside (See Appendix). There were no clear trends indicating that *T. ventricosus* favors a certain type of hard substrate; however, there is a trend showing that *T. ventricosus* appears to avoid sand as a substrate (Fig. 4). No algae were observed growing on sand during the data collection, and this could explain why *T. ventricosus* doesn't favor sand substratum.

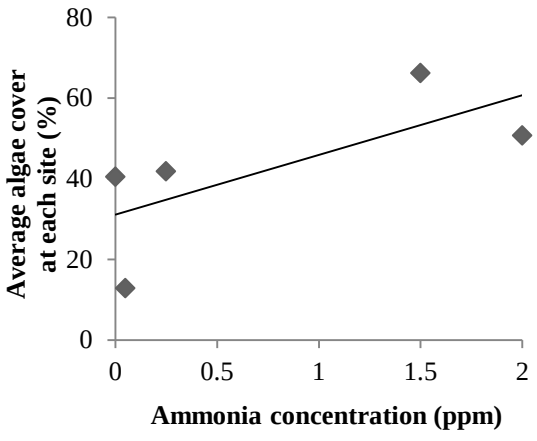

Fig. 7 Ammonia concentration compared to the average algae cover (%) at each site on the western side of Bonaire, DC (n=5) R^2= 0.4958

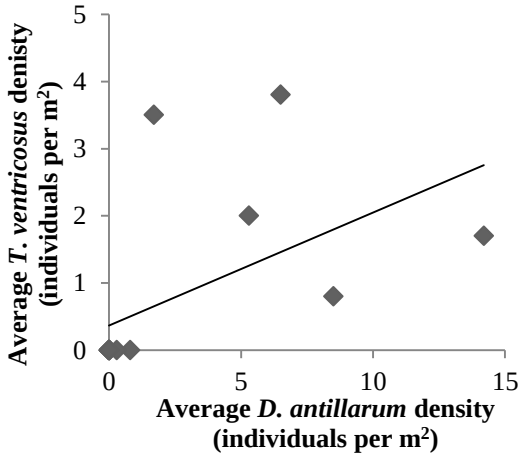

Fig. 8 Average *Diadema antillarum* density (individuals per m^2) compared to the average *Tripneustes ventricosus* density (individuals per m^2) from all transects of all sites surveyed on the western side of Bonaire, DC (n=15) R^2=0.2886

There was no significant correlation between algae cover and the abundance of *T. ventricosus* (Fig. 5, R^2=0.1149 p= 0.23). This could be due to other variables influencing the presence and abundance of *T. ventricosus* such as the availabitlity of suitable covering materials. Sea urchins residing in shallow waters, such as *T. ventricosus*, have been shown to cover themselves with material from the substratum (Millot 1975) to protect themselves from light and UV wavelengths (Verling et al. 2002), stablize the urchins in surge (James 2000), and protect them from deposits of mud and sand (Richner and Milinski 2000). These are all factors that *T. ventricosus* must deal with in the shallow, back reef environment along the waterfront. In the field, *T. ventricosus* was observed covering itself with coral rubble, small rocks, leaves, anthropogenic material such as bottle caps, and different species of macroalgae. These items, expecially coral rubble, rocks, and macroagle were abundant within the main population distribution area (See Appendix).

In addition, the number of algal species present was significantly correlated with the density of *T. ventricosus* (Fig. 6 p=0.001). Algae diversity may not only provide a varity of food sources to *T. ventricosus*; different macroalgal species may be ideal covering materials for *T. ventricosus* on the reef. Future studies could examine if *T. ventricosus* has a preffered substrate or algal covering material. Postive trends favoring the abundance and diversity of algae, along with the fact that algae coverage is significantly correlated with hard substrate (Fig. 3), could explain why *T. ventricosus* favors this particular area along the waterfront. In this area, algae diversity and abundance were higher in the sites inside *T. ventricosus'* main population distribution (See Appendix).

Ammonia concentration also appears to be positively correlated with algal abundance, however these results were not statistically significant (Fig. 7, p= 0.18). This could be due to the fact that this test was only performed once due to time constraints. However, there was a noticeable difference between the amount of nitrogen within the main population distribution compared to outside (See Appendix). Nutrients composed of nitrogen, such as ammonia, are necessary for primary production (Hatcher and Frith 1985, Anderson et al. 2002), and higher concentrations of these nutrients are correlated with higher primary production in the area and increased numbers of primary consumers (Tewfik et al. 2007). Further research should be done to investigate if nitrogen levels are different along Kralendijk's waterfront and if they are influencing algal abundance.

D. antillarum does not appear to restrict *T. ventricosus'* population, which is supported by the linear regression comparing the average *D. antillarum* density to average *T. ventricosus* density (Fig. 8, F=5.27, p=0.04). As *D. antillarum* density increases, *T. ventricosus* density also increases and there is no statistical difference between their abundances (See Appendix). *D. antillarum* does not appear to have an influence on *T. ventricosus'* distribution and abundance. Furthermore, at least four other sea urchin species, *Echinometra viridis*, *Echinometra lucunter lucunter*, *Eucidaris tribuloides*, and *Lytechinus variegatus*, and many herbivorous fish were observed in the main distribution area of *T. ventricosus*, including *Scarus vetula*, *Scarus taeniopterus*, and juvenile *Pomacanthus paru*. This could be another indicator of higher productivity in the area between the recreational pier and Karel's bar and the microhabitat's ability to support a higher diversity and abundance of species. It would be an interesting study to examine the total diversity and abundance of sea urchins and herbivores in general in this area.

There appear to be numerous factors that are playing a role in influencing the

distribution and abundance of *T. ventricosus*. Understanding these factors is important because these urchins have the potential to be the next main herbivores on the reef in the absence of *D. antillarum* (Woodley 1999; Woodley et al 1999). High impact herbivores, such as sea urchins (Ogden and Lobel 1978; Moses and Bonem 2001), are needed to maintain the health and stability of coral reefs (Edwards et al. 2010). They play a main role in grazing away the algae that reduces coral growth and recruitment (Burkepile and Hay 2008). With decreasing numbers of herbivorous fish due to overfishing (Thyresson et al. 2011; Hughes 1994), the presence of herbivorous invertebrates is vital for maintaining and protecting coral reefs. The natural processes that control their abundance and distribution must be studied and understood. Further research should be done on feeding and substrate preferences on *T. ventricosus* to better understand what influences their distribution and abundance in the reef habitat. Protecting and conserving key herbivores on the reef is paramount to protecting and conserving coral reefs for the future.

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Appendix

Benthic transect 1 m from wall

There appeared to be a difference in benthic, algal, and sea urchin density on the 1-m transect between sites. Benthic composition for sites C, D, and E contained rock while A and B did not (Fig. A1 A). Sites A and B also had a higher average density of rubble compared to sites C, D, and E (Fig. A1 A). Sites C, D, and E had a greater variety of algae compared to sites A and B (Fig. A1 B). However, algal composition appeared to vary between sites C, D, and E; there was a higher density of bare substrate with no algae (specified as "None") at site D compared to sites C and E (Fig. A1 5 B). Finally, sea urchins were present in sites C, D, and E and absent in sites A and B. There did not appear to be a significant difference between *D. antillarum* and *T. ventricosus* in the sites where they were present (Fig. A1 C).

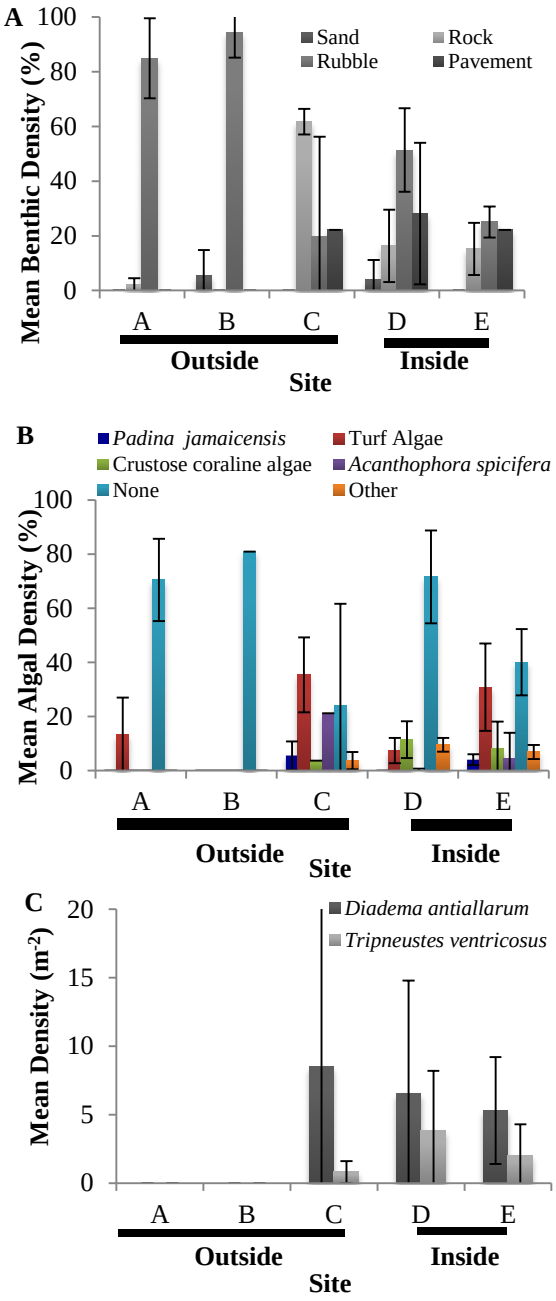

Fig. A1 Mean (± SD) benthic (A) and algal (B) composition and sea urchin abundance (C) on the transects 1 m from the wall for each site. Sites A-E correspond to distance down the waterfront (from North to South) of Kralendijk, Bonaire, DC (Fig. 1). "Outside" refers to outside the main population distribution, and "Inside" refers to inside the main population distribution. "Pavement" (A) describes an extension of the waterfront wall or any other manmade structure composed of rock

Benthic transect 5 m from wall

There was a difference seen in benthic, algal, and sea urchin density at the

5-m transect between sites. Sites B, D, and E had a higher prevalence of rock substrate compared to sites A and C, which had a higher prevalence of rubble and contained sand (Fig. A2 A). Algal composition varied widely between sites (Fig. A2 B).

There did not appear to be a significant difference in algal composition, however sites D, B, and E had the greatest variety of algal types (Fig. A2 B). *D. antillarum* was present in sites B, C, D, and E, while *T. ventricosus* was present in only sites D and E. (Fig. A2 C). There did not appear to be a significant difference between *D. antillarum* and *T. ventricosus* (Fig. A2 C).

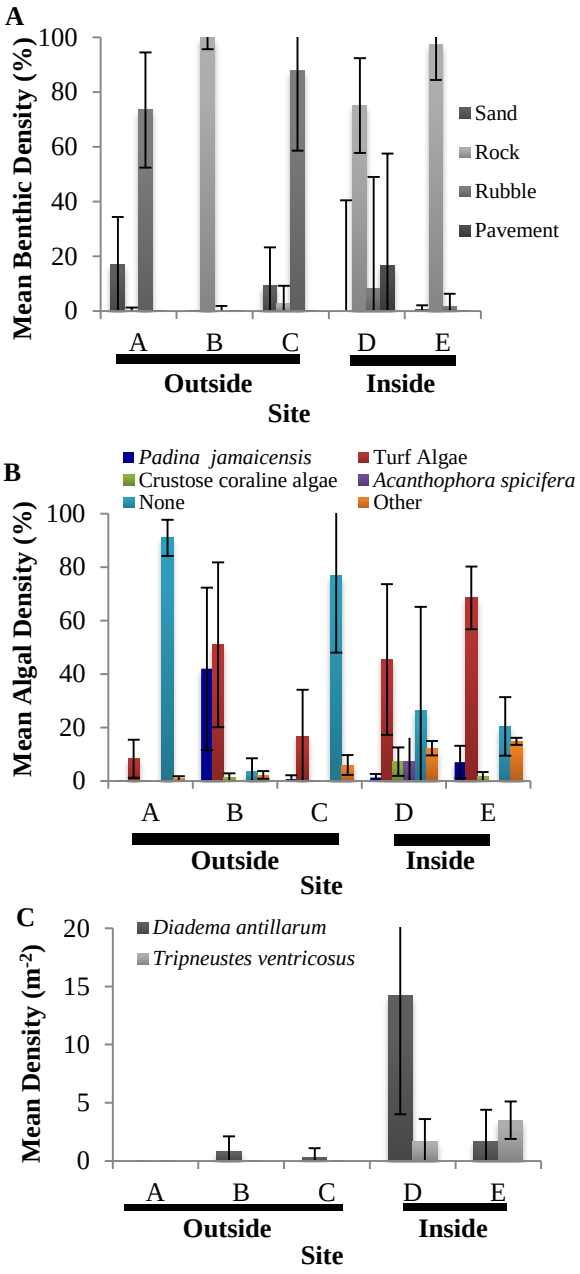

Fig. A2 Mean (± SD) benthic (A) and algal (B) cover and sea urchin (C) abundance on the transects 5 m from the wall for each site. Sites A-E correspond to distance down the waterfront (from North to South) of Kralendijk, Bonaire, DC (Fig. 1). "Outside" refers to outside the main population distribution, and "Inside" refers to inside the main population distribution. (A) "Pavement" describes an extension of the waterfront wall or any other manmade structure composed of rock

Benthic transect 10 m from wall

There was a difference in benthic, algal, and sea urchin density at the 10-m transect between sites. Sites A and B were dominated by sandy substrate while sites C and E were rubble dominated, and site D had a more equal variety of substrates present (Fig. A3 A). The majority of substrate at sites A, B, and C was bare (classified as "None"), and if algae was present at sites then it was most likely turf algae (Fig. A3 B). There were no sea urchins found at any site (Fig A3 C).

Nutrient concentrations across sites

There appeared to be a difference in ammonia concentration for the sites inside the main distribution of *T. ventricosus* compared to the sites outside (Fig. A4).

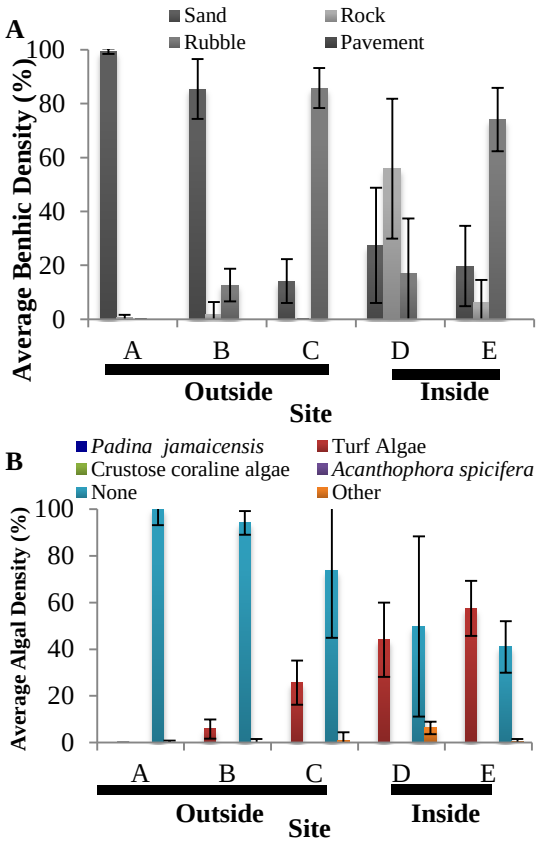

Fig. A3 Mean (± SD) benthic (A) and algal (B) cover on the transects 10 m from the wall for each site. Error bars indicate standard deviation. No sea urchins were found in any of the quadrats on the 10-m transects. Sites A-E correspond to distance down the waterfront (from North to South) of Kralendijk, Bonaire, DC (Fig. 1). "Outside" refers to outside the main population distribution, and "Inside" refers to inside the main population distribution. (A) "Pavement" describes an extension of the waterfront wall any other manmade structure composed of rock

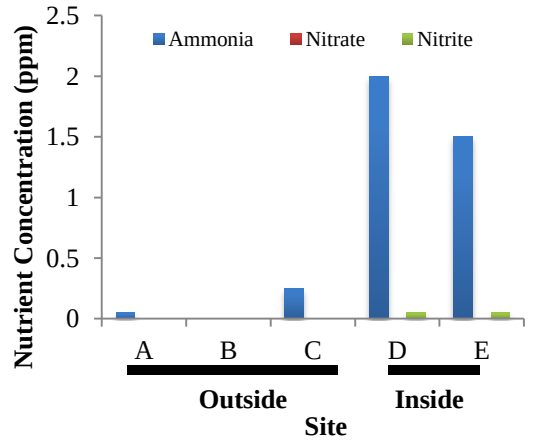

Fig. A4 Concentration of ammonia, nitrate, and nitrite outside (A,B,C) and inside (D,E) the main distribution of *Tripneustes ventricosus*

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REPORT

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The effect of predation and competition on the slow population return of *Diadema antillarum*

Abstract Populations of *Diadema antillarum* have had low densities ever since its mass mortality event in 1983. A slow population density increase has resulted from fertilization complications due to extensive distance between individuals. The relationships between *D. antillarum* and their competitors and predators as a cause for the lack of population recovery has not been directly studied. The correlation of *D. antillarum* density with the abundance of predators, competitors, and microalgae, was studied to determine additional possible explanations for the low density of individuals. There were three dives during the day at six sites. The day dives included observational fish counts and transects and quadrates to assess percent algae cover in a 10 m^2. While the night dives include observation counts of all of the urchins in the 10 m^2. No increase was found in Diadema antillarum density compared with a study in 2009 (0.005 individuals per m^2). No significant correlation was determined between *D. antillarum* density and predator density. A weak, positive correlation between competitor density and *D. antillarum* density was determined. In contrast, a strong, positive correlation between percent algae cover and *D. antillarum* density was found This study revealed additional pressures on *D. antillarum* population (e.g. competitors, percent algae cover), which could account for the slow recovery of local *D. antillarum* population in Bonaire.

Keywords *Diadema antillarum* • Coral reefs • Predation • Competition

Introduction

Before 1983, *Diadema antillarum* (Long Spined Sea Urchin) was considered a keystone species in the Caribbean (Mumby et al. 2006). After massive population morality in 1983, due to a species-specific disease affecting *D. antillarum*, the population dramatically declined. The population density of *D. antillarum* before the massive mortality event was on average 13 individuals per m^2 across the Caribbean (Randall et al. 1964). In Puerto Rico, the population fell to 0.13 individuals per squared meter after the event (Weil et al. 2005). In 2000, the population density increased to an average of 1.0 individual per meter squared in Puerto Rico and *D. antillarum* individuals were larger in size compared to before the mortality event (Weil et al. 2005). This pathogenetic outbreak in *D. antillarum* contributed to a phase-shift in the coral reefs of the Caribbean (Hughes 1994).

The slow recovery of *D. antillarum* in the Caribbean is suspected to be the result of low existing population density due to the post-mortality allee effect. *D. antillarum* on a 50-m strip in St. Thomas was completely removed to test the recovery rate of *D. antillarum* (Hay and Taylor 1985). Diadema sp. recovered to 70 percent in the course of 16 months in this study (Hay and Taylor 1985). Based off of this study, a 70% return of the original

population should be expected by 1985, 16 months after the die-off. The green sea urchin (*Strongylocentrotus droebachiensis*) was also subjected to a mortality event that resulted in zero urchins per meter squared above a depth of 20 m (Brady and Scheibling 2005). The green sea urchin two years later had a density around 70 urchins per meter squared due to a high level of recruitment and migration along a shallow subtitle zone (Brady and Scheibling 2005). If *D. antillarum* had a high level of recruitment and migration in the Caribbean the result may be a higher density than what we see now. Limited fertilization success has been causing a slow increase in the population density in *D. antillarum* (Levitan 1991). This is due to fertilization success decreasing with increasing distance of dispersal; the further the individuals are from each other, the less fertilization success they have (Levitan 1991). Like *D. antillarum*, pink abalone (*Haliotis corrugata*) has experienced a major population decline due to fishing, which has resulted in an allee effect. Pink abalone's low density, due to fishing pressures, has resulted in small aggregations composed of two individuals (Catton and Rogers-Bennett 2013). This has forced the closing of fisheries in multiple areas (Catton and Rogers-Bennett 2013). *D. antillarum* was found to retain pre-mortality genetic variability in the Caribbean (Lessios et al. 2001). The massive mortality event has resulted in an increase in the numbers of "new" genetic mutations present in the population (Lessios et al. 2001). In other words, the species has a chance of rebounding because of the genetic variability already present.

Predation and competition on *D. antillarum* has not been directly studied. Predation and competition could both be factors contributing to the slow recovery rate of *D. antillarum*. If the population of *D. antillarum* experience predation, this might cause a slow recovery in the *D. antillarum* population due to an inability to surpass the population density needed for successful fertilization. For instance, the purple sea urchin (*Parantrotus lividus*) in the Mediterranean has shown a density that is negatively correlated with predator abundance (Sala and Zabala 1996). This suggests a relationship of predation to sea urchin numbers, where the low densities of urchins could be due to high densitites of predators. It has also been observed that *D. antillarum* are mostly active during the night if there are predators in the area that hunt primarily during the day (Weil et al. 2005).

Predators of *D. antillarum* cannot consume individuals over 4 cm, thus creating an "escape size" (Clemente et al. 2007). The juvenile sea urchin, which are smaller than 4 cm, were the ones targeted for predation (Randall et al. 1964). Although it has been shown that even with juvenile *D. antillarum* being under high predation, there is still a large enough population of *D. antillarum* to prevent the population from crashing to zero (Clemente et al. 2007). Therefore, predation is a factor that influences the *D. antillarum* population. The predators that eat *D. antillarum* include triggerfish, jacks, porcupine fish, wrasses, and grunts (Randall et al. 1964).

Another aspect that may affect the *D. antillarum* population is competition intensity with other herbivores. *D. antillarum*, under healthy conditions, contribute to a healthy reef by aiding in removing 40% of the total macroalgae on the reef (Mumby et al. 2006). Macroalgae cover and *D. antillarum* are inversely correlated (Alves et al. 2001). If there is high competition for macroalgae, lower amounts of algae and a lower population density of *D. antillarum* should be observed. When *D. antillarum* was removed from a 50 m strip it resulted in an increase in parrotfish and surgeonfish grazing on that strip (Hay and Taylor 1985). 16 months after the removal, the *D. antillarum* recovered to 70% of their

original density with the competition present (Hay and Taylor 1985). Without competitor present, they could therefore recover at a faster rate.

The relationship of *D. antillarum* competition with other herbivores and existing predatory pressure has not been studied extensively to understand the underlying factors that inhibit recovery of the urchin population. The only documentation of the current Diadema sp. population in Bonaire was in Lac Bay, in a sea grass and mangrove ecosystem (Sternberg 2010). Studying *D. antillarum* would provide evidence as to why this population has not returned to its previous population density at a similar rate to that of other urchin species (e.g. green sea urchin). Thus, it is hypothesized that:

H_1: The population of *D. antillarum* is negatively correlated with the density of predators present.
H_2: The percent of algae cover is positively correlated with the density of *D. antillarum* present.
H_3: The numbers of competitors will be negatively correlated with the density of *D. antillarum*

Materials and methods

Study site and organism

This study was conducted on the Western reefs of Kralendijk, Bonaire. Three locations were chosen: Yellow Submarine (12°09'36.47"N, 68°16'55.16"W), Something Special (12°09'43.69"N, 68°17'07.79"W), and Kas di Arte (12°09'19.22"N, 68°16'45.35"W) with two sites per location, one to the north and the other to the south. Each site were located on a sloping reef and the site started 10 m from the top of the reef crest. The average maximum depth was 17 m because the AAUS maximum depth was deidicated as 17 m.

The long-spined sea urchin *(D. antillarum)* has brittle spines significantly larger than its body size. The adults have solid white, black, or alternate white and black spines while the juveniles have black and white striped spines.

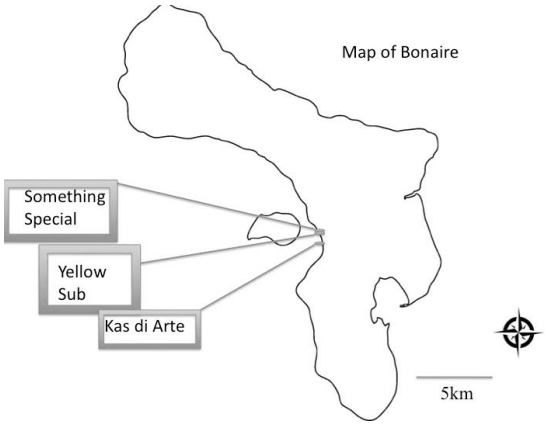

Fig. 1 Map of Something Special, Yellow Submarine and Kas di Arte where research is conducted from http://www.worldatlas.com/webimage/countrys/namerica/caribb/outline/bonaire.htm

Field research

All observations/surveys were conducted in 10x10 m plots. These plots were established during the day and marked so that the same plot could be surveyed again at night. A 10-m transect was laid out across the top of the box plot and down the slope of the reef at 90° to mark the edges of a 10-m^2 plot. The deepest point of the plot (the bottom most edge) was recorded for the night dive. Each of the observed urchin predators, competitors, and density of *D. antillarum* present during the day were recorded by all observers on SCUBA divers. An average of what all divers observed was recorded from the observational count. Fish observations took place at 8:00 for five minutes to count the predators and competitors in the 10x10 m square. During same day dive, algae percentages were calculated using our transects were laid out two meters from each other across the reef within the 10-meter plot. At 2-m intervals along the transect 0.5 quadrats at a total of 2

quadrats were surveyed for percent algae coverage. This was done by dividing a ½-m quadrat into 25 squares (10 x 10 cm^2 per square) and laying the quadrat towards the shallower side of the tape on the reef. Each square was labeled as sand/rubble, algae, dead coral (no algae), or coral/sponge based on the dominant substratum within the individual square.

At 20:00 at each site, a count of *D. antillarum* in the Plot, this was done by laying 10-m transects across the top of the plot so that the tape was visible from the deepest part of the site to mark the edges of the plot. The *D. antillarum* were counted by pacing across the reef. Each was classified in terms of their life stage (juveniles or adults) and if they were larger than 4 cm. The presence and type of other urchins was also recorded.

The height variation of the reef was taken by the use of a rugosity rope during the following weeks. Rugosity was taken three times in every plot by laying down the rugosity rope in the identical location as the three of the four transects laid across the reef.

Data analysis

D. antillarum and predator density, algae percentage, algae height, and rugosity were analyzed through the use of linear regression analysis and correlation graphs. Several linear regressions were used to examine the effect of different explanatory variables (percent algae cover, competitor density, predator density, and rugosity) on the response variable *D. antillarum* density. Each variable was paired up and the R^2 value and p-value were calculated to see if they are statistically significant.

Results

Density

Additional competitors included all other sea urchins found during the night dive, which were included in the overall competitor count (Fig. 2). The average competitor density for all of the sites was 1.92 ± 0.031 individuals per m^2 (mean ± SD). Predators of *D. antillarum* that were seen in the plot at night were also included in the density count. The overall average of the predators for all of the sites was 0.45 +/- 0.028 individuals per m^2 (mean ± SD) (Fig. 2).

D. antillarum density was observed at night in the same plot used for the competitor and predator density. The average density of *D. antillarum* for all of the sites at night was 0.05 ± 0.007 individuals per m^2 and the average *D. antillarum* density during the day was 0.005 ± 0.009 individuals per m^2 (mean ± SD). There were a total of 27 urchins observed (24 juveniles and 3 adults). Two of the 27 urchins were less than 4 cm in size. All three of the adult *D. antillarum* seen at night were also the ones seen during the day and most of the *D. antillarum* found at night were in crevices on the top of the reef.

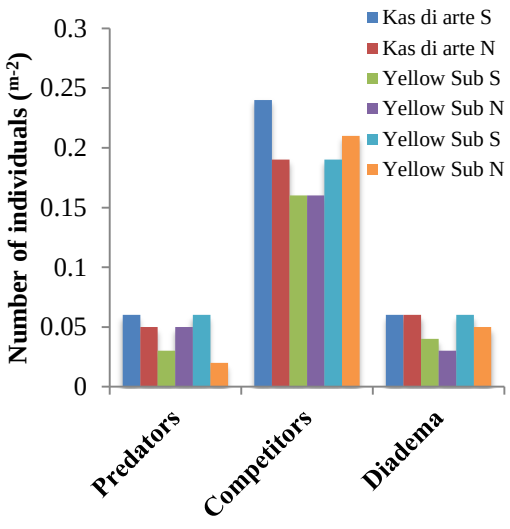

Fig. 2 Total density of competitors of *Diadema antillarum*, predators of *D. antillarum*, and *D. antillarum* in Kas di Arte, Yellow Sub, and Something Special North and South

Percent algae coverage

The average algae coverage for all of the sites was 45.76% ± 0.91 (mean ± SD). The second most dominant substrate was coral

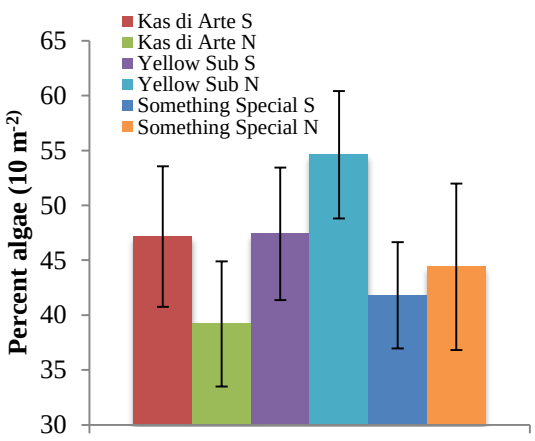

Fig. 3 Average percent algae coverage in Kas di Arte, Yellow Sub, and Something Special North and South.

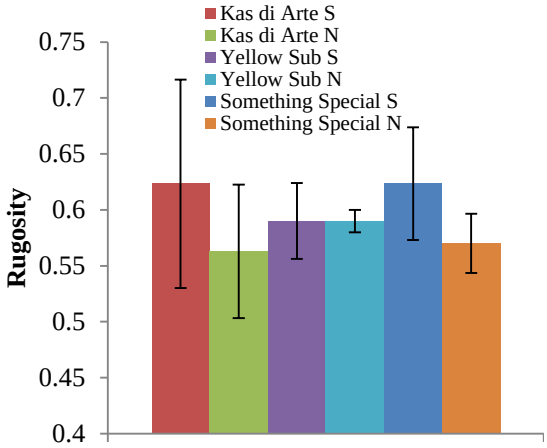

Fig. 4 Average height variation of the reef (rugosity) at Kas di Arte, Yellow Sub, and Something Special North and South. This was taken with a 10-m transect and a 10-m rugosity rope.

and sponge combined, which had an average of 28.65 ± 0.79 (mean ± SD). The third dominant substrate was sand and rubble, which had an average of 26.48 ± 1.52 (mean ± SD) (Fig. 3).

Rugosity

The average height variation of the reef was examined by the use of rugosity to determine if there is a correlation between *D. antillarum* density and competitor density with rugosity. The average rugosity of all of the sites was found to be 0.57 ± 0.03 (mean ± SD) (Fig. 4).

Correlation and linear regression analysis

The correlation and linear regression between each variable was examined to determine if the hypothesis was rejected or not. The first hypothesis stating that the population of *D. antillarum* is negatively correlated with the density of predators present, was rejected. *D. antillarum* density and predator density had no significant correlation (Table 1).

The second hypothesis stating that the percent of algae coverage is positively correlated with the density of *D. antillarum* present, was rejected. *D. antillarum* density had a strong, negative correlation with the percent algae coverage (Fig. 5, Table 1).

The third hypothesis stating that the numbers of competitors is negatively correlated with the density of *D. antillarum*. *D. antillarum* density related to competitor density was positively correlated (Table 1).

Other correlations and linear regression analyses were taken to explain the results of the hypotheses, none of which resulted in statistical significance. There was no relationship between the competitors and the percent algae coverage, *D. antillarum* density and rugosity, *D. antillarum* density and algae height, and competitor density and algae height (Table 1). There was a statistically insignificant, slight positive correlation between competitor density and rugosity (Table 1). There was a statistically insignificant slight positive correlation between juvenile *D. antillarum* density and rugosity (Table 1). There was a statistically insignificant negative correlation between juvenile density in *D. antillarum* and % the percent of algae cover (Table 1). There was no relationship between adult *D. antillarum* density and the percent algae coverage (Table 1). There was no relationship between juvenile *D. antillarum* density and

Table 1 Linear regression analysis and correlation between different variables, which are *Diadema* density, competitor density, predator density, algae height, % algae coverage, rugosity, and the total density of the other sea urchins found in the ten meter square plot. Data taken at 20:00 for the urchin densities and 8:00 for everything else listed

Variable (x)	Variable (y)	F-stat	df	p-value	r2
Diadema density	% algae	9.571	5	0.036	0.705
Diadema density	Predator density	0.696	5	0.451	0.148
Diadema density	Competitor density	0.019	5	0.978	0.523
% algae	Competitor density	0.0002	5	0.989	0.11
Rugosity	Diadema density	2.74E-08	5	0.999	0.003
Diadema density	Algae height	5.80E-07	5	0.999	0.013
Competitor density	Algae height	3.21E-09	5	0.999	0.0004
Competitor density	Rugosity	0.003	5	0.962	0.374
Jv Diadema density	Rugosity	8.79E-05	5	0.993	0.199
Jv Diadema density	% algae	0.001	5	0.973	0.773
Adult Diadema density	% algae	0.455	5	0.537	0.028
Jv Diadema density	Competitor density	0.0001	5	0.992	0.079
Adult Diadema density	Competitor density	0.006	5	0.944	0.551
Other urchin density	Diadema density	1.24E-05	5	0.997	0.015

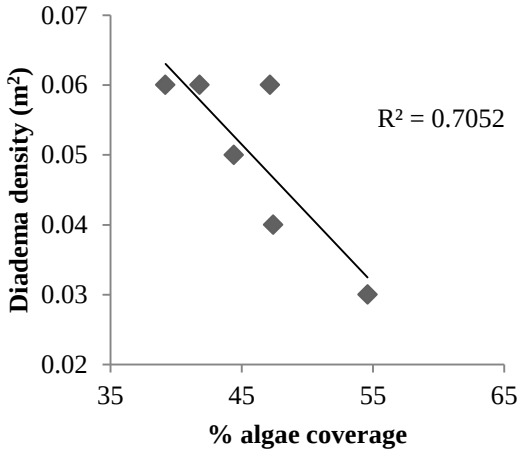

Fig 5 Correlation between the *Diadema* density per m^2 and the percent algae coverage at Kas di Arte, Yellow Sub, and Something Special North and South

competitor density (Table 1). There was a statistically insignificant slight positive correlation between adult *D. antillarum* density and competitor density (Table 1). There was no relationship between the density of other sea urchins in the plot and *D. antillarum* (Table 1).

Discussion

While *D. antillarum* have been slowly recovering from their massive mortality event in 1983, possible explanations for the recovery of mortality are coming to light. The present study found no effect of predation on *D. antillarum* density. A possible explanation for this is that there might be other sources of food for *D. antillarum* predators. In addition, these predators are very mobile, which makes their local abundance difficult to estimate. Only two of the 27 urchins seen were under the escape size so they could be eaten. There should be a larger amount of urchins under of this escape size because urchins have to start in this size class. The low *D. antillarum* count would also be a cause for why there was no significant correlation between predation and *D. antillarum* density. There simply is not a large population of *D. antillarum* less than 4 cm to be consumed by predators.

The most apparent phenomenon to explain is the relationship between *D. antillarum* density and the amount of algae present on the reef. H_2 states that an increase in percent algae coverage would lead to an increase in the density of *D. antillarum* present, but survey results

refute this. This can be explained because of *D. antillarum* consume mostly algae (Mumby et al. 2006). If a positive relationship between percent algae and *D. antillarum* density was seen, then *D. antillarum* would be considered a keystone species (Tuya et al. 2004). Keystone species are characterized by their strong contribution to the environment relative to their small population size. In this case, if *D. antillarum* was a keystone species, a drastic decrease in algae coverage would be observed from the increase of their population. If a statistically significant strong positive relationship between algae coverage and *D. antillarum* density was present, than *D. antillarum* would be a keystone species in its native habitat. However, since this relationship was not supported in this study, we can suggest that *D. antillarum* are not keystone species and the area has undergone or is undergoing a phase shift to account for the loss of this keystone species.

The third hypothesis stated that *D. antillarum* and other herbivore species would be negatively correlated. However, this was also rejected. An increase of competitor density was found in regions with more *D. antillarum.* Since a statistically insignificant, weak correlation between competitors and *D. antillarum* density was suggested by the correlation analysis there have to be other explainations for this result. The reason behind this observation resides in the observation of juvenile *D. antillarum* occupying a different niche from the much larger fish (e.g. parrotfish). Competition between adult *D. antillarum* and other herbivores is more apparent because they occupy the same niches. The large amount of juveniles that occupy different niches would have helped cause a weak positive correlation between competition and *D. antillarum* density. In addition, a past study also found that competition between herbivores and *D. antillarum* slowed down the population recovery rate (Hay et al. 1985). So it is suggested that the similar positive correlation between competitors and *D. antillarum* found in both studies could mean that the *D. antillarum* can recover in Bonaire despite the competition present.

D. antillarum populations have not increased or decreased when comparing the previous density in 2009 in Bonaire to the current density. The previous density of 0.005 individuals per meter squared was taken during the day (Steneck et al 2009). The 2009 study only sampled the larger urchins (mainly reproductive adults) that are much more apparent and easier to find due to their size. The difference in finding urchin densities during the day versus during the night is drastically unsimilar. While only 0.005 per m^2 of *D. antillarum* were seen during the day, 0.05 per meter squared were seen at night. Overall, the density recorded in this study (0.05 individuals per m^2) is not comparable to previous densities recorded by Steneck et al. (2009). The two studies evaluate *D. antillarum* density at different locations and times. Furthermore, the density in this study includes both the juvenile and adult populations together. Having a greater knowledge of the amount of juveniles on the reef might reveal the mortality rate during transition of juvenile *D. antillarum* to adult *D. antillarum*. A larger scale would mean more sites over many years to understand the survivorship of *D. antillarum.* Through further understanding of juvenile *D. antillarum* populations, this may give us further explanations for the competition and predation that takes place on the coral reef.

Healthy reefs are normally seen as having an abundant amount of live coral and little to no macroalgae (Hughes 1994). Bonaire's reefs are particularly different from the rest of the reefs in the Caribbean. This claim is supported by the large amount of live coral coverage with macroalgae (Steneck et al 2009). With an average 45.75% algae coverage on the reefs near Kralendijk, the reefs are found to be losing their coral coverage. In 1982,

the average percent algae cover at Cliff dive site was 6.4% (Stokes M, 2010). Despite these different locations, this shows the drastic increase in the percent algae coverage. With more algae present, there is a lower chance in coral recruitment success on the reef crest (Steneck et al 2009). The loss in coral coverage could be attributed to the presence of fishing on both predators and herbivores in the Kralendijk area. With the rising concern of increased algae coverage, if *D. antillarum* is no longer a keystone species at this time then a gradual decline in the coral coverage will soon lead to degradation of the habitat on the reef crest.

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REPORT

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Client choice, competition, and cleaner dependence pressure cleaner fish to cooperate in mutualistic symbiosis

Abstract Mutualistic symbiosis is a finely tuned relationship between two species in which each receives a service that increases its own fitness in exchange for providing service to another. The evolutionary stability of such a relationship is dependent on all species performing in an honest manner. However, many species that participate in mutualistic symbiosis have been observed cheating, or taking benefits beyond those evolutionarily agreed upon. This study attempted to identify factors that contribute to the frequency of cheating at cleaning stations on coral reefs. In these relationships, small fish and crustaceans clean parasites from larger host organisms. Client abundance and proximity of cleaning stations were examined as indicators for competition between cleaners and client choice. These factors put pressure on cleaners to cooperate by creating competition for clients. It was found that there was a greater abundance of clients at stations where cheating occurred less frequently, suggesting that clients may have chosen those stations for the higher quality service demonstrated. Proximity of cleaning stations did not seem to influence the frequency of cheating. Finally, obligate cleaners spent more time cleaning individual clients and cheated less frequently than facultative cleaners, demonstrating their higher dependence on the relationship. Understanding the factors that motivate cleaners and clients to cooperate at cleaning stations is an important component to comprehending community dynamics on reefs, but it is not as clear of a relationship as is commonly described.

Keywords Cleaning stations • Cheating • Obligate and facultative

Introduction

Mutualistic symbiosis is an interspecific interaction in which individuals of two or more species exhibit behaviors that provide benefits to one another. Such benefits may come in the form of food, housing, protection, or any combination thereof. As one of the most biologically diverse ecosystems on the planet, coral reefs are full of mutually symbiotic relationships, including relationships between shrimp and the anemones in which they live, gobies and shrimp that provide burrows in the sand, and the paradigm relationship of zooxanthellae and the corals they inhabit (Pearse and Muscatine 1971; Smith 1977; Lyons 2012). In these examples, the anemone provides protection and housing for the shrimp, which reciprocates through aggressive defense against invaders such as polychaetes (Smith 1977). Gobies guard the burrows of shrimp, alerting these blind arthropods of potential predators, and are rewarded with protection in the shrimp's burrow (Lyons 2012). Finally, coral provides a skeletal home for zooxanthellae, which in return provide coral with food products from photosynthesis (Pearse and Muscatine 1971). These interspecific pairs have

evolved over time as natural selection has allowed for greater fitness of both species through mutualism.

Another example of mutualism on the reefs is the phenomenon of cleaning stations. At cleaning stations, small cleaner fish or crustaceans remove old scales, mucus, and ectoparasites from larger client organisms. The cleaner species, which include gobies, wrasses, shrimp, and juveniles of residential reef fish, position themselves in a small territory, often around a coral head or divot in the ocean floor; here they wait for client organisms to come to be cleaned (Côté 2000). The client organisms' identities include, but are certainly not limited to, herbivorous fish such as parrotfish and chromis as well as carnivorous species including groupers and barracudas. When seeking cleaning, clients have been observed to assume a head-up or tail-up position, undergo a dramatic change in colors, open their mouths, or flare their opercula to solicit cleaning (Wicksten 1995; Côté 2000).

For several decades, scientists have been speculating what factors motivate clients and cleaners to engage in mutualistic relationships (Limbaugh 1961; Youngbluth 1968; Losey 1979; Losey 1987; Losey et al. 1995). However, data showing mutual benefit to both parties have been inconclusive (Cusack and Cone 1986; Gorlick et al. 1987; Grutter 1996). It seems that cleaners benefit from the interaction, as they consume ectoparasites, dead scales, and mucus from clients (Gorlick 1984). Obligate cleaner species (those whose diets consist predominantly of the food they glean from clients) procure on average 85% of their food from cleaners, making this behavior crucial to their survival (Côté 2000). Facultative cleaner species (those who do not rely on cleaning for their entire diet, but also consume other benthic food sources such as invertebrates and plankton) fulfill approximately 43% of their daily intake with parasites from cleaning (Randall 1958; Youngbluth 1968; Hobson 1971; Carr and Adams 1972; Losey 1974; Grutter 1997a). Data from the Great Barrier Reef indicate that one cleaner will interact with more than two thousand clients in a single day, and a single cleaner may clean approximately 1,200 total parasites from clients in one day (Grutter 1996). The magnitude of these values demonstrates the importance of the interaction to cleaner species.

The benefits incurred by client organisms are much more uncertain. They appear to benefit from the relationship through the removal of parasites as a means of reducing infection and disease. A number of studies suggest that when cleaners are removed, the client populations suffer notably in the form of disease, reduced growth, and reduced reproductive success (Cusack and Cone 1986; Pulkkinen and Valtonen 1999; Limbaugh 1961). Similarly, research has demonstrated significantly higher species diversity and fish abundance on reefs with cleaning stations than on reefs without cleaners (Grutter et al. 2003). Other studies, however, suggest that removal of cleaners does not cause changes in client fish distribution (Gorlick et al. 1987; Grutter 1996). Some literature also shows no significant reduction in ectoparasite loads by cleaners (Gorlick et al. 1987). Clearly the data are controversial, and understanding the true nature of the relationship between clients and cleaners may better explain patterns of diversity and population dynamics on reefs.

While cleaning stations appear to have positive effects within coral reef ecosystems, dishonest cleaning, or cheating, by cleaner fish has also been observed as a prevalent part of this interaction. Cheating consists of a cleaner biting scales or mucus, in addition to parasites, from clients. A number of recent studies have examined proximate causes of cheating by cleaners. One theory is that cleaners build up relationships with frequent clients to allow future

exploitation (Pinto et al. 2011). This hypothesis is reinforced by observations of cleaner fish expanding their home range. This behavior minimizes repeat interactions with previously exploited clients and increases interactions with unsuspecting clients (Oates et al. 2010). These strategies suggest that cleaners are generally inclined to try to clean dishonestly, and that a mutualistic relationship is maintained only by feedback on the part of the client. Forms of feedback include clients aggressively chasing a dishonest cleaner or terminating the cleaning bout early (Côté 2000). From the perspective of the clients, cheating is better-tolerated when ectoparasite load is high, suggesting that the moderate costs of cheating are compensated by the benefits of having the parasites removed (Cheney and Côté 2005).

Contributing to the theory that cleaner species are actually opportunistically parasitic, studies have suggested that cleaner fish may manipulate the client into assuming a sedentary position by grazing their skin as a form of tactile stimulation (Losey 1979, 1987). Once close, they are able to steal a bite of mucus or scales and escape before the client has time to respond (Bshary and Würth 2001).

In the present study, cheating by cleaner fish on a coral reef was observed and quantified to identify trends that may shed light on the motivation for, frequency, and control of this behavior. Factors that were examined included proximity to other cleaning stations, the abundance of clients soliciting cleaning at individual cleaning stations, and the frequency of cheating by obligate versus facultative cleaners. It was hypothesized that:

H_1: Dishonest cleaning would occur more frequently as the number of clients soliciting cleaning increases, because cleaners would have more choice in which fish they clean, and client abandonment would be less detrimental to a single cleaner.

H_2: Cheating would occur more often by facultative cleaners than by obligate cleaners, because they are not as dependent on a cooperative long-term relationship with clients as obligate cleaners are.

Data supporting these hypotheses could suggest that cleaner fish are in fact opportunistic parasites, and that the identification of cleaning stations as an example of mutualistic symbiosis may be a misnomer. Regardless of the rhetoric used to define the relationship, parasitic behavior by cleaners could influence coevolution and community dynamics between cleaner and client species.

Materials and methods

Study site

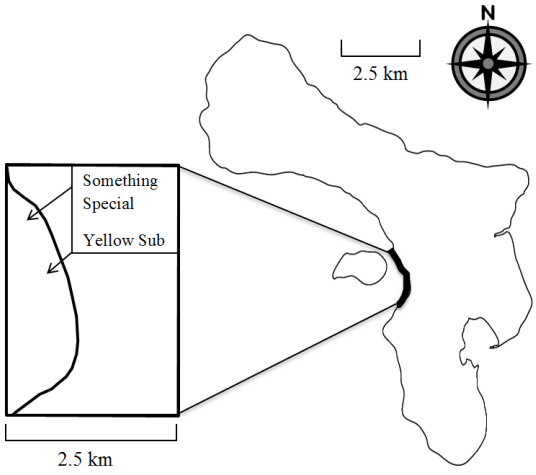

Fig. 1 Map of Bonaire, DC with close-up of the Kralendijk waterfront. The bracketed area indicates the dive sites observed in this study: Something Special (12° 9'40.9062" N, 68° 17' 0.7362" W) and Yellow Sub (12° 9' 36.648" N, 68° 16'55.578" W) ([worldatlas.com] visited 3 Nov 2013)

This study was conducted from 28 September to 3 November 2013 on the leeward side of Bonaire, Dutch Caribbean. Data for this study was collected by SCUBA divers at the dive sites Yellow Sub and Something Special. Both sites are

fringing reefs extending ~60 m to ~250 m from shore. All observations were made exclusively on the sloping fore reef of these two sites.

Study organisms

Two reef fish were observed in this study: juvenile *Bodianus rufus* (Spanish hogfish) and *Gobiosoma evelynae* (Sharknose goby). Juvenile Spanish hogfish were observed as a model for facultative cleaners, and sharknose gobies served as a model of obligate cleaners. These species were described as such in Côté's review (2000) of cleaner species. Preliminary observations showed that both species are abundant as cleaners on the reefs of Bonaire, DC, and that neither species appeared to be disturbed by the presences of divers that maintained a reasonable distance (i.e. two or more meters), which allowed for prolonged observation.

Data collection

Random cleaning stations on the fore reef between six and twenty meters deep were selected and video-taped for ten-minute intervals by a diver hovering two to three meters away. While reviewing the videos, the duration of each cleaning bout was tabulated and the numbers and species of clients and cleaners were recorded in a field notebook. Occurrences of cheating by the cleaner fish were counted. Dishonest cleaning was identified as it was in Bshary and Grutter (2002) and Grutter et al. (2003). These studies observed clients "jolting," or sharply turning away from the cleaner, when live scales or skin were taken in addition to the parasites.

Data analysis

A linear regression between abundance of clients and the percentage of interactions that resulted in cheating was run. Another regression was run between proximity of cleaning stations and the frequency of cheating. A third linear regression paired the abundance of clients and the duration of individual cleaning bouts. A student t-test was run to compare the duration of a cleaning bout with an obligate cleaner to the duration of a bout with a facultative cleaner. Finally, the percentages of interactions that resulted in cheating were compared between obligate and facultative cleaners using a student t-test.

Results

Over the course of five weeks, 202 minutes of video were gathered of cleaning stations that were observed to be active. Active cleaning stations were identified as those at which clients were soliciting cleaning from cleaner fish and cleaner fish were engaging in the interaction. A total of 26 cleaners were observed interacting with a total of 411 clients. Of the cleaners noted, five were *G. evelynae* (sharknose goby), which served as the model for obligate cleaners, and 21 were juvenile *B. rufus* (Spanish hogfish), referred to henceforth as facultative cleaners.

There was a weak negative trend between the abundance of clients and the percentage of interactions that resulted in cheating (R^2=0.06, F-crit=4.35, p=0.29; Fig. 2; Fig. 3). There appeared to be no correlation between proximity of cleaning stations and frequency of cheating (R^2=0.005, F-crit=0.05, p=0.83; Fig. 4).

Obligate cleaners spent significantly more time with each client than did facultative cleaners (t-stat=2.12, p=0.02; Fig. 5). For all cleaners, as the abundance of clients an individual cleaner interacted with increased, the average time spent cleaning each client decreased (R^2=0.16, F-crit=4.35, p=0.07; Fig. 6). Finally, there was no significant difference between obligate and facultative cleaners regarding the percentage of interactions that result in cheating (t-stat=-0.58, p=0.28; Fig. 7).

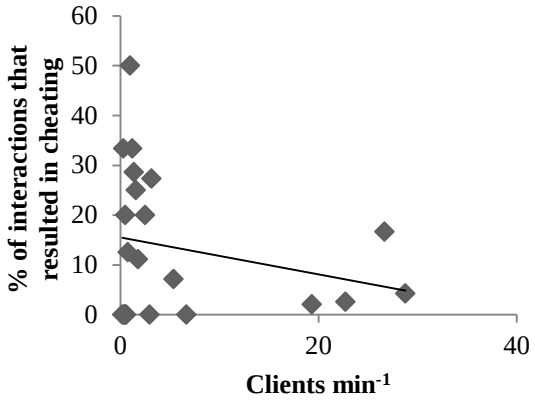

Fig. 2 Regression between the abundance of clients a cleaner interacted with (clients min^{-1}) and the percentage of interactions that resulted in cheating (R^2=0.06)

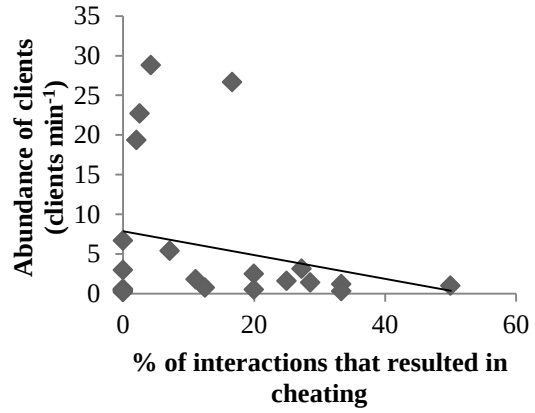

Fig. 3 Regression between the percentage of interactions resulting in cheating and the abundance of clients at the cleaning station, with percentage of interactions that resulted in cheating serving as the explanatory variable (R^2=0.06)

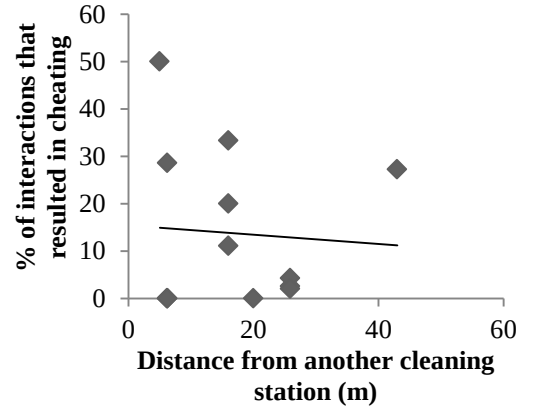

Fig. 4 Regression between proximity of cleaning stations and the percentage of interactions that resulted in cheating (R^2=0.005)

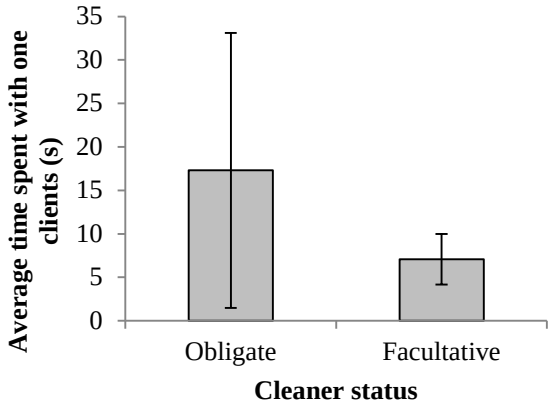

Fig. 5 Mean ± 95% confidence intervals of time spent interacting with a single client compared between obligate (n=5) and facultative (n=21) cleaners

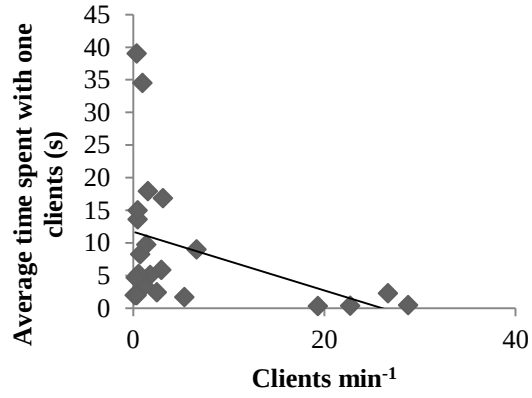

Fig. 6 Regression between the abundance of clients (clients min^{-1}) a cleaner interacted with and the average time that cleaner spent interacting with each client (R^2=0.016)

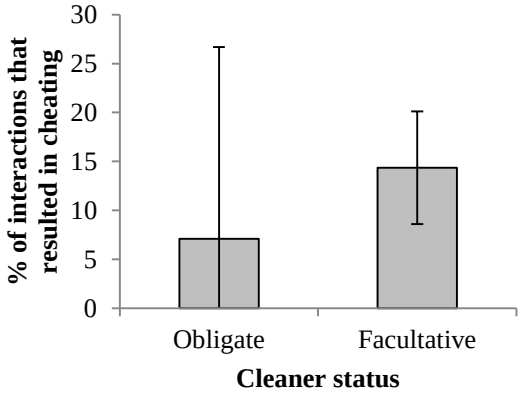

Fig. 7 Mean ± 95% confidence intervals of percentages of interactions resulting in cheating, compared between obligate (n=5) and facultative (n=21) cleaners

Discussion

The results of this study refute H_1: that dishonest cleaning would occur more frequently when the number of clients soliciting cleaning is higher. There is a negative correlation between the abundance of clients a single cleaner had access to and the percentage of interactions that result in cheating (Fig. 2). In other words, as the frequency of cheating by one cleaner increased, the number of clients soliciting cleaning from that cleaner decreased (Fig. 3). In this case, frequency of cheating would be the explanatory variable, and abundance of clients the response variable. This pattern is consistent with literature that suggests image-scoring as a deterrent for cleaners from cheating. If a client witnesses a cleaner cheating, they might be less likely to approach that cleaner (Bshary 2002). In this scenario, a cleaner's dishonesty would be easily observed when there are many potential clients around, and the slight gain from the cheating behavior may be more costly to cleaners over the long-term compared to the total gain that could be attained from an abundance of clients. Consequently, cleaners observed displaying dishonest behavior would be in lower demand than cleaners that do not display such behavior.

Further evidence of pressure exerted by selective clients was provided by Pinto and colleagues (2011), who demonstrated that the cleaner wrasse *Labroides dimidiatus* could be conditioned to cooperate more consistently when their cooperation granted them access to additional clients (Pinto et al. 2011). Modification of behavior was especially prevalent when interactions between individual client-cleaner pairs were repetitive, because the clients demonstrated recollection of which cleaners had cleaned honestly and which had not (Pinto et al. 2011). For the purpose of the present study, it was not taken into consideration whether a specific client-cleaner pair had interacted with one another before, due to the relatively short duration of each replicate trial. Each observation period was treated as a new set of interactions. In future studies, clients might be observed for longer periods to see whether they return to cleaning stations over the course of a day. A client's refusal to return to a specific cleaner, especially if that cleaner had previously been dishonest, provides negative feedback against cheating. Past studies have indicated that client-fidelity as well as aggressive reactions to cheating both discourage future dishonesty by a cleaner (Bshary and Grutter 2002).

Supplemental to client-fidelity is the concept of client choice, which is considered central to maintaining an honest mutualistic relationship (Adam 2010). Client choice is based on the assumption that a client has more than one option available for cleaning, and can therefore afford to be selective about which station it visits. Competition encouraging higher-quality service is a common biological market strategy that curbs dishonesty in many symbiotic relationships. In the case of cleaning stations, as competition for access to clients increases, it has been observed that cheating by cleaners significantly decreases (Soares et al. 2008). Though honest interaction is not as beneficial to the cleaner in the short-term, it benefits the individual in long-run by encouraging repetitive interactions (Adam 2010). Client choice provides a negative feedback on exploitation by cleaner fish by requiring a certain standard of service by the cleaners. If clients are more likely to interact with cooperative individual cleaners, the cleaners would have a higher incentive to clean honestly (Foster and Kokko 2006).

Contrary to this concept, the results of the present study showed no relationship between the distance between cleaning stations and the percentage of interactions that resulted in cheating (Fig. 4). If the results agreed with past literature, one

might expect higher frequencies of cheating at more isolated stations, as clients would have less access to other cleaners and could not be as selective about service. A potential explanation for this discrepancy is that the distances examined in the present study were not biologically significant to the client fish. The furthest distance measured between stations was 43 m, but if both stations were within the home range of a client fish, then competitive pressure to provide quality service would still apply. Isolation or relative abundance of cleaning stations is an important factor to consider, but in future studies the home ranges of client fish should also be measured to determine how many cleaning stations each client has reasonable access to.

When examining the cleaning tendencies of obligate cleaners versus facultative cleaners, the present results showed that obligate cleaners spent more time on an individual client than did facultative cleaners, as predicted by H_2 (Fig. 5). A difference due to cleaning status might be a result of less access to clients due to a cleaner's smaller home range. This explanation is supported by the trend that as the abundance of clients soliciting cleaning increased, the average duration of a cleaning bout was shorter (Fig. 6). The trend also might be attributed to greater thoroughness on the part of obligate cleaners resulting from their high dependence on ectoparasites as food. Obligate cleaners are much more dependent on the ectoparasites they clean from clients than facultative cleaners (Côté 2000), so they might be less likely to cheat based on the higher necessity of maintaining a positive relationship with clients. This explanation is supported by a difference in mean number of interactions involving cheating, with obligate cleaners cheating about 4% less than facultative cleaners (Fig. 7). This difference is not statistically significant, but appears to be due to large confidence intervals resulting from small sample sizes (t-stat=-0.58, $p=0.28$, $n_{obligate}=5$, $n_{facultative}=17$; Fig. 7) rather than a true similarity in means. Additional observations of obligate cleaners may reduce the size of the confidence intervals and reveal meaningful differences between obligate and facultative cleaners.

Although much of the data in the present study was inconclusive, some interesting trends emerged that imply the importance of proximity of cleaning stations with regards to generating competition among cleaners through client choice. Fragmentation of habitat may eliminate the clients' choice and force them to only visit one cleaning station, regardless of the quality of service available at that station. This could negatively affect the clients; if client abundance is high, cleaners may not spend as much time on each client, as indicated by the present study. Insufficient removal of parasites could result, which could be injurious to the client. With respect to the cleaners, fragmentation may prevent clients from travelling between stations, allowing cleaners to cheat more frequently because clients do not have an alternative option. Cleaning stations provide a good model for theoretically mutualistic relationships and demonstrate how negative feedbacks by one species necessitate cooperation from their partner. Even though one or both species may be inclined to cheat when possible, the stability of mutualistic symbiosis is maintained by these feedbacks through retention of higher fitness levels accrued by cooperation than by exploitation.

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REPORT

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Enterococci, a bacterial fecal indicator, and its correlation with coral disease abundance in Bonaire

Abstract Coral reef environments are diverse and productive ecosystems that supply a variety of benefits to marine species and humans alike. Unfortunately, these same reefs, including Bonaire's, are under increased stress from anthropogenic activities such as nutrient and bacteria groundwater runoff. With poor sewage control, nutrients and bacteria can leech into the groundwater and flow directly into our reefs and thus increase the frequency and intensity of coral disease and bleaching. *Enterococci*, a common bacteria found in the intestines of humans, is used as an indicator of fecal contamination in water sources. In this study, 10-m transects and *Enterococci* water samples were taken at three high human impact (HHI) sites and two low human impact (LHI) sites. Although *Enterococci* was present in the water column at four of the sites, there was no correlation between increased *Enterococci* and abundance of coral disease. The *Enterococci* concentration levels at one site were higher than what the Environmental Protection Agency deems a healthy recreational water concentration. Coral disease was present at each site, with frequencies ranging from 12.4-19%. LHI sites had 4.2% more diseased coral than HHI sites. The direct cause of many coral diseases is unknown, although there are a variety of factors that likely contribute to their outbreak and spread. Tourism, terrestrial runoff and nutrient overload all affect coral disease abundance in Bonaire. Since coral disease was present at each site, further protection and prevention must be implemented to reduce the outbreak and spread of diseases before the coral reef is degraded past repair.

Keywords *Enterococci* • Coral disease • Sewage runoff

Introduction

As diverse, productive ecosystems, coral reefs not only provide benefits to marine species but to humans as well. They provide a fishing trade, recreational activities, cultural benefits, and tourism for humans (Moberg and Folke 1999). In addition, corals offer many things to fish species: protection from predation, shelter, and a substrate or surface to hide within and be camouflaged. More importantly, coral helps to increase species biodiversity as a whole within the ecosystem (Alvarez-Filip et al. 2009). Unfortunately, human impacts are causing increased coral reef degradation and threaten to destroy many of the reefs around the world (Hughes et al. 2003). These impacts include overfishing or destructive fishing techniques, uncontrolled tourism, new coral diseases, boating accidents, increased carbon dioxide and temperature levels, and in many cases sewage contamination (Hughes 1994; Hughes et al. 2003; Jones et al. 2011).

Bonaire, a small island in the Dutch Caribbean surrounded by a fringing reef, is supported by limestone and sand bedrock formed from dead corals and plate tectonics (van Sambeek et al. 2000).

Currently, Bonaire has no effective sewage treatment plan in place. As such, bacteria, viruses, heavy metals, active chemical compounds and nutrients that are dumped into unlined trenches on the island, can be easily absorbed into the groundwater that flows beneath it (Jones et al. 2011). This is a major problem for Bonaire's reefs because the contaminated groundwater is flowing directly into them.

With nutrient overload, the primary production and biomass of benthic algae can increase causing the amount of light reaching the corals to diminish and an increase in competition for space. Zooxanthellae, one of the sources of energy in corals, will be directly affected and expelled if energy production is too low (Pastorok and Bilyard 1985). With continuous overloading of contaminants and nutrients onto coral reefs, the frequency and intensity of coral disease and bleaching will further increase and intensify (Voss and Richardson 2006). The most common diseases identified by Steneck et al. (2011) in Bonaire were Yellow Band disease, followed by Dark Spot disease and Red Band disease. These diseases have been linked to increased abundances of fecal bacteria, including *Enterococci* (Kaczmarsky et al. 2005).

One way to test for sewage contamination in waterways and from runoff is to check for the presence of *Enterococci* by collecting water samples. *Enterococci* is a bacteria that is found in the intestines of humans and thus a good indicator of human sewage. Lipp et al. (2002) sampled bacterial fecal indicators (fecal coliform and *Enterococci*) in the heavily populated area of the Florida Keys. Similar to Bonaire, the Florida Keys lie on a porous limestone substrate and almost exclusively relies on on-site sewage disposal. By testing concentration levels in water samples directly above the coral and from the coral mucus itself, they found that the bacterial fecal indicators were present. Patterson et al. (2001) conducted a similar study in the Florida Keys where a direct relationship was found between White Pox disease in the elkhorn coral, *Acropora palmata*, and the abundance of *Serratia marcescens*, a bacteria found in the human gut. Rini (2008) and Lipshultz (2010) both conducted student-based research on the concentration levels of *Enterococci* in the water column and above coral reefs in Bonaire. Higher concentrations of *Enterococci* were found at resort based sites compared to non-resort sites (Lipshultz 2010). Thus, the following hypothesis was tested:

H_1: Areas of high human impact (HHI) will have higher concentrations of the bacterial fecal indicator, *Enterococci*, and thus a higher percent area coverage of coral disease compared to areas of low human impact (LHI)

Materials and methods

Study site

Study sites in Bonaire were selected based on two categories: high human impact (HHI) areas and low human impact (LHI) areas. All sites are classified as a fringing reef (*i.e.* reefs close to shore). Sites were chosen based on accessibility and proximity to commercial establishments (*i.e.* a SCUBA dive resort, office building, restaurant). High human impact sites include Something Special, Yellow Submarine, and Kas Di Arte. These three sites are located off the main road of Kaya J.N.E Craane, one of the main oceanfront roads in Kralendijk. Low human impact sites include Tori's Beach and Margate Bay. These two sites are located at the southern tip of the island, past the salt pans (Fig. 1). These locations are farther away from housing developments, resorts and commercial establishments.

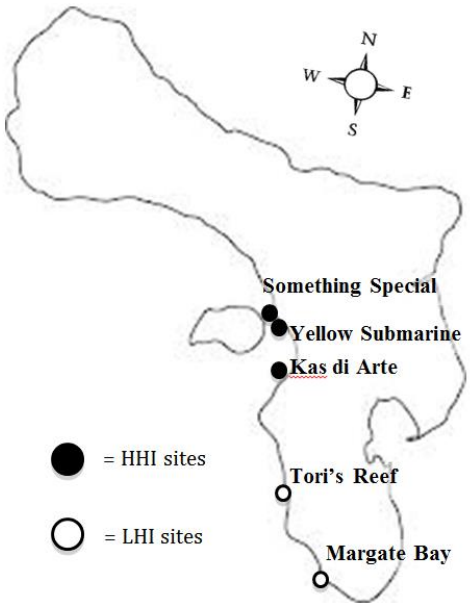

Fig. 1 Map of Bonaire indicating the locations of sampling sites. Low human impact (*i.e.* LHI) sites include: Something Special (12°09' N, 68°17' W), Yellow Submarine (12°09' N, 68°16' W), and Kas di Arte (12°09' N, 68°16' W). High human impact (*i.e.* HHI) sites include Tori's Reef (12°04' N, 68°16' W) and Margate Bay (12°03' N, 68°16' W)

Coral testing

A coral/benthic survey was conducted at each site, in order to get a better representation of the coral coverage and the abundance of coral disease. At each site, two 10-m transects were laid at depths of 10.67 m and 13.72 m to the north and two 10-m transects at the same depths to the south of the entry point (Fig. 2). Coral species, disease and disease coverage were recorded for all corals that fell directly below the transect line, and size (height, length and width) was recorded using a T-bar. Each site was only surveyed once with the assumption that coral disease abundances did not change significantly in a 5-week period. At each site, the distance from the shore to the reef crest was measured online using Wikimapia, to examine how far bacteria and nutrients need to travel before coming in contact with the corals and reef.

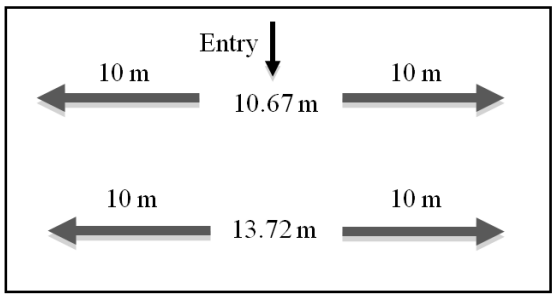

Fig. 2 Example of methods used when laying transects and collecting water samples. Water samples were taken at the beginnings of each transect. Depths are stated in *parentheses*

Coral analysis

The data collected from the coral surveys were combined and analyzed in a variety of ways. The frequency coral disease at HHI sites and LHI sites were combined and grouped separately to help compare disease prevalence at the two types of sites. The mean frequency of disease present at each site was determined including the standard deviation. A one-way ANOVA test was used to examine the effect of the sites on coral disease abundance.

Enterococci testing

A water sample was taken 0.5 m above the substrate, at the end of each transect, in order to test for the presence of *Enterococci.* Samples were taken using 100-mL, sterile plastic containers. Prior to descending, the samples were filled with water from the surface, emptied and refilled at the depth of each transect. After completion of the dive, samples were put on ice and returned to the lab and processed according to the Enterolert *Enterococci* detection protocol (IDEXX 2008). First, the sample was diluted 10 times and an Enterolert packet was then added into the container and mixed. The mixture was then poured into IDEXX Quanti-trays and sealed. This procedure was repeated for each water sample and all Quanti-trays were put into an oven at 41^{o} C ±0.5^{o} C and left for 24-28 hrs. Positive results, indicated through blue light

fluorescence, were counted and recorded. The most probable number (MPN) of *Enterococci* colonies were calculated using the IDEXX Enterolert MPN table. The concentration levels were then multiplied by 10 to represent the dilution process and determine an accurate concentration level of each 100-mL water sample.

Enterococci data analysis

A correlation analysis was used to examine the effect of *Enterococci* concentration on coral disease abundance. The mean MPN values at each depth were determined by combining concentration values measured at the same depth. To determine the average coral disease coverage, the two averages that were measured at the same depth were combined. This test was conducted to examine if there was any connection between *Enterococci* concentrations and frequency of disease at each site.

Results

Coral findings

Low human impact (LHI) sites had 4.2% more healthy coral coverage with 85.7% compared to high human impact (HHI) sites that had 81.5% healthy coral. All four identified diseases (*i.e.* Yellow Band, Black Band, Red Band and Dark Spot) were found at HHI sites compared to only two diseases (*i.e.* Yellow Band and Black Band) at LHI sites (Fig. 3 & 4). Out of the 35 diseased corals recorded, 57% were present on the coral species *Orbicella annularis*. These diseases included Yellow Band, Red Band and Black Band disease. *Siderastraea siderea* was the second most diseased coral species with an abundance of 31%, followed by *Orbicella faveolata* with 9% and *Montastaea cavernosa* with 3% (Fig. 5). The mean frequency of coral disease was higher at two of the three HHI sites than those of the LHI sites (Fig. 6a). Among transects, there was no difference in coral disease (One-way ANOVA: $F=0.215$, $df=11$, $p= 0.811$).

The distances between the shoreline and reef crest for each site varied. Tori's Reef had the longest distance with 153 m, followed by Margate Bay with 107 m, Kas Di Arte with 86 m, Yellow Submarine with 71 m and finally Something Special with the shortest distance of 60 m.

Enterococci findings

The range of MPN values found at a depth of 10.67 m was 0-120/100 mL with a mean of 18/100 mL while the range at

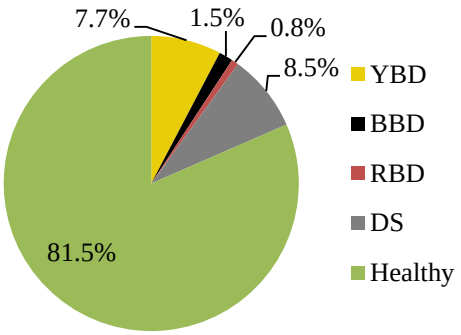

Fig. 3 Frequencies of coral disease at all High Human Impact (HHI) sites (*i.e.* Yellow Submarine, Something Special and Kas Di Arte). Four diseases were identified throughout the entire experiment including Yellow Band disease (*i.e.* YBD), Black Band disease (*i.e.* BBD), Red Band disease (*i.e.* RBD) and Dark Spot disease (*i.e.* DS)

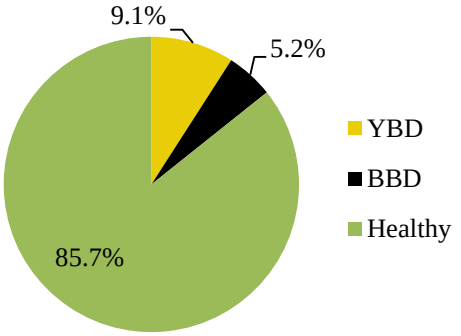

Fig. 4 Frequencies of coral disease at all Low Human Impact (LHI) sites (*i.e.* Margate Bay and Tori's Reef). Four diseases were indentified throughout the entire experiment including YBD, BBD, RBD and DS. Dark spot and Red Band disease were not present in either LHI site and thus had a frequency of 0%

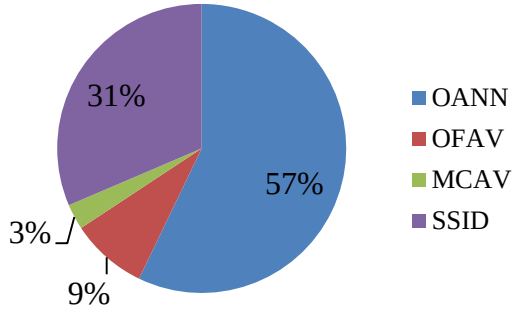

Fig 5. Coral disease abundances based on what coral species were observed as diseased. Four coral species were affected by disease including *O. annularis* (*i.e.* OANN), *M. faveolata* (*i.e.* OFAV), *M. cavernosa* (*i.e.* MCAV) and *S. siderea* (*i.e.* SSID)

13.72 m was 0-41/100 mL with a mean of 6/100 mL (Fig. 6b). Tori's Reef had the largest mean MPN concentration value with 40/100 mL followed by Kas di Arte with 13/100 mL, Yellow Submarine with 5/100 mL and Margate Bay with 3/100 mL. All water samples at Something Special tested negative for the presence of *Enterococci* (Fig. 6b).

Through a correlation test between the mean *Enterococci* concentrations and mean frequency of coral disease, the R^2 coefficient at a depth of 10.67m was 0.0916 while the R^2 coeffcient for the data at 13.72m was 0.23458 (Fig. 7).

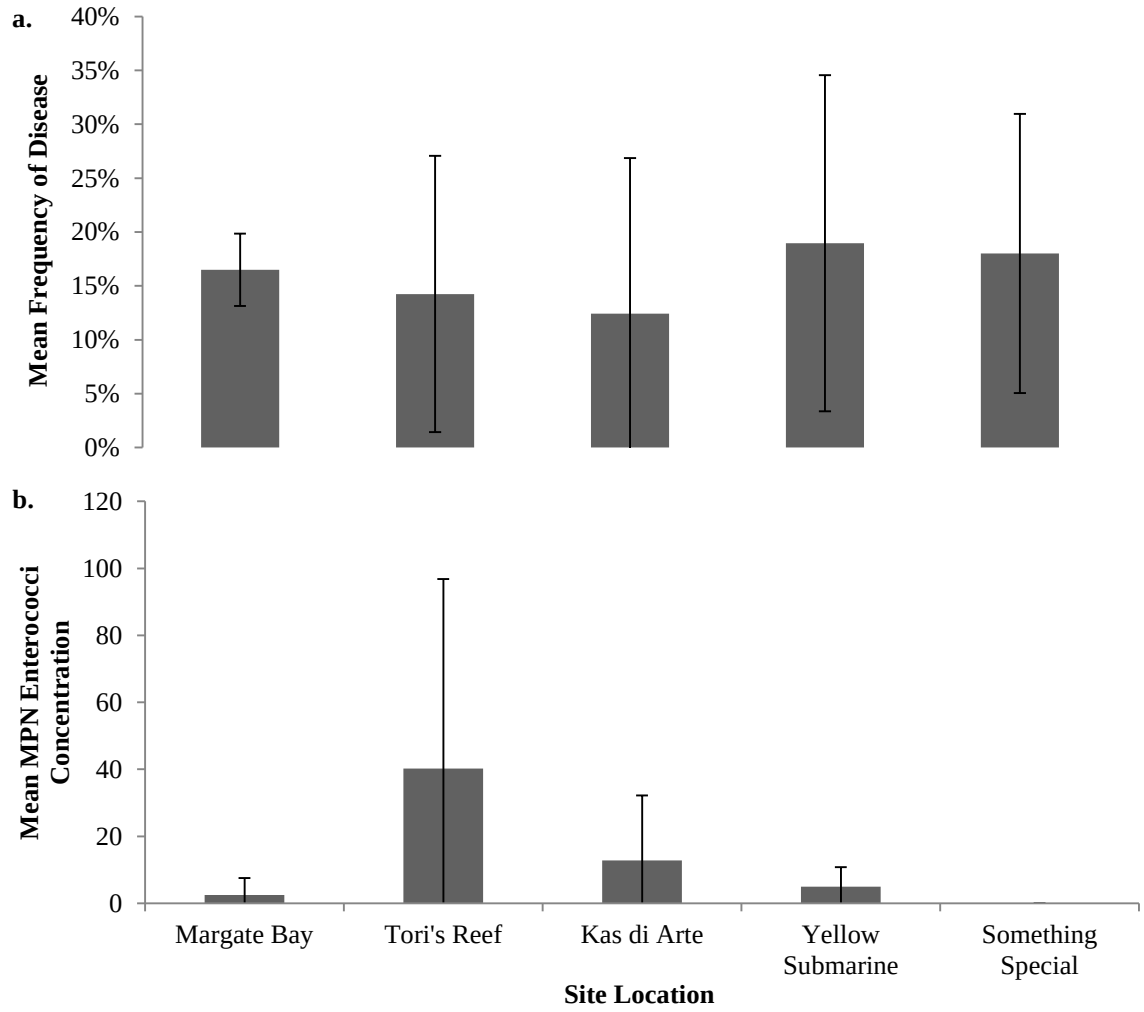

Fig. 6 a The mean (±SD) frequency of coral disease at each sampled site over the course of five weeks shown. The frequency of coral disease was calculated per transect and the mean (±SD) of all four transects per site were calculated. **b** The mean (±SD) frequency of *Enterococci* concentrations at each sampled site. The mean probable number values at each transect were collected through water samples and the mean (±SD) was calculated

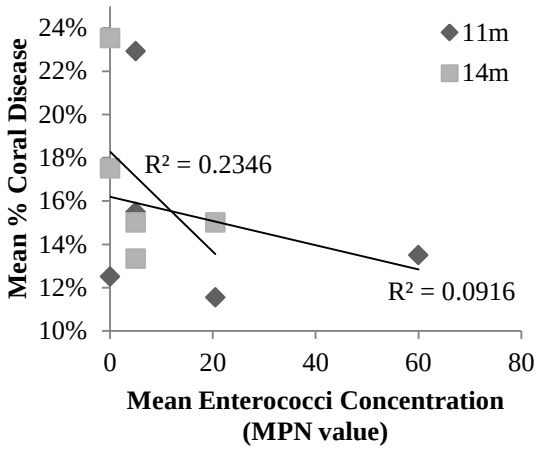

Fig. 7 Mean *Enterococci* MPN concentrations compared to the mean percent of diseased coral at 11m (*diamonds*) and 14 m (*squares*) across all sites. Each depth is represented by five points- one from each site tested

Discussion

Based on the results of this study, higher concentrations of the bacterial fecal indicator, *Enterococci*, are not related to higher frequencies of coral disease at HHI sites compared to LHI sites. Although there may have been no correlation, *Enterococci* was still present in the water column at four of the five sites tested and there were higher frequencies of coral disease at two of the three HHI sites compared to the LHI sites.

As seen in Figures 3 & 4, high human impact sites have a larger mean percentage of diseased coral coverage than at the low human impact sites. Although *Enterococci* concentrations may not be correlated to disease abundance in this study, there are a variety of factors that may have contributed to outbreak and spread instead. As found in the study by Lamb & Willis (2011), SCUBA diving tourism can be a factor in coral disease abundance. This may be caused by increasing stress in corals by divers directly touching or harming them. Something Special and Yellow Submarine have the two highest frequencies of coral disease out of the five sites. This may be attributed to a larger amount of divers accessing the two sites due to their close proximity to the capital of Kralendijk and easy shore entry access. In addition, the distance measured from the shore to the coral reef crest was shorter at Something Special and Yellow Submarine compared to Margate Bay and Tori's Reef. With this shorter distance, terrestrial runoff that may contain chemicals, pathogens, and small amounts of *Enterococci*, could reach the corals in higher concentrations and impact the amount of coral disease present. It is surprising, however, that Kas Di Arte, a HHI site, has the lowest frequency of coral disease out of all five sites. While collecting data at this site, a large amount of dead coral, rubble and sand was observed. Although this doesn't contribute to the value of coral disease frequency that was found, it may help understand why it was lower than other sites. With more coral reef degradation as a whole, corals that were diseased before may have already died prior to data collection and thus would not be reflected in this data.

It is important to note that *Montastraea* spp., *Orbicella* spp., and *S. siderea* were the primary corals affected by disease in this study. According to the IUCN (*i.e.* International Union for Conservation of Nature) Red List, both *O. faveolata* and *O. annularis* are listed in the endangered category due to such rapid population declines from coral disease. Although direct causes for coral disease are still generally unknown, this study helps show how affected these coral species are and gives proof that further testing and research should be conducted to search for a cause to these diseases. If corals are left unprotected, coral disease can spread and could eventually wipe out the reefs and fish species that inhabit them. Bonaire would be drastically impacted if this happened, because it depends so heavily on the reefs diversity for tourism and fishing.

Enterococci were present in the water column at all sites except Something Special. Although this is surprising

because of its proximity to a large condominium complex, it could be due to low terrestrial runoff prior to data collection. Little to no rain during the weeks' prior to data collection may have contributed to the lack of positive *Enterococci* results. Yellow Submarine, Kas Di Arte and Margate Bay all had comparable concentrations, while Tori's Reef was the outlier of the group with a mean MPN of 40/100 mL, nearly 30/100 mL higher than the other sites. Tori's Reef is located next to an outwash flow from the salt pans at the South of the island. Many warm-blooded mammals occupy these salt pans and thus could contribute feces and *Enterococci* into the waters that flow into Tori's Reef. With a concentration level of 40/100 mL, Tori's Reef has a concentration that is 5/100 mL points higher than the concentration that the Environmental Protection Agency deems suitable and healthy recreational salt water (35/100 mL). With these high levels, increased filtration or monitored drainage must be put into place to protect Tori's Reef from further nutrient runoff.

This study had several limitations that should be addressed and if re-done, should be altered. With a limited data collection time of five weeks, it was difficult to get multiple sets of data from each site. If this study is replicated, several sets of data should be taken per site so that potential data outliers (*e.g.* mean MPN values at Tori's Reef) and environmental variables (*e.g.* increased rainfall/runoff and current changes) could be taken into account. In addition, a better representation of each site could be formulated and a more accurate mean of coral disease and *Enterococci* concentrations could be found. Another limitation was access to sites. The LHI or southern sites were only accessed once due to a lack of transportation. It would be beneficial to the study to test more sites. This would give the study a broader scope of where sewage and terrestrial runoff and coral disease is high and what sites need greater protection.

Although there seems to be little to no correlation between high *Enterococci* concentrations and high frequency of coral disease, this study does show that coral disease is abundant throughout Bonaire's reefs. When corals are diseased, there is a greater likelihood of coral mortality and reduced coral reproduction (Santavy et al. 2005), which causes increased degradation of the reef as a whole. Therefore, with coral disease present at each site, further protection and prevention is needed to try and reduce the outbreak and spread of these diseases before the coral reef is degraded past repair.

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REPORT

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Effects of shore proximity and depth on the distribution of fish larvae in Bonaire, Dutch Caribbean

Abstract A vast majority of marine fish species, both reef and pelagic, are bipartite, meaning that they have a pelagic larval stage distinctly separate from their juvenile and adult stages. The survival of larval fish recruits results in the number of fish that make it to adulthood, which directly correlates to reef and pelagic fish population sizes and diversities that have significant biological and commercial importance. In response to being highly vulnerable to predation in the photic zone of open waters or reefs, fish larvae swim to deeper, darker pelagic waters where they can remain relatively unseen. This study examined whether a greater abundance and diversity of fish larvae would be found further from shore and at deeper depths off the island of Bonaire, Dutch Caribbean. To investigate this, research was conducted at a site close to the fringing reef in front of Kralendijk and a site roughly a kilometer offshore, between Klein Bonaire and Flamingo Airport. At each site, oblique plankton tows were conducted at three depths (≈1.83 m, ≈1.52 m, ≈0.61 m). Samples were analyzed for fish larvae abundance and individual fish larvae were identified to family in order to determine fish larval diversity using Simpson's Diversity Index. Proximity to shore and depth were shown to have statistical significance on fish larval density. However, the same variables were not shown to have statistical significance on fish larval diversity. This study gives insight into the nocturnal vertical distribution of reef and pelagic fish larvae, which had not been previously studied on Bonaire.

Keywords Fish larvae • Depth • Abundance • Plankton • Diversity

Introduction

Many marine fish species, particularly those residing in the deep sea and on coral reefs, are bipartite, meaning they have a pelagic larval stage distinctly separate from juvenile and adult stages. This larval life phase begins when the larvae hatch out of their eggs and can last from weeks to months depending on the species (Johnson et al. 2007). Pelagic fishes have been known to migrate great distances across the Gulf of Mexico and the Caribbean, and therefore distribute their eggs and larvae over great distances across this area (Glazer B, et al. 2007). The vast majority of reef dwelling bony fishes are also bipartite (Leis et al. 2002). In response to being highly vulnerable to predation out in open waters or on the reef, fish larvae swim out to deeper, darker pelagic waters where they can remain relatively unseen (Cowen 2002). To further decrease exposure to predators, fish larvae also avoid residence in the photic zone of the water column during the light of day (Victor 1986). Both pelagic and reef fish larvae will stay in this deep open ocean environment for a majority of the pelagic larval phase. However, larvae will move to protected habitats such as coral reefs for brief periods around the time of the new

moon when there is no moonlight being produced, thereby reducing predator exposure (Victor 1986).

This pelagic larval phase of reef fish is extremely important to the vitality of the coral reef ecosystem because the survival of larval recruits results in the number of fish that make it to adulthood, which directly correlates to fish population size and diversity (Victor 1986). In fact, because of the patchy nature of coral reef ecosystems and the tendency of reef fishes to remain sedentary, the only significant population growth that takes place comes from larval settlement onto the reef (Green et al. 1988). In terms of pelagic fish, the distribution of larvae plays a large role in the resulting pelagic fish populations, which often have high commercial and recreational importance. Many of these large pelagic fish species, such as wahoo, king mackerels, yellowfin tuna, bigeye tuna, and skipjack tuna are harvested by many Caribbean nations and are often fully- or over-exploited (Glazer and Acosta 2007).

However, it is very difficult to directly measure the distribution and abundance of planktonic fish larvae because of their microscopic and discreet nature (Victor 1986). Identification of the larvae is often challenging because the resemblance to their adult phase is minute. In recent years, there has been an increase in research conducted concerning this larval phase of reef fish and their distribution (Cowen 2002; Johnson et al. 2007; Leis 2002) of several coastal locations, but none have been conducted on the island of Bonaire.

This study aims to provide basic information about the vertical larval distribution of pelagic and reef fishes after sunset off the coast of Bonaire, DC. Considering that fish larvae avoid predation by evading exposure to light, including that given off by the moon and stars, it is hypothesized that:

H_1: A higher abundance of fish larvae will be found offshore at deeper depths.

H_2: A greater diversity of fish larvae will be found offshore at deeper depths.

Studying the abundance and diversity of reef and pelagic fish larvae at different depths and distances from shore may give insight into their nocturnal distribution in the water column during their pelagic larvae life stage, which is not currently known in Bonaire.

Materials and methods

Study sites

Plankton tows were conducted at two sites off the west coast of Bonaire, DC (Fig. 1). Site 1 was located just off the fringing reef west of Kralendijk. Tows conducted at site 1 were conducted parallel to shore in both NW and SE directions. Tows conducted at site 2 were located a kilometer offshore out in the open ocean, between the southern tip of Klein and Flamingo Airport on the mainland of Bonaire. Tows conducted at site 2 were conducted in both SW and NE directions.

Plankton tows

Data was collected through plankton tows at sites 1 and 2 (Fig. 1) on the nights of October 10th 2013 and October 23rd 2013. At each site, tows were conducted at three different depths: deep (≈1.83 m), shallow (≈1.52 m), and surface (≈0.610 m). Time constraints limited the number of tows that could be conducted at each site on each date. Six tows (two deep, two shallow, and two surface) were conducted at site 2 and tows were conducted for each depth (deep, shallow, and surface) at each site (1 and 2) after combining tows from October 10th and October 23rd.

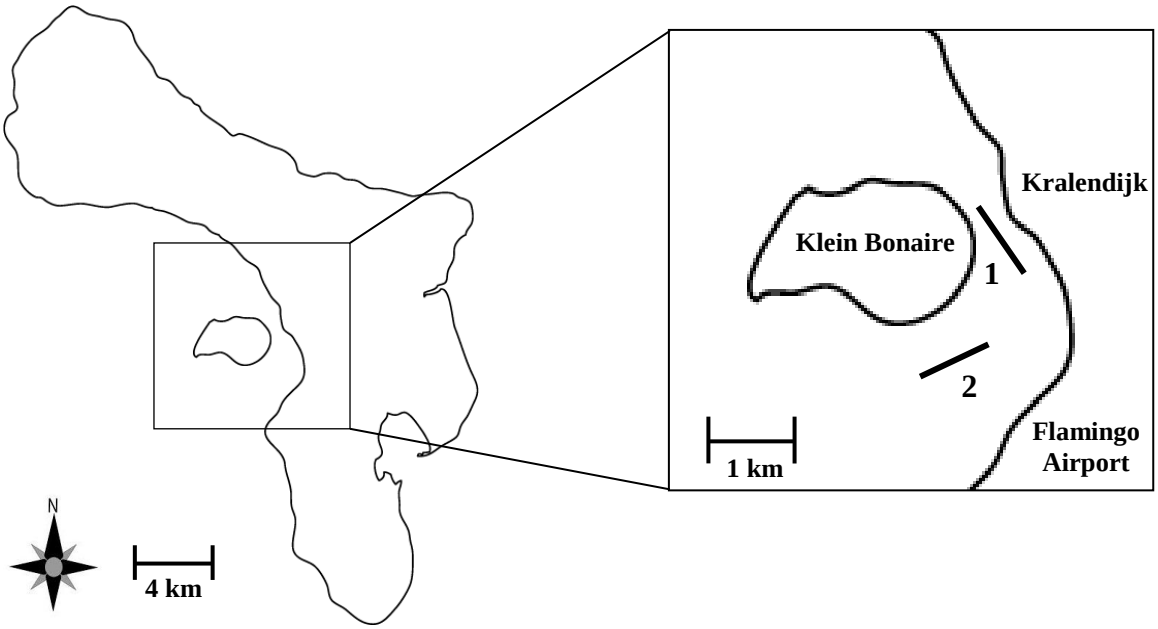

Fig. 1 Map of Bonaire, Dutch Caribbean. *Lines 1* and *2* indicate paths where plankton tows were conducted, which correspond to study sites 1 and 2, respectively (Modified from www.worldatlas.com)

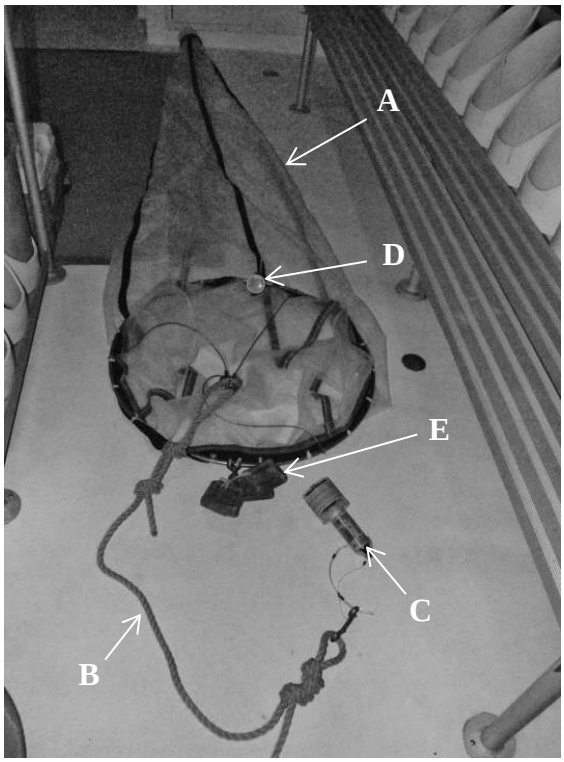

Fig. 2 Setup used for plankton tows. Plankton net (A) has a diameter of 75 cm with a mesh size of 500 µm. Net was towed with a 35 m rope (E) with a flowmeter (D), dive computer (B), and dive weights (C) attached

A 500-µm mesh plankton net with a diameter of 75 cm was used for the tows (Fig. 2). A flowmeter was attached to a portion of the rope used to pull the plankton net (Parra 2008). However, the flowmeter data was not used because the device was not meant to perform under the high speeds of the research vessel. A dive computer was attached to the top of the net to give accurate depth values and dive weights were attached to the bottom of the net to ensure that the net was placed at the appropriate depths: 4.54 kg for the deep depth, 2.67 kg for the shallow depth, and 0.00 kg for the surface depth.

Each tow was conducted for seven min at the speed of seven KTS (minimum speed of research vessel). After each tow, the net was quickly pulled to the surface in order to prevent fish larvae from escaping and the contents of the cod-end of the plankton net were collected. Date, site, and depth were recorded, and the inside of the cod-end was rinsed with saltwater to ensure that all contents were collected. The remaining volume of each sample container was filled with a 70% ethanol solution to preserve the samples for later

lab analysis. Dive computer readings for average depth and max depth were recorded for every tow.

Sample sorting

Fish larvae were separated from the rest of the plankton in each sample using a magnifying glass and a dissecting probe in 10-ml increments. The number of larvae found in each sample was recorded and organized according to date, site, and depth. Fish larvae were photographed individually under a dissection scope with an AmScope (Microscope Digital Camera; Model #MD1800). Photographs were taken using above and below lighting to provide different angles and levels of contrast to allow accurate identification. Photographs of larvae were organized according to date, site, and depth, and individual larvae were preserved in small vessels of 70% ethanol according to date, site, and depth for later use if necessary.

Fish larvae identification

The fish larvae found within each sample were identified (if possible) to their lowest taxonomic level. Resources used to identify the larvae include: Personal observations from Dr. Benjamin Victor, Dr. Benjamin Victor's larval fish identification website (Victor 2008), and Richards (2005). Each fish larva was separated by family and, where possible, a lower taxonomic level.

Fish larvae analysis

Each depth sample was analyzed under a microscope to determine total fish larvae abundance and diversity. Abundance was determined by counting the number of larvae within each sample and displayed in a bar graph according to site and depth. Fish larvae densities were determined for each depth and site by first multiplying the area of the plankton net opening by the distance the net was dragged at a certain speed to calculate the volume of water sampled. The total fish abundance found at each depth was then divided by the volume of water sampled to yield the larval fish density (fish per m^3) for each sample. Fish larval diversity was determined at each depth and site using Simpson's Diversity Index (D):

$$D = \Sigma (n / N)^2$$

In the diversity formula, n = total number of organisms of a particular family, N = the total number of organisms of all families and D = probability that two individuals randomly selected from a sample will belong to the same species. The D value from the index is subtracted from 1, where a higher value represents greater diversity.

Statistical analysis

Two one-way ANOVA's were performed to look at the effect of the explanatory variables (site and depth) on the response variable (fish larvae abundance). Two one-way ANOVA's were also performed using the same explanatory variables on the response variable (fish diversity).

Results

Larval fish abundance

A total of 303 fish larvae and 14 families (See Appendix) were found distributed throughout the contents collected from 18 plankton tows (Table 1). Of these 303 larvae, 252 (83.2%) were able to be identified, leaving 51 larvae (16.8%) unidentified because they were too underdeveloped or damaged from the collection process.

Total larval abundances were determined by averaging the number of larvae found in the three samples taken at each depth and site (Fig. 3). The abundance of fish larvae was significantly

Table 1 Total number of fish larvae found at deep, shallow, and surface depths according to family and site off the coast of Bonaire, Dutch Caribbean. Identifiable fish larvae abundances per family arranged in descending order from most abundant to least abundant. Fish larvae abundances are cumulative values for all three tows (each filtering 665.28 m^3 of water) performed at each depth at sites 1 and 2. "*" indicates reef fish

		Site 1			Site 2			Total #
Fish Families	Common Name	Deep	Shallow	Surface	Deep	Shallow	Surface	
Carangidae	Jacks and Scads	16	4	6	38	19	15	**98**
*Gobiidae	Gobies	9	10	7	10	18	10	**64**
Myctophidae	Lanternfish	11	5	2	2	8	4	**32**
Scombridae	Tunas	8	3	2	4	2	3	**22**
*Pomacentridae	Damselfish	0	0	0	7	1	3	**11**
*Sciaenidae	Drums	1	0	0	5	0	0	**6**
*Apogonidae	Cardinalfish	1	1	1	2	1	0	**6**
*Scaridae	Parrotfish	1	0	1	0	1	0	**3**
Clupeidae	Herrings	0	0	1	0	0	2	**3**
Paralichthyidae	Flounders	0	0	0	1	1	0	**2**
*Eleotridae	Sleeper Gobies	0	0	1	0	1	0	**2**
Gempylidae	Snake mackerels	0	0	0	1	0	0	**1**
*Callionymidae	Dragonets	0	0	0	0	0	1	**1**
Serranidae	Sea basses and Groupers	1	0	0	0	0	0	**1**
Unidentifiable		4	5	5	11	11	15	**51**
Total # of fish larvae found at each depth and site		**52**	**28**	**26**	**81**	**63**	**53**	**303**

higher at site 2 when compared to site 1 (one-way ANOVA, F= 4.16, p= 0.0582).

The abundance of fish larvae was significantly different at deep, shallow, and surface depths (one-way ANOVA, F= 3.29, p= 0.0652) (Fig. 3).

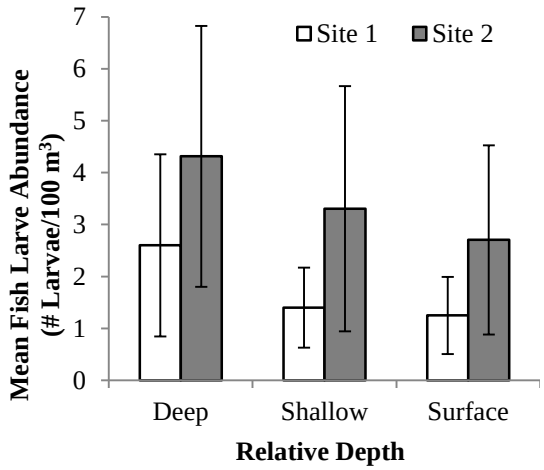

Fig. 3 Mean (± SD) fish larvae abundance per ~100 m^3 at deep, shallow, and surface depths (2 m, 1 m, and 0.5 m respectively) at sites 1 and 2 off the western coast of Bonaire, DC. Data were taken from 18 plankton tow samples in which three tows were conducted at each depth and site. Larval abundances for tows conducted at each depth were averaged to get mean fish larvae abundance for each site and depth

The highest larval densities were the Carangidae, Gobiidae, Myctophidae, Scombridae, and Pomacentridae families (Fig. 4, 5 and 6). For site 1, the deep depth showed the highest abundances of Scombridae, Carangidae, and Myctophidae, while the shallow depth showed the highest abundances of Gobiidae. No fish larvae from the Pomacentridae family were present at site 1. For site 2, the deep depth showed the highest abundance of Carangidae, Scombridae, and Pomacentridae, while the shallow depth showed the highest abundance of Gobiidae and Myctophidae.

Fish larvae density

Using the speed of the research vessel (≈7 KTS) and the time each tow was conducted for (≈7 min), the approximate distance of each tow was calculated to be

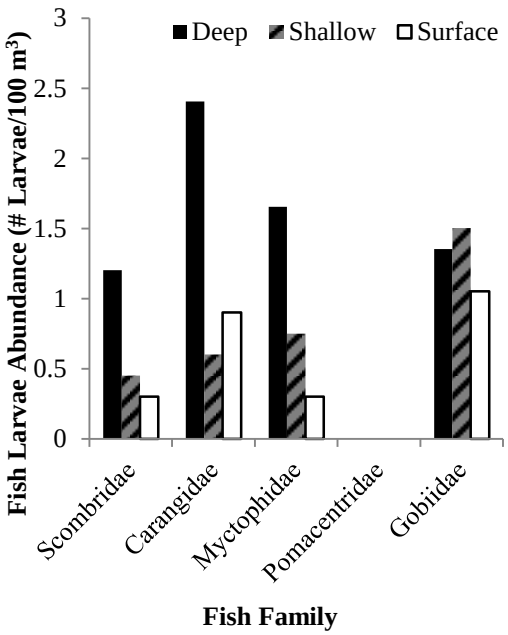

Fig. 4 Total fish larvae abundance (# larvae/100 m^3) per family found at deep, shallow, and surface depths (2 m, 1 m, and 0.5 m respectively) at site 1 off the coast of Bonaire, Dutch Caribbean. Families displayed are of the highest abundances found at sites 1 and 2

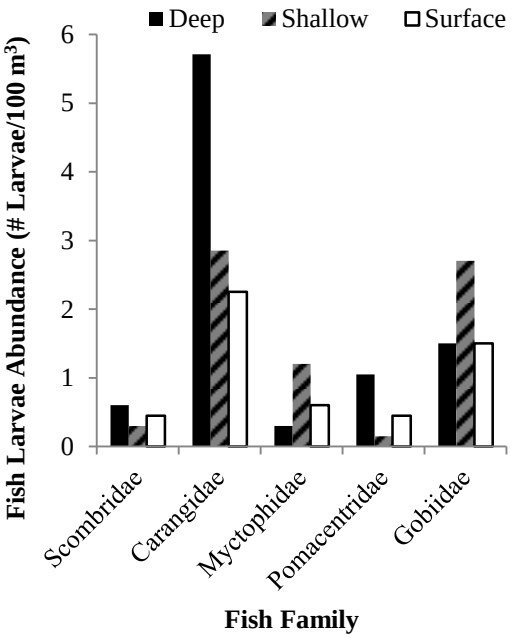

Fig. 5 Fish larvae abundance (# larvae/100 m^3) per family found at deep, shallow, and surface depths (2 m, 1 m, and 0.5 m respectively) at site 2 off the coast of Bonaire, Dutch Caribbean. Families displayed are of the highest abundances at sites 1 and 2

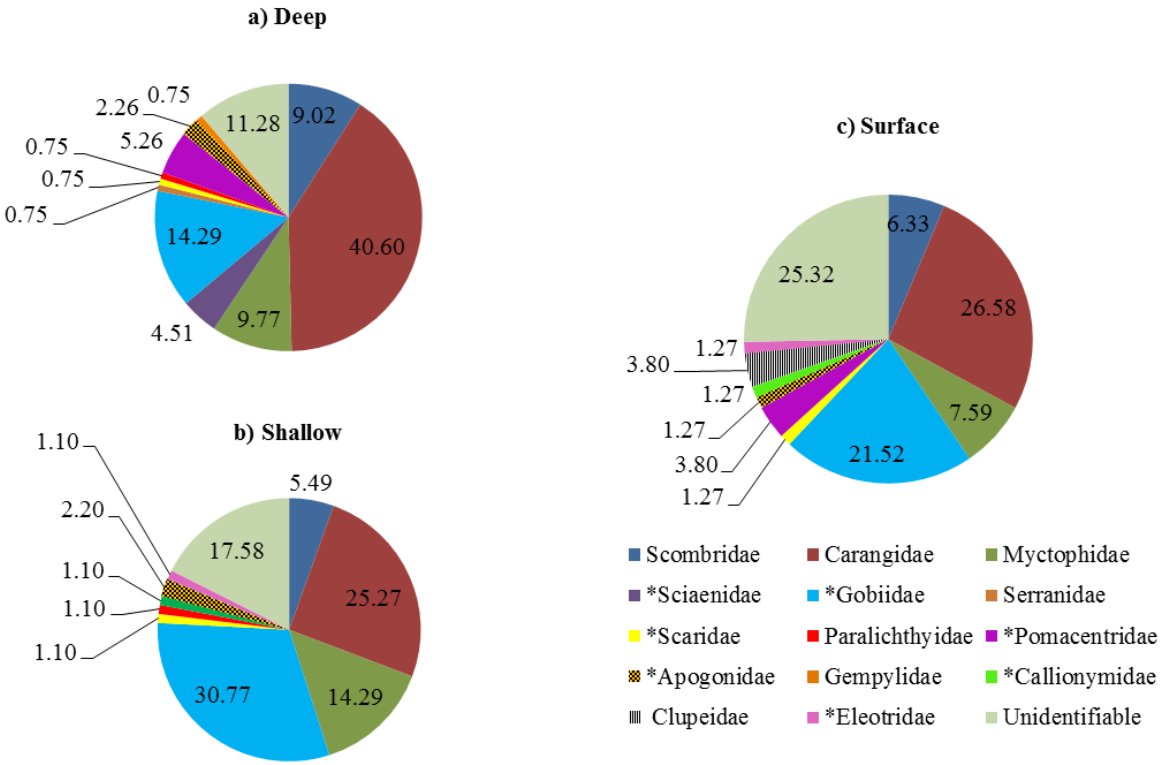

Fig. 6 Pie charts displaying the percent abundance per 665.28 m^3 (volume of water filtered by one plankton tow) of fish larvae from 14 families, including unidentifiable, at three different depths off the coast of Bonaire, Dutch Caribbean; a) deep, b) shallow, and c) surface

≈1512 m. The area of the plankton net opening (πr^2) was calculated to be 0.442 m^2. The volume of water filtered by the plankton net for every tow was calculated to be ≈665.28 m^3 by multiplying the area of the plankton tow net (≈0.442 m^2) by the distance of the individual tows (≈1512 m).

The deep depth samples have the highest fish larvae density per 100 m^3 (10.0 ± 1.76 fish larvae/100 m^3), followed by the shallow depth (6.84 ± 0.77 fish larvae/100 m^3), and finally the surface depth (5.94 ± 0.83 fish larvae/100 m^3), which was shown to have statistical significance (one-way ANOVA, F= 3.33, p= 0.0637). The samples from site 2 (1 km offshore) have the highest fish larvae density per 100 m^3 (9.87 ± 2.01 fish larvae/100 m^3) compared to site 1 (5.31 ± 1.22 fish larvae/100 m^3), which was shown to have statistical significance (one-way ANOVA, F= 3.77, p= 0.0699).

Fish larvae diversity

Fish larval diversities for each sample were determined using Simpson's Diversity index (Itô 2007). Diversity indexes for the three samples at each depth for sites 1 and 2 were averaged in order to get average diversity indexes for each depth at each site. The highest fish larval diversity at site 1 was found at the shallow depth (0.749 ± 0.041), followed closely by the surface depth (0.731 ± 0.080), and lastly the deep depth (0.682 ± 0.077). The highest larval diversity found at site 2 was found at the deep depth (0.701 ± 0.081), then the surface depth (0.681 ± 0.150), and lastly the shallow depth (0.681 ± 0.097). There was slight evidence, shown by statistical testing (one-way ANOVA, F= 3.078, p= 0.0758), that depth (regardless of site) has an effect on fish larval diversity. However, fish larval diversity found at varying sites (regardless of depth) was also not found to have statistical significance (one-way ANOVA, F= 1.15, p= 0.299).

Discussion

A higher abundance, and therefore density, of fish larvae was found at site 2 (1 km offshore) and at deeper depths which supports hypothesis (H_1). Statistical testing weakly supported the significance of the effect of depth on fish larval diversity. Proximity to shore was not shown to have a significant effect on fish larval diversity. Based on the lack of statistical significance, H_2 was rejected, which states that a higher diversity of fish larvae will be found offshore at deeper depths.

The distribution pattern of fish larvae on Bonaire was found to follow the same basic distribution pattern as those found off of other reef locations (Cowen 2002; Johnson et al. 2007; Leis 2002), which is that they tend to concentrate in deeper waters further away from the reef (Johnson et al. 2007). However, the depths at which this research was conducted were less varied in vertical depth than those performed in other experiments (Cowen 2002; Johnson et al. 2007; Leis 2002), which measured fish larval distribution at depths from zero down to 50 m. The variance in the distribution of fish larvae found by this research throughout a vertical difference of only ≈ 1.8 m was found to be statistically significant, which shows that depth has a very large effect on fish larval distribution even on a small scale.

The most likely explanation for why higher abundances of fish larvae were found far from shore and at deeper depths is because the reef habitat is extremely hazardous for larval fish. Due to the need for coral to grow in relatively shallow photic zones, the reef habitat limits the fish larvae's ability to escape light exposure, which makes them visible to predators which increases predation (Johnson el at. 2007). Reef habitats host a much higher density of predatory fishes than in open waters (Hixon 1991). Hixon (1991) found that 8-53% of the fish found in an area of

the reef consume other fishes, including fish larvae, which greatly decreases the changes of survival for larval fish. The fish species that spend their larval stage far off the reef and in deeper, aphotic waters, have a much higher chance of avoiding predation and increasing their chances of survival (Victor 1986).

The fact that fish larvae distributions were analyzed at night when there was no sunlight did not change their vertical distribution pattern from the one that is expected during the day, in terms of relative depth and abundance. The light produced by the moon and stars still has a significant effect on fish larvae distribution (Victor 1986) because both produce a significant amount of light, which still creates a photic zone. Although the moonlit photic zone does not extend down as far as when created by sunlight due to the fact that the transmittance factor of moonlight is six to seven times lower in magnitude than sunlight (Baker and Smith 1990), it still creates a lit shallow zone that fish larvae avoid in order to reduce exposure to predators.

Proximity to shore may not have been found to be a significant factor for fish larvae diversity because of the collection methods used in this experiment. Pulling a slow net when reef fish aren't settling could explain why a majority of the fish caught were early stage pelagic fish larvae and not reef fish entering their settlement stage (Dr. Benjamin Victor, personal communication). The pelagic fish larvae collected were most likely the larvae that are consistently present in offshore waters (Victor, personal communication). A faster net, the appropriate time of the month, and a more successful settlement season may have yielded a higher abundance of reef fish larvae, which would have most likely also yielded a higher diversity due to the corresponding high fish diversity found on the reef. However, it is worth mentioning that although depth was not shown to have statistical significance on the level of fish larval diversity, it did present a possible trend which could have resulted in statistical significance if more samples were taken.

There are a number of aspects of this experiment that could be modified for improvement. First, an increased number of samples taken at each depth would have greatly improved the accuracy of the results of this research. Second, a greater vertical distance between the depths of the tows would have given a better, more definite representation of the vertical distribution of fish larvae. This could be achieved by either adding more weight to the plankton net or pulling the net at slower speeds. Third, the effect of lunar cycle on the distribution of fish larvae could be observed by conducting numerous plankton tows throughout the lunar cycle.

The information provided by this research has given new insight into the vertical distribution pattern of fish larvae (both reef and pelagic) on the island of Bonaire, DC. Results could be used to predict future reef and pelagic fish populations which have great importance for the health of the reef ecosystem and for commercial and recreational fishing industries. The methodologies from this experiment could also be applied to other coastal locations and be used to determine approximate fish larvae abundance and diversity.

Acknowledgements I would like to first thank the CIEE Research Station as well as all CIEE professors and staff for providing me with the opportunity to fulfill my lifelong dream of studying abroad and study marine biology. I want to thank my advisor Dr. Patrick Lyons and interns McCrea Sims and Estelle Davies, with their advice, guidance, encouragement, and enthusiasm I was able to successfully conduct my research and produce a formal research paper. I also want to thank our favorite boat captain, Menno de Bree, for the use of his boat for the conduction of my plankton tows and to all my CIEE colleagues who helped me carry gear, pull in numerous nets, and offer positive support during my research boat trips. I also want to thank my research buddy, Celeste Moen, for her dedication to helping me in every aspect of my project including spending long

hours in the lab sorting plankton samples and assisting me during my plankton tow trips. I want to offer a special thanks to Dr. Benjamin Victor in helping me with the identification of my fish larvae. Lastly, I would to thank my parents for their unwavering support and encouragement, without them this research would not have been possible.

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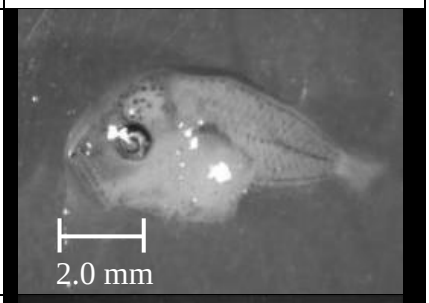
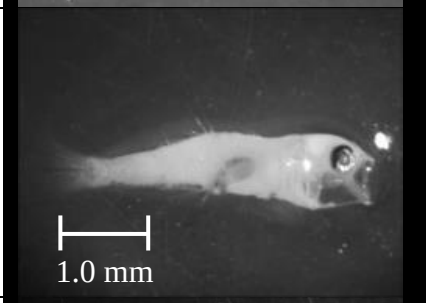
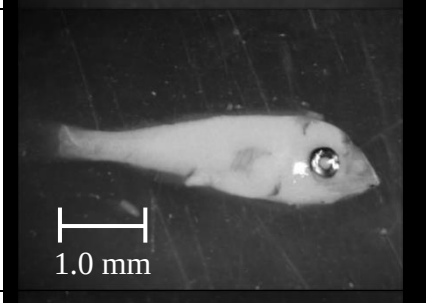
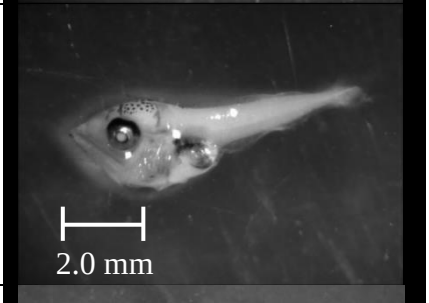
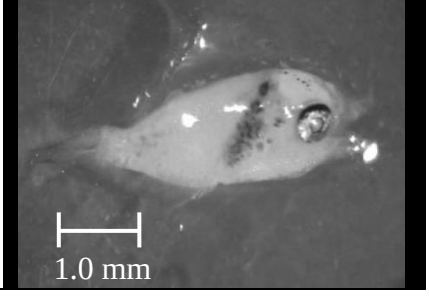
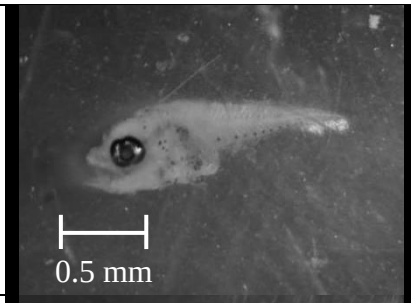
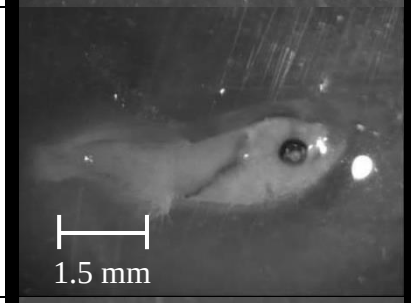
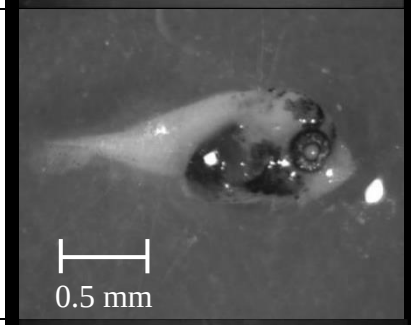
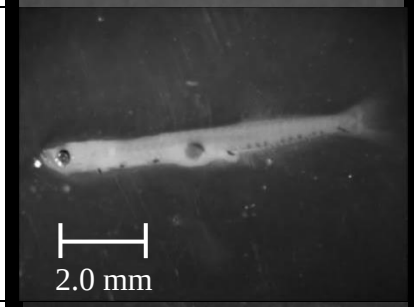
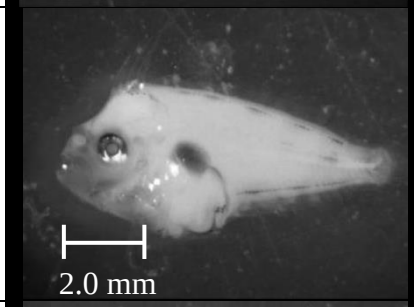
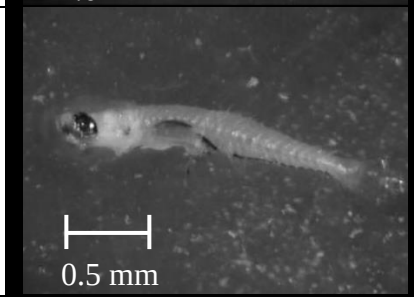
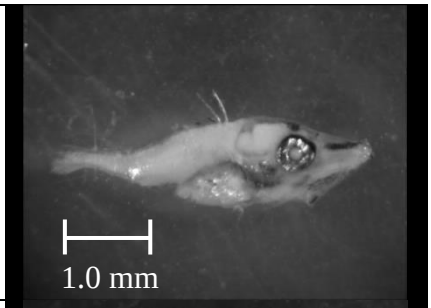
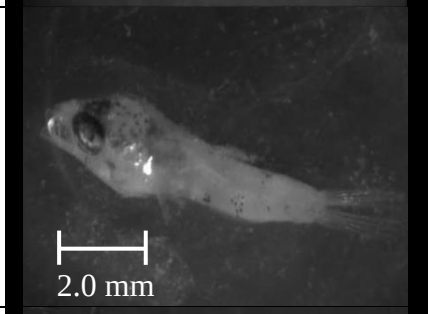
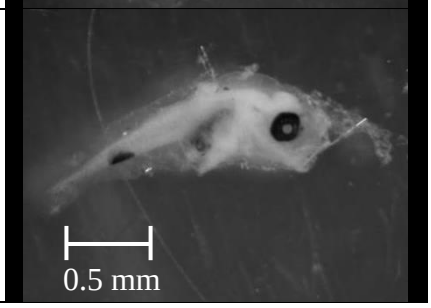
Appendix

Fish larvae representatives for each family found in plankton tow samples on Bonaire, Dutch Caribbean. "*" indicates reef fish families.

Reference #	Fish Family	Common Name	Larvae Representative
1	Carangidae	Jacks and Scads	
2	*Gobiidae	Gobies	
3	Myctophidae	Lanternfish	
4	Scombridae	Tunas	
5	*Pomacentridae	Damselfish	

6	*Sciaenidae	Drums	
7	*Apogonidae	Cardinalfish	
8	*Scaridae	Parrotfish	
9	Clupeidae	Herrings	
10	Paralichthyidae	Flounders	
11	*Eleotridae	Sleeper Gobies	

12	Gempylidae	Snake Mackerels	
13	*Callionymidae	Dragonets	
14	Serranidae	Sea Basses and Groupers	

Physis (Fall 2013) 14:61-68

REPORT

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Distribution, substrate preference and possible host benefits of the tropical polychaete *Spirobranchus giganteus* on a reef in Bonaire

Abstract The Christmas Tree worm, *Spirobranchus giganteus*, is a sessile polychaete found on coral reefs worldwide. Its larvae display photopositive behavior and use chemicals excreted from live coral as a settlement cue. After settlement, the worm grows in concert with the coral for the remainder of its life. *S. giganteus* displays variable preference for corals as substrate worldwide. It has been shown that the presence of *S. giganteus* on corals can lessen the effects of disease and coral bleaching on polyps surrounding the worm's tube, as well as lessening predation by certain corallivores. Due to its potential to maintain healthy corals, it is important to learn more about the ecological role and distribution of *S. giganteus*. This study aimed to determine at what depth *S. giganteus* densities are highest, as well as its preferred coral substrate on the fringing reef surrounding Bonaire, Dutch Caribbean. It also intended to demonstrate that the presence of *S. giganteus* could lessen the effects of disease and bleaching on the corals on which it lives. Using benthic transects at three different depths at two study sites, it was found that *S. giganteus* density was highest at shallower depths. The preferred substrate was found to be the boulder star coral, *Orbicella annularis*. Data regarding the effects of *S. giganteus* on coral disease and bleaching was too limited to determine any relationship between them, but it is recommended that this relationship be investigated in depth due to its possible role in recovering from coral bleaching.

Keywords *Spirobranchus giganteus* • Distribution • Substrate preference • Bonaire

Introduction

The Christmas Tree worm, *Spirobranchus giganteus*, is a sessile polychaete worm found in tropical waters worldwide. Its name derives from the visible portion of the worm, which comprises two spiraling branchial extensions used to filter nutrients from the water (Waldrop and Kier 2007). It has a larval stage that lasts from 9-12 days. During the larval stage, it exhibits non-habituating photopositive behavior (Marsden 1984). This behavior is likely a result of larvae spending the majority of their time relatively shallow in the water column. *S. giganteus* larvae prefer live coral as a substrate for settlement (Hunte et al. 1990), although it has been found living on coral rubble and other sturdy substrates, such as anchors or barrels (Williams 2009, personal field observations). They build a calcium carbonate tube on the surface of the corals. The worm adds to its tube as the coral grows and coral polyps surround the tube.

S. giganteus is known to prefer certain corals for settlement (Marsden et al. 1990). However, the preferred coral seems to vary in different areas; in Japan, it was found to prefer different species of *Porites* (Nishi and Kikuchi 1996), in Taiwan, the preferred corals were three species of *Porites* and the acroporid *Montipora informis* (Dai and Yang 1995), in

Barbados it preferred *Diploria strigosa* (Hunte et al. 1990), and in South Africa it preferred *Acropora clathrata* (Floros et al. 2005).

Prior research has shown that the relationship between *S. giganteus* and the corals on which it lives is mutualistic. DeVantier et al. (1986) found that coral polyps adjacent to living *S. giganteus* individuals were protected from predation by the Crown of Thorns sea-star, *Acanthaster planci*. Water circulation created by the filtering action of the worm's two branchial extensions increases the amount of food available to coral polyps. Higher circulation rates may also decrease the coral's chances of contracting diseases as well as reducing the possibility of coral bleaching (Ben-Tzvi et al. 2006). Because of the potential for improved coral health due to the presence of *S. giganteus*, it is important to understand more about the settlement habitats of this species.

S. giganteus is frequently found on the fringing reef surrounding the island of Bonaire, Dutch Caribbean. Previous research concerning *S. giganteus* on the reefs of Bonaire has found that it could be used as a bioindicator for the health of Bonaire's reefs (Williams, 2009). The aim of the current study was threefold: whether or not *S. giganteus* will display a substrate preference among the corals found on the reef in Bonaire; whether or not there will be a difference in the density of *S. giganteus* associated with depth; and whether or not there is evidence of *S. giganteus* lowering the chances of disease and bleaching for its chosen substrate.

To address these questions, the following hypotheses were tested:

H_1: The density of *S. giganteus* will decrease as depth increases, based on the photopositive behavior of its larvae and their response to chemical cues for settlement

H_2: *S. giganteus* will display a preference for *Orbicella annularis*

H_3: There will be a negative correlation between the presence of *S. giganteus* on coral substrates and the presence of disease or coral bleaching on those corals

Materials and methods

Study site

The research took place at two common dive sites on the island of Bonaire, Dutch Caribbean: Yellow Submarine (12.160053, -68.2822) and Something Special (12.161367, -68.283624). Both sites consist of a fringing reef roughly 50 meters from the shore in Kralendijk, the capital of Bonaire. Something Special is located roughly 200 meters north of Yellow Submarine on Kaya J.N.E. Craane (Fig. 1). The reef itself begins at roughly 7.5 m. of depth, and extends to between 27 and 30 m., where it ends in a wide sand flat with scattered corals.

Fig. 1 Map of Bonaire, Dutch Caribbean and the two study sites, Yellow Submarine (12º9'37" N, 68º16'56" W) and Something Special (12º9'41", 68º,17'0" W) Modified from Google Maps [www.maps.google.com]

Data collection

Data collection took place over a five-week period between October and November of 2013. Three 30-m transects were laid end-to-end at three separate depths; three at 10 m., three at 12 m., and a final three at 15 m. (Fig. 2), for a survey area of 180 m² at each depth, 540 m² at

each study site, and 1080 m² total. In an attempt to avoid startling the worms and causing them to hide, a two-meter wide section of the reef was surveyed by two divers (one meter each) on the first pass, using the transect line as the mid-point. Any coral 20 cm^2 or larger in size showing colonization by *S. giganteus* was identified to the lowest taxonomic level possible and the total number of *S. giganteus* on each coral was counted. In order to determine overall abundance, all corals >20 cm² were counted as well. At Something Special, any instance of coral bleaching or coral disease in the study area was recorded to determine the relationship between the worms and these coral stressors.

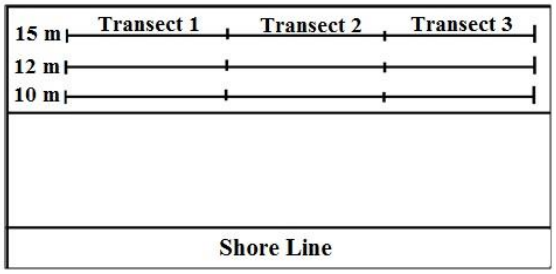

Fig. 2 Experimental set-up for both locations

Data analysis

To ensure that data from transects at the same depth could be combined, a one- way ANOVA (factor: transect position) was performed to determine whether there were any differences in the number of worms observed per transect. After pooling data from different depths, Student's t-tests were performed comparing the densities of worms at different depths.

Analysis of coral cover consisted of determining an estimate of surface area, the percent cover of the area surveyed, and the number of coral colonies at each study site, using the methods employed by Hunte et al. (1990).

Data concerning the presence of bleaching and disease on corals were analyzed using Pearson's statistical correlations.

Results

Depth

Density of *S. giganteus* at depth was calculated using the total number of worms observed at each depth, divided by the total survey area (540 m² for each site). A one-way ANOVA (factor: study site) revealed that the two study sites were not similar enough to allow for pooling of the data, so density was measured separately for each study site (Fig. 3). Statistically significant differences were observed between all depths at Yellow Submarine ($p<0.01$for each depth). Only the difference between worms at 10 m. and 15 m was significantly different at Something Special ($p=0.008$).

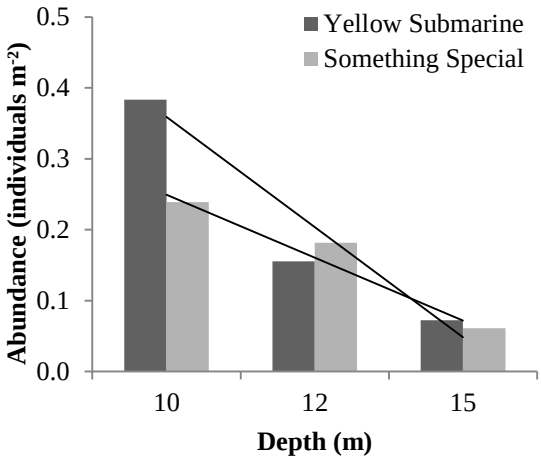

Fig. 3 Abundance of *Spirobranchus giganteus* (individuals/m²) at depths of 10, 12, and 15 m at Yellow Submarine and Something Special. Yellow Submarine: n=207, 84, 38 for 10, 12, and 15 m, respectively.R^2=0.933. Something Special: n=129, 98, 33 for 10, 12, and 15 m, respectively. R^2= 0.9599

Coral preference

Table 1 shows the total number of coral colonies, number of colonized colonies, percent of total colonization, number of worms, and worm abundance for 15 coral species over 540 m² at each study site. Percent of colonization was determined by dividing the number of colonized corals in each species by the total number of

colonized corals. Worm abundance was calculated by dividing the number of worms found on each coral species by the total area surveyed (540 m²), then extrapolating that number to individuals per 100 m². *Undaria agaricites* was the most abundant coral species at both sites, followed by *Orbicella faveolata* at Yellow Submarine and *O. annularis* at Something Special. *O. annularis* was the most frequently colonized coral, both by the number of colonies that contained worms (Yellow Submarine=70 colonies, Something Special= 60) as well as the percent of colonization (Yellow Submarine=36.65%, Something Special =33.15%). It also had the highest number of total worms (Yellow Submarine=144, Something Special=91) and the highest number of worms/100 m² (Yellow Submarine=27, Something Special=17). Fig. 4 and Fig. 5 show the number of colonies observed on each coral species and the total number of worms found. At both sites, *O. annularis* displayed the highest numbers of worms, regardless of the abundance of coral species. Relative worm densities varied

Table 1 Coral colonies (n), number of colonized colonies (C), percent coral species colonized (%C), number of worms on each species (worms), density of worms (/100 m²). Species organized from highest to lowest densities as observed at Yellow Submarine

Yellow Submarine					
Coral Species	n	C	%C	worms	/100 m²
Orbicella annularis	168	70	36.65	144	27
Dead Coral	93	35	18.32	56	10
Undaria agaricites	373	30	15.71	40	7
Porites astreiodes	124	20	10.47	27	5
Orbicella faveolata	197	12	6.28	21	4
Millepora complanata	19	13	6.81	17	3
Orbicella cavernosa	118	8	4.19	9	2
Porites porites	6	2	1.05	13	2
Siderastrea siderea	44	1	0.52	1	0
Millepora alcicornis	4	0	0	1	0
Diploria strigosa	10	0	0	0	0
Colpophyllia natans	7	0	0	0	0
Diploria labyrinthiformis	15	0	0	0	0
Eusmilia fastigata	81	0	0	0	0
Meandrina meandrites	60	0	0	0	0
Total	1319	191	100	329	4

Something Special					
Coral Species	n	C	%C	worms	/100 m²
Orbicella annularis	134	60	33.15	91	17
Dead Coral	118	21	11.6	25	5
Undaria agaricites	301	23	12.71	24	4
Porites astreiodes	126	33	18.23	36	7
Orbicella faveolata	98	14	7.73	32	6
Millepora complanata	50	12	6.63	29	5
Orbicella cavernosa	112	10	5.52	3	1
Porites porites	18	1	0.55	6	1
Siderastrea siderea	47	4	2.21	7	1
Millepora alcicornis	3	0	0	0	0
Diploria strigosa	17	1	0.55	4	1
Colpophyllia natans	7	1	0.55	1	0
Diploria labyrinthiformis	18	1	0.55	1	0
Eusmilia fastigata	97	0	0	0	0
Meandrina meandrites	68	0	0	0	0
Total	1214	181	100	259	3

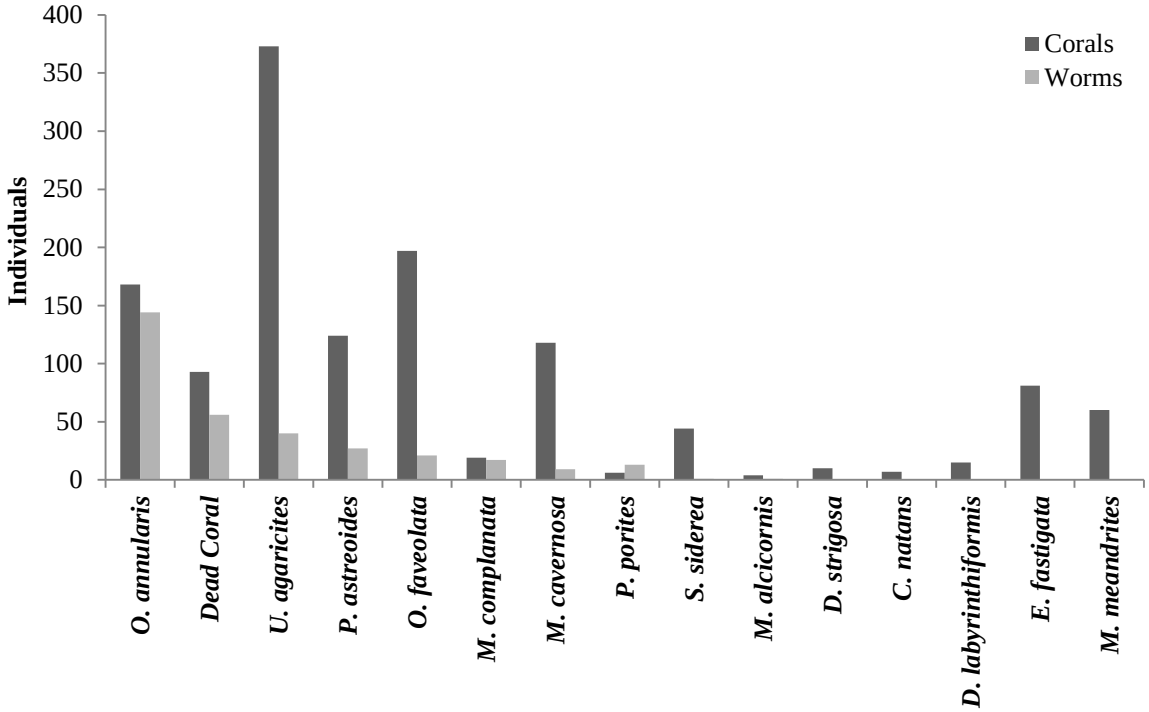

Fig. 4 Total number of coral colonies of each species and total number of *Spirobranchus giganteus* individuals found on each coral species; data from Yellow Submarine

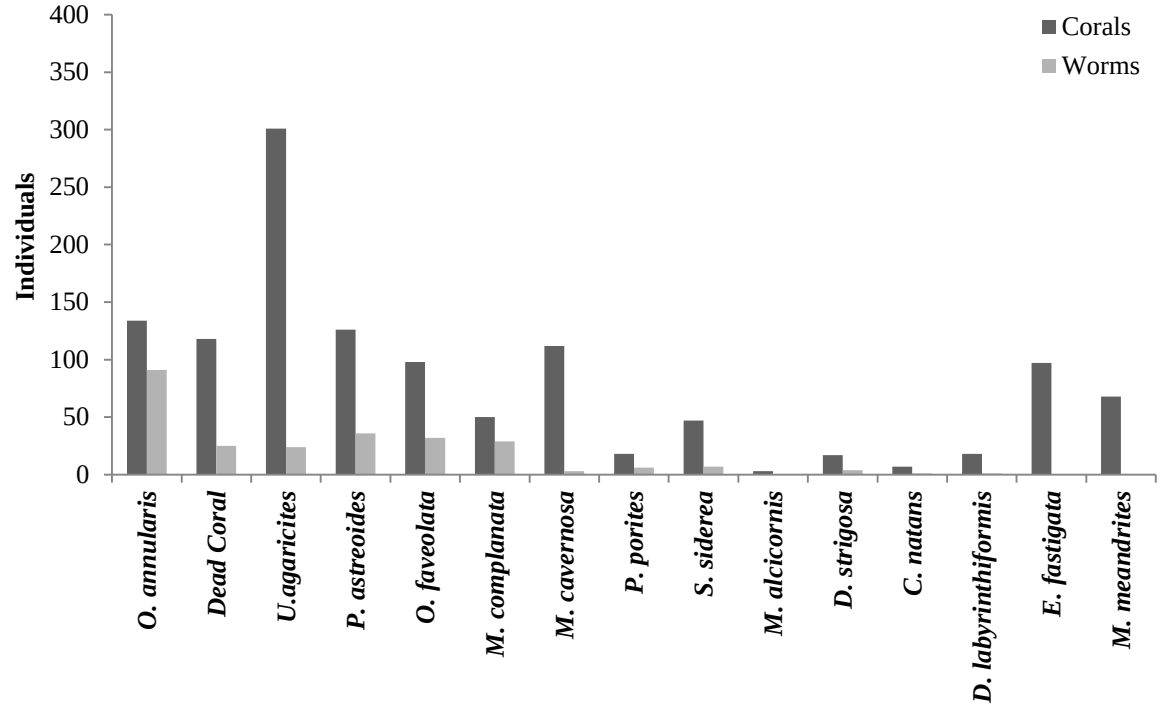

Fig. 5 Total number of coral colonies of each species and total number of *Spirobranchus giganteus* individuals found on each coral species; data from Something Special

between sites for all other coral species observed.

There was not enough data concerning disease and bleaching to obtain any meaningful results regarding the effects of the presence of *S. giganteus* on stressed corals. Preliminary results are shown in Table 2.

Table 2 Preliminary results showing presence/absence of worms on bleached/diseased corals at Something Special

Something Special		
Species	Stressor	Worms Present?
	10 m	
S. siderea	Dark spot	No
O. annularis	Dark spot	No
O. annularis	Bleaching	No
	12 m	
S .siderea	Dark spot	No
	15 m	
None	None	N/A

Discussion

Depth

From the data collected, it is clear that *S. giganteus* is more densely populated at shallower depths, supporting the first hypothesis tested. The differences in abundance at Yellow Sub were all statistically significant, and the R^2 value of 0.933 shows that the differences observed are strongly correlated with the changes in depth. The only statistically significant difference in abundance vs. depth at Something Special was found between 10 m and 15 m. Despite the lack of statistically significant differences between 10 m and 12 m and between 12 m and 15 m, the relationship between depth and abundance is clearly shown from the high R^2 value obtained.

This contradicts the results found by Floros et al. (2005), where they found a highly variable bathymetric distribution of *S. giganteus* on reefs in South Africa. Dai and Yang (1995) also found that the distribution was variable on the reefs of Taiwan. However, Hunte et al. (1990) found higher densities on reefs from 10 to 16 m vs. reefs found between 16 to 22 m on reefs in Barbados. The similar findings between this research and that of Hunte et al. (1990) could be explained by the fact that both study sites are in the Caribbean. It seems that the relationship between depth and distribution of *S. giganteus* varies depending on the geographic location of the reefs in question. In the Caribbean, density seems to be higher at shallower depths. This could be as a result of many factors, from a variation in the size of food particles to anthropogenic factors such as increased nutrient loading on reefs. As such, further research examining this phenomenon is recommended.

Coral preference

As seen in Table 1, *O. annularis* was the most frequently colonized coral, and the coral species with the highest observed densities of *S. giganteus* at both Yellow Submarine and Something Special. *O. annularis* was also one of the most frequently occurring coral species overall, which could account for its high colonization rate. However, Fig. 4 and Fig. 5, which show the total number of coral colonies for each species and the total number of *S. giganteus* found on each coral species contradicts that theory. If colonization were based solely on which corals were most abundant, *U. agaricites*, would be expected to have the highest observed densities of *S. giganteus*. In addition, it could be extrapolated that the number of worms would be higher on other frequently occurring corals as well. However, no other coral species approaches the densities observed on *O. annularis*, even corals that occur as frequently as or more frequently than *O. annularis*, such as *O. faveolata* at Yellow Submarine and *U. agaricites* at both sites. In addition, there were no *S. giganteus* individuals found on two moderately occurring corals, *Eusmilia fastigata* and *Meandrina meandrites* (Fig. 4 and Fig. 5), which Hunte et al. (1990) also observed on reefs in Barbados. The data collected strongly supports the hypothesis that *O. annularis* is the preferred coral substrate

for *S. giganteus* on reefs in Bonaire. However, the reasons for coral preference are still relatively unknown. Coral species and genera that have been found to be a preferred substrate in separate experiments were present in this study as well, yet the colonization of these corals differed strongly between studies. *D. strigosa* was found to be the preferred substrate in Barbados (Hunte et al. 1990), but displayed one of the lowest colonization percentages in this study (Table 1). In Taiwan, Dai and Yang found that *Porites* species were the preferred substrates for settlement (1995), while in Bonaire, the two *Porites* species found on the reef *(Porites astreoides* and *Porites porites*) were moderately colonized (Table 1). This suggests that substrate preference can change depending on the location of the reef in question. Further research examining the reasons for coral choice are suggested due to this relative lack of knowledge.

Bleaching and disease

Due to time constraints, data regarding coral bleaching and disease was only collected from Something Special. The data collected was limited; only four total instances of bleaching or coral disease were observed during the surveys of the reef. There was one instance of a *S. giganteus* individual on a bleached coral observed outside of the study time and area (Appendix, Fig. 6). However, this is still a very interesting and important subject, and further research into this area of study is important in helping to further understand the relationship between *S. giganteus* and the corals on which it lives.

S. giganteus is found throughout the tropical and subtropical oceans of the world, yet its distribution seems to vary among the different environments it lives in. Relatively little research has been done to investigate the factors that determine where *S. giganteus* settles and how it decides on a substrate. Much of the research has focused on larval response to chemicals excreted by live corals, while other studies have merely examined the distribution of the worms as opposed to the reasons for their distribution. The corals preferred have been found to vary in different locations, both globally (Caribbean Sea vs. Western Pacific) and on a smaller scale (Barbados vs. Bonaire). Further research investigating the factors that determine substrate preference and bathymetric distribution of these worms is a logical next step in understanding as much as is possible concerning these organisms.

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Appendix

Fig. 6 Bleached coral containing *Spirobranchus giganteus* individual; note unbleached polyps surrounding worms tube. Photo credits to Yannick Mulders

Physis (Fall 2013) 14:69-78

REPORT

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Utilization of smaller grouper species (*Cephalopholis cruentata, Cephalopholis fulva, Epinephelus guttatus, Epinephelus adscensionis*) densities as a coral reef health indicator

Abstract Serranidae, the grouper family, are common carnivorous fish that inhabit Bonaire's coral reefs. Smaller grouper species are commonly spotted along the lower layers and crevices under the complex coral reef structure. These carnivores control population levels of lower trophic level omnivores (e.g. damselfish). Four smaller grouper species (*Cephalopholis cruentata*, *Cephalopholis fulva*, *Epinephelus guttatus*, and *Epinephelus adscensionis*) densities were used as indicator to evaluate current coral reef health on Bonaire. Maximum reef relief was estimated to evaluate for consistency in reef complexity across box transects. AGGRA fish methodology was used to survey densities of targeted Serranidae species. Recorded densities were compared to previously reported grouper densities from 2003 to 2011. A significant increase in density for *C. cruentata* (9.58 individuals/100 m^2) and *E. guttatus* density (4.17 individuals/100 m^2) was found since 2011. *E. adscensionis* and *C. fulva* densities were found to be consistent since 2003. Overall, a healthy coral reef was supported by the evidences of an increase in *C. cruentata* and *E. guttatus* since 2011. This increase in Serranidae density is controversial to commonly proposed competition between lionfish and native carnivores. Lionfish removal efforts are hypothesized to positively affect smaller Serranidae density, leading to a higher density in 2013. A biocontrol mechanism is proposed as a long-term solution following lionfish invasion. Future directions are discussed in regards to maintaining a resilient Serranidae population on Bonaire's coral reef by establishing marine reserves and continued removal of *Pterois* spp. individuals.

Keywords Serranidae • Density • Reef health indicator • Lionfish • Bonaire

Introduction

Serranidae, also known as the grouper family, are common carnivorous species that dwell along the tropical coral reef habitat. In shallow fringing reefs, smaller grouper species are commonly sighted hidden along the lower level of coral reef structures (Nagelkerken et al. 2005). Four of the common groupers species surveyed by Steneck et al. (2011) in Bonaire are found along the fore reef crest, including *Cephalopholis cruentata* (Graysby), *Cephalopholis fulva* (Coney), *Epinephelus guttatus* (Red hind), and *Epinephelus adscensionis* (Rock hind). Despite the relatively smaller body size of these grouper species, they are high trophic-level carnivores in a coral reef system (Steneck et al. 2007; Steneck et al. 2011). Groupers territorial nature allows utilization of their population density as a health indicator of reefs habitat (Kramer 2003; Toller et al. 2010). The high abundance of groupers in an ecosystem signals an intact trophic network (Toller et al. 2010). In addition, groupers are found

to regulate intermediate trophic-level fish species that are generally omnivores (Stallings 2008). The behavior and the trophic niche of these grouper species provide coral reef scientists with an effective indicator group for monitoring ecosystem health (Kramer 2003; Steneck et al. 2011).

Steneck et al. (2007) developed trends to explain the implications of population changes in individual indicator groups in a coral reef ecosystem. Among the indicators, *C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis* are categorized as large carnivorous fishes. In theory, population fluctuation of one indicator group will exert positive or negative selection pressure on another indicator population (Steneck et al. 2007; Steneck et al. 2011). The increase in grouper population positively impacts coral recruitment, coral cover, and population of larger herbivores (Steneck et al. 2007; Steneck et al. 2011). On the other hand, population of territorial damselfish, macroalgae abundance, and nutrient abundance will experience a reduction (Steneck et al. 2007; Steneck et al. 2011). The increase in large carnivorous fishes will result in a positive trend that enhances a coral-based ecosystem (Steneck et al. 2007; Steneck et al. 2011).

Opposite to the "positive" trend, Steneck et al. (2007, 2011) also described a negative progression on coral reef ecosystem. In the case of a reduced population abundance of large carnivorous fishes, herbivory, coral recruitment, and coral cover would decrease (Steneck et al. 2007). The reduction in abundance of large carnivores facilitates an increase in territorial damselfishes (Steneck et al. 2007). The reef system results in a shift towards an algal-based ecosystem due to increased nutrients and reduced herbivory (Steneck et al. 2007, Steneck et al. 2011). An exploited population of Serranidae is said to also result in a lower recruitment of lower trophic-level organisms. Stallings (2008) proposed the mechanism for a lowered recruitment as a consequence of the increased abundance of intermediate omnivores (damselfishes), which prey on lower trophic-level fishes (gobiidae) or smaller mobile invertebrates.

The wide ecological implications developed from the population density of the Serranidae family closely relates to that of the coral reef (Toller et al. 2010). Being commercially important species, overfishing has been the leading cause for the local reduction of Serranidae populations (Nagelkerken et al. 2005; Stallings 2008; Toller et al. 2010). Regions of low carnivore density (e.g. Serranidae) are said to be an indicator of human-induced fishing pressure (Kramer 2003). Despite the historically reduced grouper population, the degree of reef degradation has been found to negatively impact grouper density (Nagelkerken et al. 2005). The reduction in reef complexity was found to be the primary factor driving migration of *C. cruentata* into deeper reefs (Nagelkerken et al. 2005). Thus, reef complexity is essential for housing groupers. Furthermore, Steneck et al. (2013) concluded that systematic monitoring on indicator groups is essential to pre-determine the health and problems of a coral reef. This study aims to provide a comparison between the years of smaller grouper species (*C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis*) densities in 2013 to previously documented densities from past reports (Steneck and McClanahan 2004, 2005; Steneck and Arnold 2009; Steneck et al. 2011). Steneck et al. (2011) has found an increase in snappers, grunts, and other large carnivorous fish following their observations in previous years since 2009. Therefore, it is hypothesized that:

H_1: Densities of smaller grouper species are expected to increase compared to densities from previous years (2003-2011)

The increase in population of large carnivorous fish supports the existence of an intact trophic system in the coral reef ecosystem of Bonaire. This study will provide further evidence on the health of Bonaire's coral reef ecosystem using Serranidae as the primary indicator species.

Materials and methods

Study site

Yellow Submarine (Yellow Sub) was targeted for surveying grouper species densities. The site is located on the western coast of Bonaire (12°9'40.98" N, 68°17'1.87" W). Similar to adjacent reef systems around Bonaire, Yellow Sub is a fringing reef with an extended sand flat region that extends to a depth of 7 to 9 m prior to the edge of reef crest. The reef crest consists of a steep drop off reaching a maximum depth of 30 m, where a sandy bottom with minimal rugosity and low reef coverage starts.

Maximum reef relief

Complexity of the reef habitat was measured with AGGRA maximum reef relief survey method (Lang et al. 2010). A total of 16 transects were laid along the

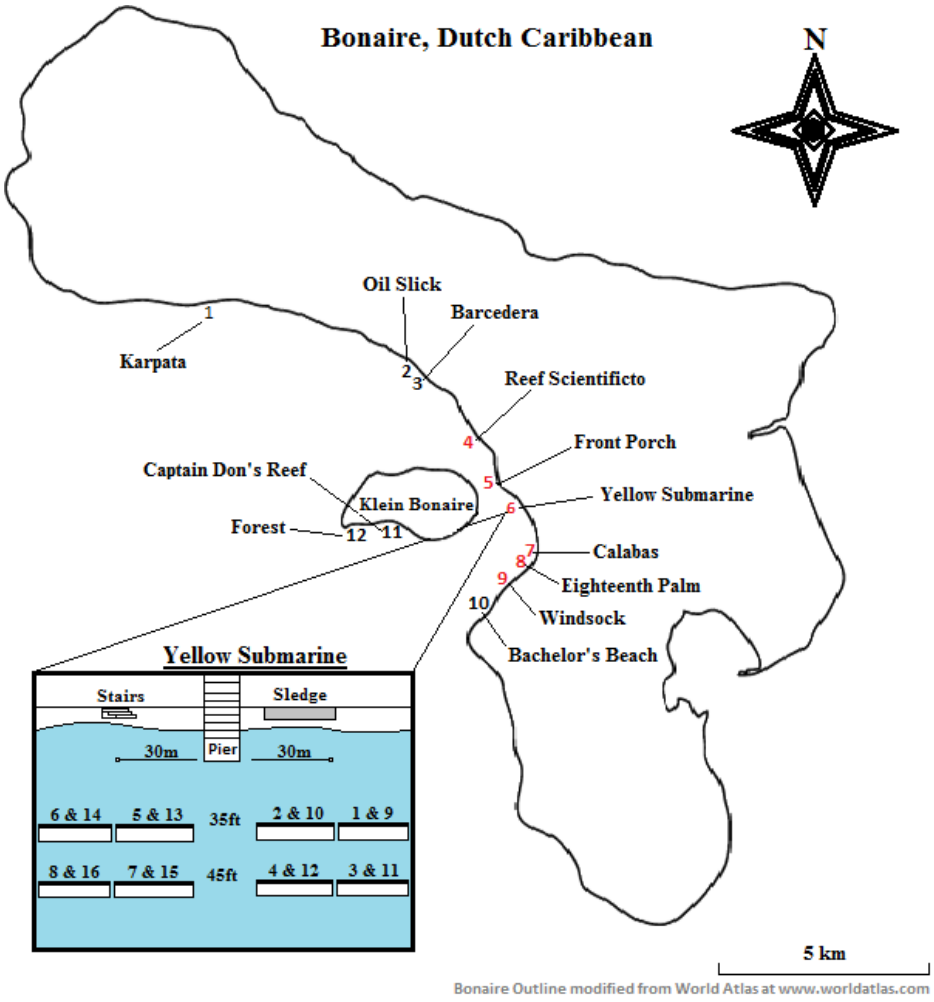

Fig. 1 Map of Bonaire with yellow sub study site (6). Numbers represent all the dive sites with documented grouper densities (Steneck and McClanahan 2004, 2005; Steneck and Arnold 2009; Steneck et al. 2011). Red numbers indicate adjacent dive sites to yellow sub (4,5,7,8,9). Study site layout is provided in the bottom left of the map with light blue portion representing the water. Box Transect locations are labeled with corresponding transect numbers above it

reef crest at depths of 11 m and 14 m (eight at each depth). Eight transects were surveyed at 10:30 h, and eight transects were surveyed at 14:00 h. The transects were laid from the mid-point of the Yellow Sub dive site towards North and South for a distance of 30 meters at both depths. Transect locations were identical for the two survey times (Fig. 1). Reef relief was measured every 3 meters along the transects by measuring the point intercepted substratum from the bottom to the highest point of the tallest hard substrate structure (e.g. live coral, dead coral, massive sponges, etc.). A standard 1-meter PVC T-bar was used to estimate the reef relief.

Reef relief was analyzed by averaging individual maximum reef reliefs surveyed from each transect at 10 point-intercepts (3, 6, 9, 12, 15, 18, 21, 24, 27, 30 m) into a single Average Maximum Relief Index (AMR). The AMR was then averaged across 8 transects surveyed at 11 m and 8 transects surveyed at 14 m into two averages of AMR that represent the reef complexity at each depths. A two-way T-test was performed between the AMR of the two survey depths to confirm absence of rugosity driven density distribution of grouper species.

Grouper species density

The densities of all four grouper species (*C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis*) were surveyed with AGGRA fish survey methodology (Lang et al. 2010). Count of targeted grouper species along depths of 11 m and 14 m were recorded following sixteen 30-meter box transects. A 2-meter wide area was surveyed at the deeper side of each transect tape, covering a total area of 60 m^2. Grouper counts were conducted along the same transects as reef relief measurements, with eight box transects surveyed at 10:30 am and other eight at 2:00 pm (See Fig. 1). Approximation of survey area was performed by standard 1 meter PVC T-bar.

Total grouper density was extrapolated to 100 m^2 area for each survey depths. A two-way T-test was performed to evaluate significance between depth-driven density distributions. In addition, densities of individual grouper species (*C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis*) were averaged and extrapolated into 100 m^2 area. Two-way t-tests were used to evaluate significant difference between mean population densities of each targeted grouper species.

Past reports

Past densities of *C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis* were analyzed along with recorded species densities observed in this study. Data was consolidated from Bonaire coral reef data from 2003 to 2011 (Steneck and McClanahan 2004; 2005; Steneck and Arnold 2009; Steneck et al. 2011). Previous reports from Steneck et al. studied *C. cruentata*, *C. fulva*, *E. guttatus*, and *E. adscensionis* densities along the western coast of Bonaire, and two other dive sites on the eastern coast of Klein Bonaire at a depth of 10 m. Grouper densities surveyed at Yellow Sub were compared to previously documented densities across adjacent dive sites (Fig. 1). Two-way T-tests were performed on densities of *C. cruentata* between all documented years (2003 - 2013) to evaluate for significant difference in density.

Results

Grouper density is not dependent on depth or rugosity

To determine independent distribution of grouper species, total grouper density was evaluated for significance across depth (Fig. 2). Total grouper density was

calculated by combining the total count of all four species (*C. cruentata, C. fulva, E. guttatus*, and *E. adscensionis*). A higher average value was found at deeper transects, but there was no significant difference (p = 0.058) between total grouper density at 11 m (13.75 individuals per 100 m^2, df = 7, SD = 2.64) and 14 m (17.50 individuals per 100 m^2, df = 7, SD = 3.45) (Fig. 2).

To exclude structure driven distribution of grouper species, AMR was evaluated across two survey depths. The combined AMR average across all box transects (n = 16) was 0.644 meter (min. 0.50 m, max. 0.86 m, SD = 0.10). There was no significant difference (p = 0.26) found between the AMR averages at the two survey depths (Table 1).

These results demonstrated that grouper densities were independent of depth, and reef structure. Grouper distribution along studied reef suggested other potential factors that affect grouper density at the study site.

Table 1 Average maximum relief (AMR) for each depth

Depth (m)	AMR (m)	SD	df
11	0.609	0.078	7
14	0.681	0.112	7

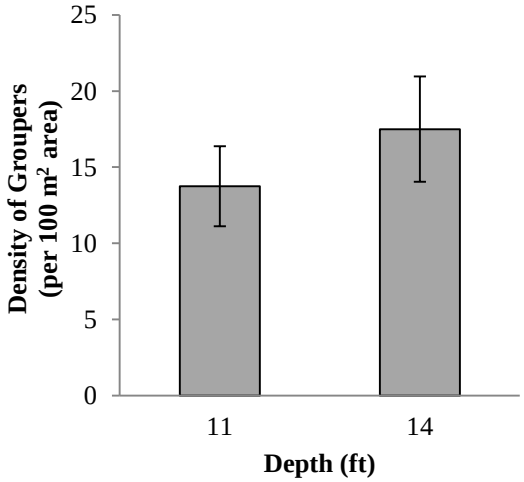

Fig. 2 Mean (± SD) total density of targeted grouper species (*Cephalopholis cruentata*, *Cephalopholis fulva*, *Epinephelus guttatus*, and *Epinephelus adscensionis*) per 100 m^2 area (n = 16) in relation to surveyed depth

Dominance of *C. cruentata* at study site

To observe for changes in densities, four species of groupers were surveyed. AGGRA fish surveys were used for documentation of sighted grouper individuals. Individual grouper species density were averaged across all box transects (n = 16) and depths. Most abundant grouper species found was *C. cruentata* (11.46 individuals per 100 m^2, df = 15, SD = 4.17), followed by *E. guttatus* (4.17 individuals per 100 m^2, df = 15, SD = 2.79). No individuals of *C. fulva* and *E. adscensionis* were found during surveys, thus no densities were documented for those two grouper species (Fig. 3). There was a significant difference (Heteroscedastic T-test, p = 0.0003) found between average densities of *C. cruentata* and *E. guttatus* (Fig. 3).

This data shows significantly higher densities of *C. cruentata* over *E. guttatus* and suggests dominance of single grouper species inhabiting the study site.

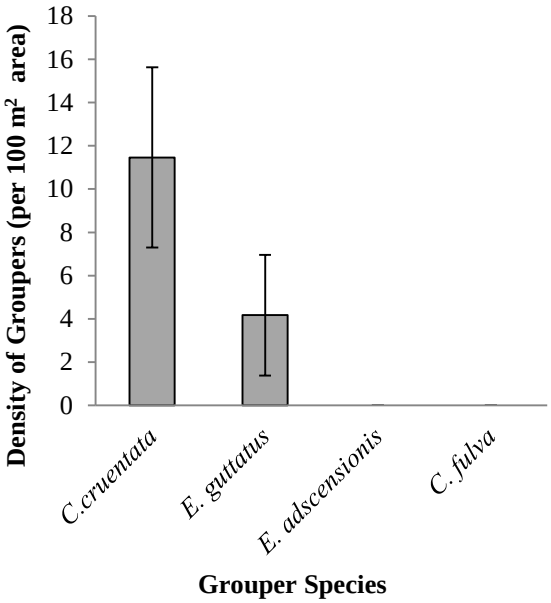

Fig. 3 Mean (± SD) **g**rouper species densities found in 100 m^2 area at yellow sub (n = 16). Two-way T-test for mean densities of *Cephalopholis cruentata* and *Epinephelus guttatus* showed significant difference (P = 0.0003)

Increased *C. cruentata* densities since 2011

To determine the current health of coral reefs on Bonaire, *C. cruentata* density was compared to previously documented density.

Density of *C. cruentata* was averaged across all adjacent sites (Fig. 1) for each documented report by Steneck and McClanahan (2004, 2005); Steneck and Arnold (2009); Steneck et al. (2011). Documented density from 2013 was averaged across all shallow box transects (11 m) for consistency in comparison. Density from 2003 (4.70 individuals per 100 m^2, df = 1, SD = 0.42) was highest prior to documentation in 2013 (9.58 individuals per 100 m^2, df = 7, SD = 3.65) *C. cruentata* density (Fig. 4). Averaged densities from 2005 (2.44 individuals per 100 m^2, df = 1, SD = 0.90) and 2011 (3.16 individuals per 100 m^2, df = 4, SD = 1.40) had no significant difference in density when compared to 2003 (p = 0.18; p = 0.22) and 2009 (p = 0.21; p = 0.23) (Fig. 4). There was a significant decrease in *C. cruentata* densities between 2003 and 2009 (Heteroscedastic T-test, p = 0.02) (Fig. 4). *C. cruentata* density from 2013 was significantly higher than previous densities from 2005 (p = 0.049), 2009 (p = 0.001), and 2011 (p = 0.003). However, t-test statistic showed no significant difference in *C. cruentata* density between 2003 and 2013 (p = 0.11) (Fig. 4). The significant increase (Fig. 4) documented in 2013 presents the record-high *C. cruentata* density around selected adjacent sites (4-9) (Fig. 1). This comparison illustrated significant increase in density of single grouper species (*C. cruentata*) since 2011.

Low densities of *E. guttatus*, *E. adscensionis*, and *C. fulva* since 2003

To determine further evidence supporting the indication of current coral reef health in Bonaire, the three other targeted species (*E. guttatus*, *E. adscensionis*, and *C. fulva*) densities were also compared to previously documented reports from Steneck et al. (Steneck and McClanahan 2004; 2005; Steneck and Arnold 2009; Steneck et al. 2011). However, statistical analysis was not conducted for these three grouper

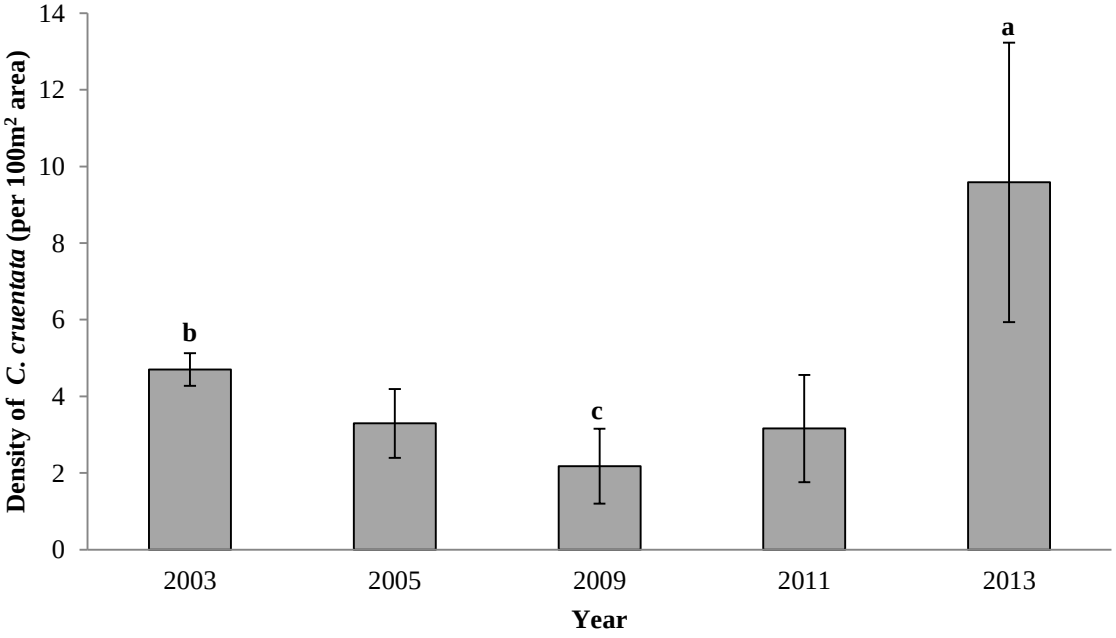

Fig. 4 Mean (± SD) *Cephalopholis cruentata* density averaged across adjacent sites (Fig. 1) from 2003 to 2013. Two-way T-test for mean density of *C. cruentata* between 2003 and 2009 showed significant difference (**b** & **c**, P = 0.02). Two-way T-test of *C. cruentata* mean density between 2009 and 2013 showed significant difference (**c** & **a**, P = <0.01)

species across the years due to the absence (0 individuals per 100 m^2 area for *E. guttatus*, *E. adscensionis*, and *C. fulva*) recorded in past reports (Steneck and McClanahan 2004; 2005; Steneck and Arnold 2009; Steneck et al. 2011). *E. guttatus* density (4.17 individuals per 100 m^2 area, df = 15, SD = 2.79) was documented in this study, showing an increase from previous reports. In contrast, *E. adscensionis* and *C. fulva* densities were consistent to previously reported densities (0 individuals per 100 m^2 area) (Fig. 3).

Discussion

Positive increase of Serranidae density in 2013

The densities observed from targeted Serranidae species (*C. cruentata, E. guttatus, E. adscensionis*, and *C. fulva*) were not dependent on depth (Fig. 2) or reef complexity (Table 1). Despite the insignificant statistical value between 2003 and 2013 grouper densities, biological difference between the two years may be accounted by the 2-fold increase in mean densities between 2003 and 2013 (Fig. 4). The hypothesis was supported by the recorded *C. cruentata* density in 2013, which was significantly higher than density recorded in 2009 (Fig. 4). In addition to the observed increase in *C. cruentata*, the presence of *E. guttatus* (Fig. 3) further supports this hypothesis. The higher recorded densities of *C. cruentata* and *E. guttatus* may account for the progression of a coral-based ecosystem according to past mechanism proposed by Steneck et al. (2011).

Positive progression of Bonaire's coral reef ecosystem since 2011

As a coral reef health indicator, Serranidae abundance directly influences other indicator groups in the reef ecosystem. According to the cascade mechanism described by Steneck et al. (2007), the high abundance of "large carnivorous fishes", such as Serranidae, facilitates removal of trophic omnivores (e.g. territorial damselfishes) and in turn promotes an increased herbivory by larger herbivores. The removal of intermediate omnivores by Serranidae was also supported in a field experiment (Stalling 2008). This mechanism trickles down into an enhanced removal of macroalgae, which opens up substratum for coral settlement and coral growth (Steneck et al. 2007). Steneck et al. (2011) also concluded that strong evidence of a healthy reef revolve around a constant or increasing coral cover with high intensity of herbivory, and coral recruitment. Thus, a healthy Serranidae population will contribute to the positive trend leading to a healthy coral reef ecosystem. In addition to the positive trend, Toller et al. (2010) mentioned that the presence of Serranidae indicates an intact trophic network in the reef ecosystem. As a result, a high abundance of Serranidae species also indicates positive population growth on lower trophic level that supports the increased carnivore population. All in all, the increase in *C. cruentata* and the presence of *E. guttatus* indicates a positive progression towards a healthy coral-based reef ecosystem along the west coast of Bonaire.

Methodological considerations

A couple of limitations regarding the methodology of this study must be considered. First, this study was only able to cover one coral reef site (Yellow Sub) along the Western coast of Bonaire due to time constraint. The limited site surveyed by this study resulted in comparison of Serranidae densities with only adjacent reef sites previously surveyed by Steneck and McClanahan (2004, 2005); Steneck and Arnold (2009); Steneck et al. (2011). This comparison of targeted Serranidae densities may be insufficient for

generalization on the coral reef health across all Bonaire's coral reefs. Secondly, standard AGGRA fish survey was modified to fit the purpose of this study, and focused only on four Serranidae species (*C. cruentata, E. guttatus, E. adscensionis,* and *C. fulva*). This modification may result in a more precise surveying method than previous surveys, where all AGGRA fish species were counted simultaneously by Steneck and McClanahan (2004; 2005), Steneck and Arnold (2009), and Steneck et al. (2011). In addition to modification of AGGRA fish survey, personal field observations suggest that juvenile grouper individuals (< 5cm) tend to be disregarded in the surveys due to their opportunistic hunting behavior. The cryptic nature of juvenile groupers may result in their exclusion from the grouper count. These considerations may have influenced the recorded Serranidae densities by this study.

Implication of present-day Serranidae density on future management

The observed increase in targeted grouper species (e.g. *C. cruentata*) may imply a healthy coral reef ecosystem, but this study captures only a snapshot on the ecological time scale. Steneck et al. (2009) recorded a significantly lowered Serranidae density at 2009. This observation was suspected to be the result of lionfish (*Pterois* spp.) invasion in 2009 (STINAPA 2010). Previous simulation models suggested a drop in Serranidae population from increased presence of *Pterois* spp. (Arias-Gonzalez et al. 2011; Cote et al. 2013). The fast growth rate suggests a high potential for *Pterois* spp. to become predators of slower-growing native groupers; in addition to a potential competition for shelter (Cote et al. 2013). According to the recorded abundance of *C. cruentata*, there is a significant increase in their density from 2009 (post lionfish invasion) to 2013 (Fig. 4). This controversy to previous projections on *Pterois* spp. impacts is currently not documented. The mechanism behind this controversy may be indirectly supported by Hackerott et al. (2013), in which invasion success was not influenced by native groupers. Native Serranidae may not be directly competing with invasive *Pterois* spp.; therefore, no decline in smaller grouper population was experienced as shown by 2013 densities. Despite documented increase in *C. cruentata* density in this study, the positive impact of *Pterois* spp. on native Serranidae population is not supported by field observations. During grouper density surveys, a juvenile lionfish was spotted on the same patch reef as another juvenile *C. cruentata* in shallow water. The competition for shelter between native Serranidae may be the prime conflict with regards to *Pterois* spp. invasion. Furthermore, the active removal effort of *Pterois* spp. must also be taken into account. STINAPA (the Bonaire National Marine Park authority) has started overseeing the removal of *Pterois* spp. since their invasion in 2009 (STINAPA 2010). The proactive strategy may enhance the resilience of native groupers by minimizing possible explosion of *Pterois* spp. population. In terms of enhancing resilience, facilitating a healthy population of smaller Serranidae in Bonaire may be the first step to maintaining a healthy coral reef post *Pterois* spp. invasion.

The active removal solution performed by the lionfish hunters in Bonaire can only be implemented as a short-term solution to the invasion. Many limitations are bounded by the human physical capacity (e.g. depth limit, underwater time) during removal of lionfish on Bonaire's reef. A long-term solution would revolve around the self-sustaining removal of *Pterois* spp. individuals. Mumby et al. (2011) concluded that a self-controlling system is possible under the condition of a high smaller Serranidae density on coral reef. The idea of biocontrol has been widely

discussed (Cote et al. 2013; Mumby et al. 2011); however, most of the proposed biocontrol fails under prey naiveté exhibited by native groupers (Hackerott et al. 2013). Present day studies do not show conclusive evidence of active predation on *Pterois* spp. by native groupers (Cote et al. 2013; Hackerott et al. 2013). According to principle of precautionary, maintaining native grouper density serves as a buffer to minimize negative impact of lionfish on native reef ecosystem. Realistic long term solution may lie on the establishment of marine reserves to preserve native carnivorous fish population (e.g. Serranidae).

Marine reserves may be a double-edged solution if it is not followed by active removal of lionfish. Patchy reserve establishments with active monitoring of Serranidae and *Pterois* spp. population will be essential for meeting minimal biocontrol density. Peak increase in density of Serranidae was found inside a marine reserve at Pamilacan Island in the Philippines, with an 8.4-fold increase (Russ 2002). Implemented marine reserves were found to positively impact surrounding reef systems by providing a refuge for targeted species (Russ 2002). Site-specific implementation of marine reserves could potentially provide a safe-haven for areas with high Serranidae density. With reserves areas dedicated to native Serranidae, existing smaller grouper populations would be more resilient to the presence of *Pterois* spp. by eliminating anthropogenic impact (e.g. fishing mortality). Dedicated marine reserves should always be monitored for ecosystem health through various indicators (e.g. primary producers, corals, herbivores, omnivores, etc.). Active management will be the key for establishing sufficient Serranidae stock for biocontrol. This study indicates a healthy population of Serranidae along the western coast of Bonaire. Furthermore, study results suggest a non-significant impact of *Pterois* spp. on existing Serranidae species on Bonaire under current removal effort. The popularly suggested biocontrol mechanism for invasive lionfish will serve as the next step following actively managed marine reserves. Self-sustaining long-term removal of *Pterois* spp. by Serranidae agent may be the future direction for controlling negative invasion impact on Bonaire.

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REPORT

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Anthropogenic influence on sedimentation and hydrocarbon concentration by terrestrial run-off near a drain in Bonaire, Dutch Caribbean

Abstract The anthropogenic effect of terrestrial run-off on coral reef ecosystems is a topic of high concern to marine ecologists. In the Caribbean, coral cover is decreasing at an alarming rate, and phase shifts to an algae-dominated reef have been documented. Studies have shown a correlation between densely populated coastal communities and high levels of substances known to be detrimental to marine ecosystems. This study focuses on two contaminants common in waters affected by terrestrial run-off: fine sediment and UV reactive hydrocarbons. Fine sediment cannot be removed easily from the tissues of corals and can prevent corals from receiving enough light. UV reactive hydrocarbons can embed themselves in tissue membranes and cause oxidative damage upon exposure to UV light. The presence and effects of these contaminants were determined near a drain in Bonaire, Dutch Caribbean. The percent distribution of sediment grain sizes was determined at increasing distances from the drain. The results revealed that the percentage of fine sediment is highest close to the drain and decreases with increasing distance from it. The presence of UV reactive hydrocarbons was determined using bioassays of *Artemia* sp. The results of the bioassays suggest that run-off from the drain contains UV reactive hydrocarbons. The effect of these contaminants on the abundance of organisms in benthic communities was analyzed using endobenthos technique but results were inconclusive. This study determined the presence of fine sediment and UV reactive hydrocarbons due to a point source of terrestrial run-off.

Keywords UV reactive hydrocarbons • Fine sediment • Pollution

Introduction

The health of coral reefs is important both to marine ecologists and to many coastal communities who depend on coral reefs as a source of income from resources or tourism. For example, in the 1990's an estimated $140 billion in revenue was generated annually from reef-related tourism globally (van Dam et al. 2011). The relationship between coral reefs and human populations is one that is of high importance to marine scientists, because human-caused stress has been shown to cause degradation of reefs close to coastal communities on local and regional scales (Dubinsky and Stambler 1996). Coral reefs have been deteriorating globally at an alarming rate, with an observed loss of 80% of coral coverage in the Caribbean over the last 30 years (Kline et al. 2003). Many studies attribute this loss of coral coverage to terrestrial run-off containing sediment, nutrients, and carbon compounds. In the Caribbean, dredging associated with the construction of hotels, roads, etc. has been correlated with accelerated run-off of eroded soils that has been shown to cut down on light available for photosynthesis as well as increasing sediment load on corals (Rogers 1990). Tissue damage caused by sediment load is

more detrimental with increasing organic content of the sediment and decreasing grain size (Fabricius 2005). Exposure to sedimentation, especially when associated with a high concentration of fine sediment, can cause long-term effects in populations of corals by removing cohorts of young corals, which are highly susceptible to damage by sedimentation (Fabricius 2005). Few examples of recovery after sediment stress have been observed, most likely because sediment stress is usually accompanied by other stresses such as sewage (Rogers 1990). Many coastal communities near coral reefs do not have adequate methods for containing sewage on land, so the sewage is released into the ocean waters. Drainage of sewage into ocean waters provides an opening through which any terrestrial run-off produced by coastal communities can flow into the ocean.

Although the input of nutrients from terrestrial run-off has been the most commonly studied type of chemical pollution to coral reefs, hydrocarbons and heavy metals are also important (Fabricius 2005). The effects of hydrocarbon leaking into the ocean may not be immediate, as they embed themselves in marine tissues (Whitehead 2013). The toxicity of some polycyclic aromatic hydrocarbons (PAHs) may be enhanced by UV radiation. These PAHs can absorb UV light and become photo activated, then transfer their energy to molecular oxygen, forming reactive superoxide anions capable of oxidative damage (Bellas et al. 2008). Bellas et al. (2008) performed an experiment using a battery of marine larvae in a variety of concentrations of intermediate weight PAHs and found that in their presence, UV light inhibited larval development. A study testing a variety of PAHs showed that most intermediate PAHs at natural levels do not cause oxidative damage under UV light, but at levels that could exist in areas polluted with oils cause oxidative damage to marine tissues (Fathallah et al. 2012).

Terrestrial run-off is known to cause harm to aquatic ecosystems, but the impact of contamination depends on chemical composition and the species composition of the affected area (Van Eal et al. 2012). The distribution of waste products from the point of contamination is related to the velocity of the sea currents (Tomassetti and Porrello 2005). In aquatic ecosystems, organic pollutants are mostly concentrated in the sediments, which act like a sink for organic compounds and are an important exposure pathway to aquatic species (Van Eal et al. 2012). Small invertebrates living within contaminated sediment are excellent indicators of environmental stress because they are directly exposed to high concentrations of pollutants and are sessile or have limited range due to their small size (Tomassetti and Porrello 2005). Benthic assemblages cannot easily avoid exposure to stressful conditions, therefore they can respond in a variety of ways including a quantitative composition change in the community (Tomassetti and Porrello 2005).

The island of Bonaire, Dutch Caribbean is known for its coral reefs and marine conservation efforts, but does not have a system for regulating terrestrial run-off. Following an observed disturbance near a specific drain in Bonaire, this study analyzed the contents and effects of the drainage. Corals, algae, and herbivorous fishes inhabit the area are affected by this drain, but it is also an area that is commonly used by people. The drain is located in the middle of an area of Bonaire popular to tourists next to swimming lanes and across the road from a restaurant. Because the drain of interest lies in a highly trafficked area, it is of great importance to the island of Bonaire due to its high reliance on tourism as income. The waters flowing through this drain have a visible effect on the ocean waters close to the drain, but the flow rate of the drain is currently unregulated. One week prior to the start of this study, construction work was conducted near the drain, and it was

expected that chemicals and organic material disturbed by the construction entered the water through the drain. This disturbance could have potentially caused the release of contaminants and fine sediment from the drain into the ocean; an effect that could be intensified by frequent rains in the upcoming rainy season.

The purpose of this study was to test for fine sediment and UV reactive hydrocarbons content near the unregulated drain and any effect that the run-off may have on the fauna living nearby. Analysis of the contents and effects of this particular drain in Bonaire answers the following questions: 1) How does the sediment composition differ in areas closer to the drain from areas far from the drain? 2) Does the sediment that runs from the drain contain UV reactive hydrocarbon compounds? 3) Does the benthic fauna composition differ in areas closer to the drain from areas far from the drain? This study analyses the following hypotheses:

- H_1: Sediment collected from areas near the drain will contain a higher percentage of fine sediment than sediment far from the drain
- H_2: UV reactive hydrocarbon compounds will be present in water samples close to the drain
- H_3: There will be a difference in benthic community structure between areas close to the drain and areas far from the drain

If the sediment collected near the drain has a higher concentration of fine sediment, it will be more harmful to coral health than sediment far from the drain. chemicals into the ocean environment and aims to call attention to this source of contamination in Bonaire. This study builds on current knowledge of anthropogenic release of known harmful

Materials and methods

Study site

This study focuses on one particular drain in Bonaire, Dutch Caribbean. The drain is located next to the dive site Kas di Arte (12.155472 N, 68.27914 W) in the downtown area of Kralendijk, the most densely populated city on Bonaire. The drain is adjacent to swimming lanes, across the road from a few homes and a restaurant (La Barca) and down the street from a recreational park (Fig. 1). The drain runs under a main road, and the water runs through a cement-bottomed trough that collects run-off from the surrounding houses and restaurants (Fig. 1). During heavy rains, there is an increase in effluence through the drain and run-off into the ocean. The area under investigation begins 10 m from the drain and extends 30 m from the drain towards the reef. The substrate near the drain is rocky and has sparse patches of sand, becomes sandy 20 m from the drain, and the reef drop off starts 80 m from the drain. Corals are patchily distributed in the area near the drain, but are not clustered together and are mostly overgrown with algae. A rebar was hammered into the sediment 10 m, 20 m, and 30 m from the center of the drain and visited once a week for 5 weeks to record observations and take sediment and water samples for analysis in the laboratory.

Field observations

Effluence through the drain is highly dependent on rain and disturbances, therefore the number of days since the last rain or observed disturbance was recorded each week that samples were collected. The waters surrounding the drain are naturally clear but there is a visible plume of dark deposition when high amounts of water flow through the drain. This study began one week after construction work, which caused a visible black ring around

the drain. Observations on the clarity of water surrounding the drain, as well as recent rains and time elapsed since the construction work were recorded. Observations on water visibility, current, and waves were also recorded each week.

Granulometry

To analyze sediment composition and proportion of fine sediment at varying distances from the drain, sediment samples were taken each week within a 1-m radius of each rebar, starting from the 10-m marker and moving away from the drain. In a simular study study performed by Duplisea and Drgas (1999), 8-cm cores were taken within 1m of a marker to a depth of greater than 12 cm. This study used a core sampler with a 15-cm diameter to take samples to a depth greater than 12 cm. Because the 10-m marker is in a rocky area, rocks often prevented sampling of sediment to the top of the core sampler (~40 cm). In the case that sediment could not be sampled deeper than 12 cm, the samples at the 20-m and 30-m markers were taken to the same depth as the first marker. The samples were taken to the laboratory in plastic bags and placed in aluminum tins for several hours outdoors to remove excess water and subsequently placed in a dry oven set to 65.56°C for 48 h. When the samples were completely dry, they were weighed and placed in a 7-chamber sieve to be separated based on grain size. Each size of the sieve corresponded to different classifications of sediment. The chambers and their classifications are as follows: 2000 μm (rocks and shells), 1000 μm (coarse sand), 500 μm (medium sand), 250 μm (fine sand), 125 μm (very fine sand), 63 μm (silt and clay), <63 μm (fine clay) (Bian and Zhu 2009). The weight of sediment in each chamber was then recorded for data analysis.

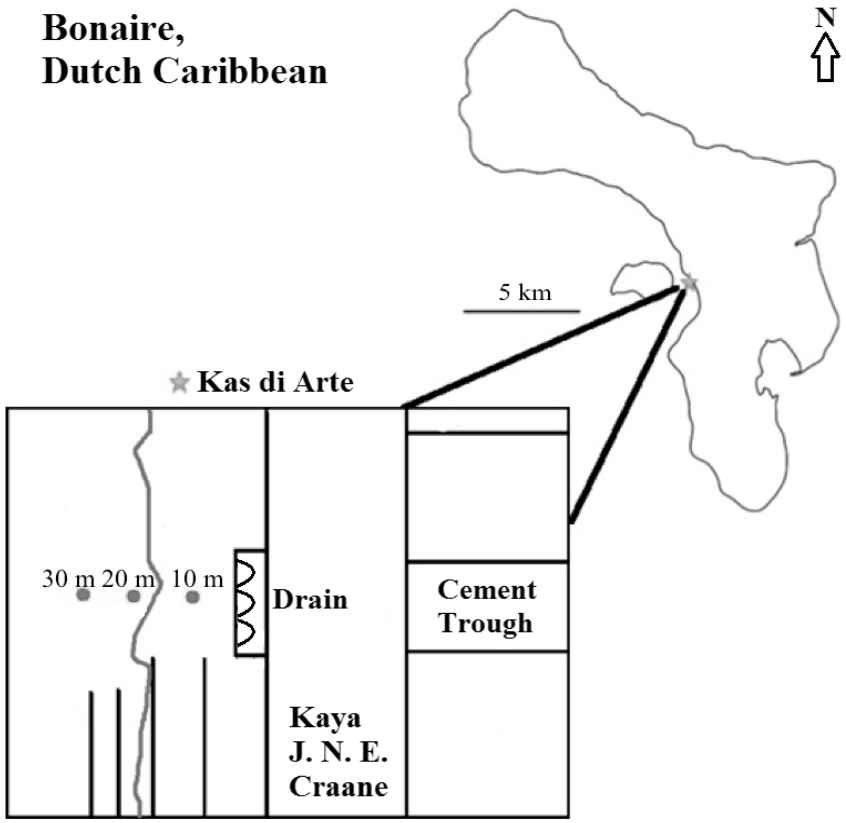

Fig. 1 Map of Bonaire with the location of Kas di Arte in relation to the island and a close-up of the study site. Dots indicate sampling sites

Bioassay

To test for the presence of UV reactive hydrocarbons, water samples were taken from within the benthos at the 10-m marker each week of sampling. Water was collected using a 60-ml syringe wrapped in 250-µm mesh to prevent sand clogging the syringe. The water was then placed in plastic air-filled bottles that were previously cleaned using 10% HCl and Milli-Q water. The water samples were taken back to the lab and diluted with filtered seawater to create a 1:3 dilution, a 1:1 dilution, and a 1:0 pure sample. The different concentrations of sampled water/filtered seawater along with a control of pure filtered seawater were used to perform a bioassay on *Artemia* sp. This shrimp is routinely used as a test organism for screening in ecotoxicological studies (Lu et al. 2012). The *Artemia* were hatched each week three days prior to the day of the bioassay. To perform the bioassay, 50 *Artemia* were placed into a 15-mL petri dish filled with 10 mL of water dilution. The *Artemia* were left for four hours and then exposed to a standing UV light. Eight petri dishes with *Artemia* were created in total, four of these received UV treatment, one dish for each solution (0:1,1:3,1:1,1:0), and four of these were placed under glass to shield them from UV light. The no-UV treatment was the negative control of this experiment and represents the percentage of individuals that died of natural or unrelated causes. Four petri dishes with 50 *Artemia* and dilutions (0:1, 1:3, 1:1, 1:0) of filtered seawater with four drops of motor oil served as a positive control and were placed in the UV treatment. One petri dish with 100% motor oil solution was placed under glass. This positive control experiment was replicated once. After exposing the *Artemia* sp. to UV light for 14 minutes and 59 seconds, they were left in a dark room overnight. The following day, the numbers of dead *Artemia* sp. in each petri dish were counted and recorded for data analysis. Toxicity results were expressed as percentage mortality adjusted relative to the negative control, following Abbot's formula:

$$\%\text{Toxicity} = (I_t - I_0)/(1 - I_0) \times 100$$

where I_t denotes the observed mortality of the UV treatment and I_0 represented the natural mortality of negative control (Lu et. al 2012).

Endobenthos

To test the effect of the pollution from the drain on some of the organisms living within the surrounding ecosystem, the number of fauna living within the benthos was determined using endobenthos technique. A core sample was taken each week near each of the markers with the 15-cm core sampler to a depth of >12 cm. The samples were brought to the surface in plastic bags and transferred to a plastic container if the bag was leaking. The benthos samples were treated with a relaxing solution ($MgCl_2$) then brought back to the laboratory. The samples were stained using a Rose Bengal/ethanol(10%) solution then placed in the refrigerator for 1-2 days before analysis. The samples were run through a 500-µm sift and placed in a plastic container with fresh water. Fauna within the samples were extracted using forceps and placed in 70% Ethanol solution for counting and analysis under a microscope. One 100-mL dish of sand from each sample was analyzed under a microscope to determine if fauna remained in the sand after the initial extraction.

Data analysis

Granulometry

The grain size proportions of each sample were organized into a bar graph for visual comparison of the three sampling sites. The percentage of silt and clay were each organized into one bar graph to analyze

the differences between the three groups and compare the change over time to the rain and disturbance observations.

Bioassay

Scatter plots were made comparing the percent of dead individuals versus the concentration of sampled water for the UV treatment and the negative control for each week's bioassay experiments and analyzed with linear regression analysis. One scatter plot comparing the percent toxicity of each week's experiments versus the percent sampled water was made and each week was analyzed with linear regression. A scatter plot of the percent dead individuals versus percent motor oil solution was made for the positive control and analyzed with linear regression. A bar graph of the percent toxicity in pure sampled wáter each week was made to analyze the difference in the toxicity over time and compare to rain and other disturbances.

Endobenthos

A table of the number of organisms counted in the different samples (10-m, 20-m, 30-m) was created to compare the sites over the four weeks against the rain and disturbance observations.

Results

Due to the open and unregulated nature of the drain and the shape of the cement trough leading into the drain, rain and disturbances have a large impact on the run-off coming from the drain. Observations on the appearance of the water around the drain, recent disturbances, and the strength of waves and currents were recorded (Table 1). No samples were taken into the lab during the first week of observation, so this week is labeled as week 0. Weeks 1-4 correspond to the weeks during which samples were brought into the lab for analysis. A black plume around the drain was observed until week 3 after the construction work, and observed again during week 4 of sampling after a heavy rain event (Table 1).

Granulometry

The percent of silt in the sediment samples was highest in the sediment 10 m from the drain each week except for week 2 when the 30 m site contained the highest percent of silt (Fig. 2). The sediment 20 m from the drain contained nearly the same percent of silt each week (Fig. 2). The sediment 30 m from the drain contained very little silt during week 1 and no silt during week 3, but more silt during weeks 2 and 4 (Fig. 2). The site which contained the highest percent of clay every week was the 10-m sampling site (Fig. 3). The 30-m sampling site contained no clay during week 3 and very little clay during week 4 (Fig. 3). The percent of clay present in the samples from the 20-m site varied little each week except week 3, when there was no clay present (Fig. 3).

Bioassay

The numbers of dead individuals in the

Table 1 Observations on recent rain/disturbance each week and surface observations of the water around the drain and strength of waves/current

Week	Days since last disturbance	Type of disturbance	Surface Observations	Waves/current
0	10	Construction	Thick black plume	Mild
1	17	Construction	Light black plume	Mild
2	24	Construction	No plume	Strong
3	5	Light rain	No plume	Strong
4	0	Heavy rain	Thick Black plume	Mild

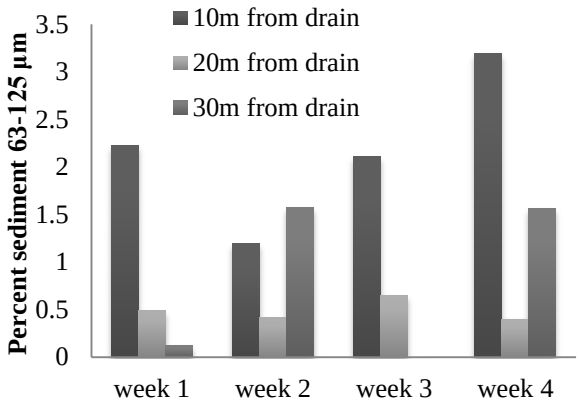

Fig. 2 Percent of the sediment at each of the sampling sites that consisted of silt (63-125 µm) each week

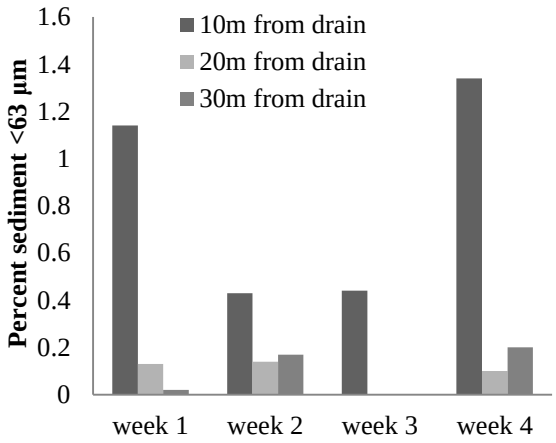

Fig. 3 Percent of the sediment at each of the sampling sites that consisted of clay (<63 µm) each week

UV treatment and the no-UV treatment counted in each concentration of contaminated water were analyzed for the first 3 weeks. The data for the bioassay during week 4 and the second test in motor oil solution were not included due to contamination in the *Artemia* hatching tank. The positive control in motor oil solution showed a positive correlation (R^2=0.93903) for the UV treatment and 10% of the individuals died in the 100% no-UV treatment (Fig. 4). A scatter plot was constructed comparing the percent toxicity vs the percent sampled water for all three weeks (Fig. 5). Week 1's data showed a positive correlation (R^2=0.91859), week 2's data showed positive correlation (R^2=0.90494), and week 3's data showed a positive correlation (R^2=0.98387) (Fig. 5). The percent toxicity in the 0% sampled water solution had a value of -21% during week 1 and -11.1% during week 2. A bar graph was constructed to show the percent

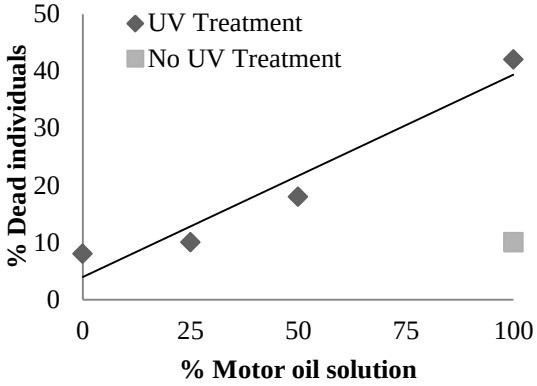

Fig. 4 Percent of dead individuals with respect to the percent of motor oil solution for the UV treatment and the no-UV treatment with linear regression (R^2=0.939)

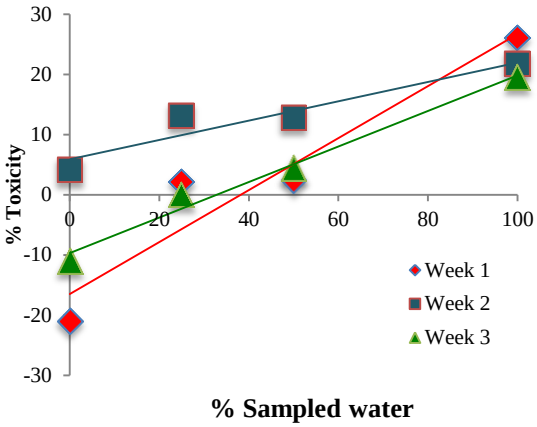

Fig. 5 Percent sample water vs percent toxicity with linear regressions for week 1 (R^2=0.91859), week 2 (R^2=0.98387), and week 3 (R^2=0.90494)

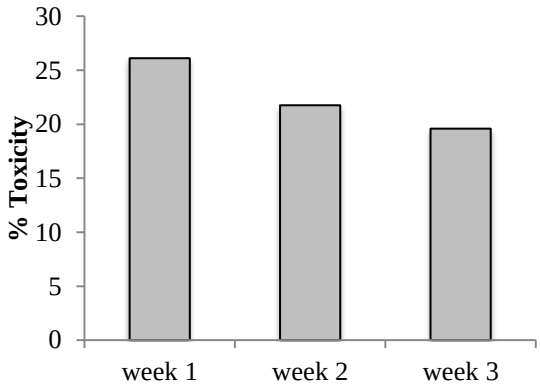

Fig. 6 Percent toxicity of pure sampled water each week

toxicity in the 100% contaminated solutions each week (Fig. 6). The percent toxicity was 26.1% during week 1, 21.7% during week 2, and 19.6% during week 3 (Fig. 6).

Endobenthos

The number of organisms counted from the sites were not consistently different from each other throughout the sampling period. The sample from the site 10 m from the drain contained 10 organisms during week 1, 39 during week 2, 20 during week 3, and 12 organisms during week 4 (Table 2). The highest number of organisms counted was 39 from the 10-m sampling site during week 2, and the lowest number of organisms counted was 7 from the 20-m sampling site the same week (Table 2). No organisms greater than 500 μm were observed in any of the petri dishes of sand taken from each sample.

Table 2 Numbers of organisms counted in the benthos samples collected at each of the sites every week

	Number of organisms counted		
Week	10 m from drain	20 m from drain	30 m from drain
1	10	10	13
2	39	7	24
3	20	18	13
4	12	26	10

Discussion

The sediment sampled near the 10 m marker contained an observably higher proportion of silt and clay each week than the sediment sampled near the 20-m and 30-m markers (Fig. 2, Fig. 3). The percentage of clay in the sediment 10 m from the drain was highest during weeks 1 and 4 of the study, which were the weeks when disturbances had the greatest affect on the drain (Fig. 3). The sediment contained 1.14% clay in the first week of sampling, 2 weeks after construction work, and decreased to a value near 0.45% until week 4 when, due to heavy rain, a value of 1.34% was measured (Fig. 3). These observations suggest that the abundance of clay in the sediment near the drain is highest when a recent disturbance has occurred. The percentages of silt showed a more uneven distribution between the three sampling sites than the percentages of clay, but the 10-m sampling site also showed a higher percentage of silt during weeks that were affected by disturbances (Fig. 3). The differences in percentages of silt and clay during weeks without disturbance and absence of fine sediment in the 20-m and 30-m sites during week 3 could be due to the strong currents and waves observed during these weeks. Fine sediment is more likely to become suspended in fast moving fluid than coarser, heavier sediment and is more likely to disperse over long distances (Bian and Zhu 2009). The presence of fine sediment in the 20-m and 30-m sampling sites was observed again after heavy rain. This data suggests that fine sediment flows through the drain after a disturbance and that this fine sediment is suspended into the water column and swept away with currents. Although a difference in sediment composition between the 3 sites is observed, the hypothesis regarding fine sediment proportions cannot be supported using statistical analyses due to the time-dependent nature of this study.

The results of the bioassay showed a positive relationship between percent sampled water and percent toxicity with above 90% goodness-of-fit each week. Increasing mortality with decreasing dilution of the sample water suggests that UV reactive hydrocarbons present in the sample water caused deaths in the *Artemia* sp. The elevated mortality in the negative control with 0% sample water compared to the UV treatment in 0% sample water during the first two weeks suggests that unrelated factors caused death in the *Artemia* sp. The results of the positive control confirm that the percent of dead individuals increases with increasing

concentration of hydrocarbons. Analysis of the percent toxicity in the 100% sample water dishes over time showed that toxicity was highest during the first week of sampling and decreased during weeks 2 and 3 (Fig. 6). This observation follows a similar trend to the percentages of clay in the sediment samples from the same site over time. The water samples contained some of the small particles that were absorbed from the benthos layer and a higher toxicity was observed when more fine sediment (<63 μm) was present. UV reactive hydrocarbon adsorption into marine sediment varies with sediment size, and small particles (<50 μm) have the highest capacity to carry hydrocarbons (Hiraizumi et. al 1979). The water collected from near the drain that resulted in phototoxicity in the *Artemia* sp. is likely to contain UV reactive hydrocarbons that flowed from the drain along with fine sediment.

The results of the endobenthos organism count did not show any trend that suggests that the pollution from the drain is correlated to a change in the abundance of benthic fauna. However, the results did show that the numbers of organisms found in the samples taken from the 10-m sampling site were lowest during weeks of high disturbance and high fine sediment percentage. The lower numbers observed during these weeks could be because increased sediment load containing UV reactive hydrocarbons from the drain caused the death of the less tolerant organisms living within the benthos. Given a longer period of study time, identification and analysis of the species diversity of the fauna may provide stronger evidence to analyze the impact of the run-off on the benthic organisms. Future studies analyzing the relationship between benthic communities and the effluence from this drain over time could determine what effect this drain has on benthic organisms.

The data collected during this study suggests that the run-off from the drain contains fine sediment and UV reactive hydrocarbons and is sensitive to rain and other disturbances. The fine sediment released from the drain can have negative impacts on the surrounding ecosystem because of its impact on turbidity and its ability to adsorb harmful chemicals. The hydrophobic nature of UV reactive hydrocarbons causes them to have a higher affinity for molecules that are less polar than water, and hydrocarbons will settle into other materials when suspended in water. Hiraizumi et al. (1979) showed that hydrocarbons suspended in water had a higher affinity for fine sediment than other sediment sizes, but had the highest affinity for the membranes of zooplankton. The chemical properties of hydrocarbons, such as their chemical stability and hydrophobicity, make them persistent in the environment and give them the ability to accumulate easily into the tissues of biota, to enrich throughout food chains, and to eventually cause toxicological effects (Van Ael et. al 2012). Fine sediment released from the drain is likely to contain hydrocarbons that will transfer to organisms living within the benthos and lead to bioaccumulation throughout trophic levels.

The area surrounding the drain up to a distance of 20 m contains corals and algae that are growing on the rocky substrate. Fine sediment released from the drain can increase turbidity and settle on the membranes of corals, decreasing light available to the corals. Sediment containing hydrocarbons that settles on corals is likely to transfer the chemicals into their membranes, making them vulnerable to phototoxicity. Damage to the corals causes the algae to outcompete the corals for space, and reduce coral cover in the area.

Pollutants released from the drain can cause damage to the marine ecosystems in many ways, and this damage can also have detrimental impacts on humans. Decreased coral cover in an area popular to tourists is important to prevent in Bonaire because of

its high dependence on tourism as a source of income. Bioaccumulation of toxic chemicals through trophic levels of the marine ecosystem can negatively impact humans who consume fish that have accumulated harmful chemicals. Regulating the source of effluence can lower the amount of pollution that has an effect on marine life and human populations. This study and others like it aim to call attention to the negative impact of pollution on marine ecosystems because the only solution to anthropogenic impacts on ecosystems is awareness and action by the citizens inhabiting offshore areas.

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REPORT

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Invasive lionfish obesity in Bonaire

Abstract Indo-Pacific lionfish (*Pterois* spp.) have spread to and established sufficient numbers throughout the Caribbean. They are extreme generalists that feed on ecologically and economically important species, they can reduce recruitment of native fishes by up to 79%, and can occur in densities in orders of magnitude greater than in their home range. Because prey do not recognize lionfish as predators (prey naiveté), lionfish prey on fish and invertebrates using little energy. The purpose of this study was to test if lionfish in Bonaire were obese and if obesity was more pronounced in males over females. Because of limited research on invasive species obesity, the presence of interstitial fat and fat in the liver was used to determine if a lionfish was obese or not. A total of 161 lionfish for interstitial fat and 74 lionfish for liver fat were analyzed. All males in this study were obese (they all had both interstitial and liver fat) however, not all females had interstitial and liver fat. Females also possessed interstitial and liver fat in lesser quantities than males probably because of allocation of energy towards reproduction. This study highlights the importance of studying obesity on invasive species, an open topic in marine science that has not been addressed thoroughly.

Keywords Lionfish • Prey naiveté • Invasive species • Obesity

Introduction

Native to the Indopacific, lionfish (*Pterois* spp.) are the first nonnative marine fish to become established along the east coast of the United States and the Caribbean (Morris and Akins 2009) most likely because of aquarium releases off of the southeastern coast of Florida in 1992 (Courtenay 1995). *Pterois* spp. are extreme generalists that feed on ecologically and commercially important species (Morris and Akins 2009) and can reduce recruitment of native fishes by an average of 79% (Albins and Hixon 2008).

Marsh-Hunkin et al. (2013) conducted a study looking at how a popular food source (different members of Gobiidae) of *Pterois* spp. reacts to a lionfish, a grunt and a grouper. They found that gobies react to lionfish as if they are grunts, a fish that feeds primarily on crustaceans such as krill. Another study was done by L*ö*nnstedt and McCormick (2013) where they found that even by training fish that lionfish are associated with danger, they still have no change of behavior when lionfish are present. Prey naiveté allows this invasive species to hunt for food with little effort in their nonnative range.

Feeding without restriction has potential repercussions for invasive species. Wolf and Wolfe (2005) assessed liver damage in fish stating that fish in the wild typically do not have fat in their livers (lipid-type hepatocytes) unless they are elasmobranchs (with the absence of a swim bladder it is suggested that a high density of lipids in their livers helps them maintain neutral buoyancy) or a select few

other species such as cod. Fish in captivity showed extensive lipid-type hepatocytes (e.g. flounder) which tends to be uniformly distributed presumably due to imbalances in energy intake and expenditure caused by artificial feeding and housing conditions. When released back to the wild and re-caught a few months later, fish showed to go back to having livers with little to no fat in them. Penrith et al. (2006) also suggested that variation of diet and avoidance of overeating appear to be the most important factors in preventing high hepatocellular vacuolization in aquarium fish.

Costello et al. (2012) showed that male lionfish acquire more interstitial fat than female lionfish probably because females allocate more energy toward reproduction, however they did not link amount of interstitial fat to hepatic steatosis (i.e. an oversupply of lipids in the liver not broken down into energy (Garg and Misra 2002)). Studies such as Dixon et al. (2001), Marceau et al. (1999) and Matteoni et al. (1999) suggest that hepatic steatosis is linked to obesity in humans. Because no literature was found on what makes a fish obese, hepatic steatosis and amount of interstitial fat were used as factors to determine obesity in lionfish. Males were expected to be more obese than females because females allocate more energy toward reproduction. Based on these findings, this study hypothesizes that:

H_1: Lionfish in Bonaire are obese

H_2: Males will be more obese than females

Materials and methods

Study site

This study was conducted on Bonaire, Dutch Caribbean (Fig. 1). The reefs around Bonaire form a narrow fringing reef with a shallow terrace that extends from the shore to the drop off which starts at a depth of approximately 10-15 meters. From the drop-off the fore reef slopes with an inclination between 30° and 60°. Klein Bonaire, located 805 meters west from the western most point of Kralendijk, shares the same reef profile as Bonaire except for on the northeastern coast of Klein where there is no shallow water terrace and the drop-off begins between 2-5 meters (STINAPA, 2013).

In the field

Wednesdays and Saturdays, SCUBA divers went out to Klein Bonaire to hunt lionfish. They hunted at a different site every day eventually covering the entire perimeter of Klein. Lionfish were speared at different depths and then taken back to

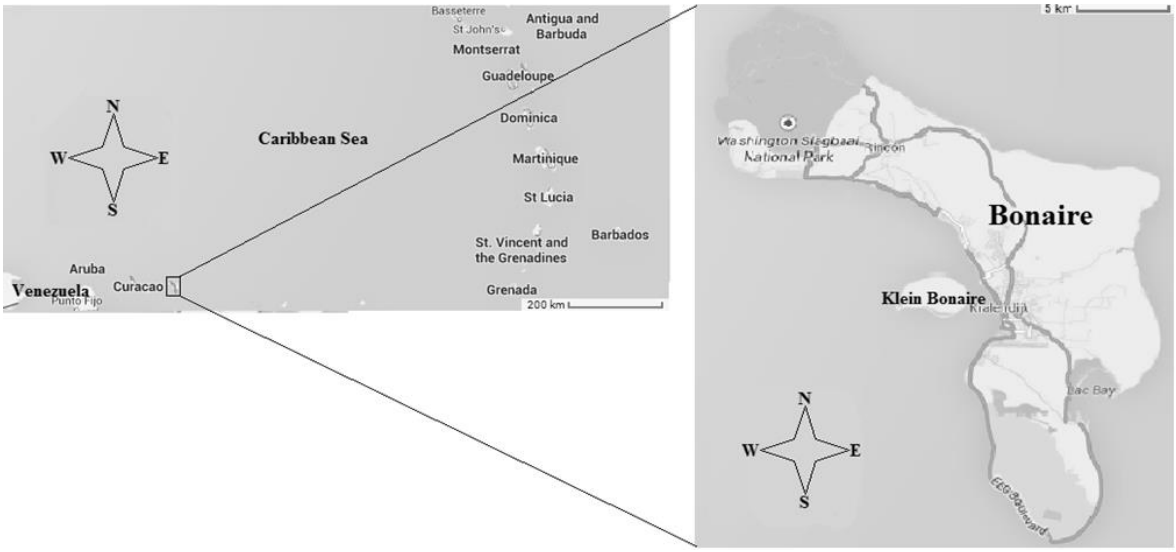

Fig. 1 Map of the southern Caribbean with a close up of Bonaire (modified from Google maps)

Kralendijk's Recreational Pier to be processed. Sex, standard length (head to the beginning of the caudal fin), total weight, and weight of interstitial fat were measured and recorded. Length parameters were measured to the nearest millimeter and weight parameters were measured to the nearest gram. The liver of each lionfish was bagged and taken back to the lab. Additional lionfish were used for this study that were caught by recreational divers at various dive sites on the western side of Bonaire. The same information was recorded and livers were also collected.

In the laboratory

Livers were stored for further use at 4°C. Interstitial fat coating the livers was trimmed off and then the livers were weighed. Weight parameters were measured to the nearest 0.001 milligram. Livers were cut until they appeared to be slurries and then placed in 50 mL polyethylene centrifuge tubes filled halfway with water and partially submerged in a rapid boiling bath (Fig. 2). After 15 minutes of submersion with frequent stirring, centrifuge tubes were taken out of the rapid boiling bath and set aside at room temperature for 15 minutes. Caps were put on the tubes and then they were stored at 4°C overnight. Boiling the livers separated the fat from the liver tissue and refrigerating them allowed the fat to settle and solidify on the surface. The following day, fat was carefully picked out and weighed to the nearest 0.001 milligram.

Fig. 2 Experimental setup with polyethylene centrifuge tubes submerged in a rapid boiling bath. Liver slurries were placed in individual polyethylene centrifuge tubes, stirred frequently and removed from the rapid boiling water bath after 15 minutes

Data analysis

To get percent of interstitial fat (% IF), weight of interstitial fat (WIF) was divided by total weight (TW) and then multiplied by 100%. To get percent of fat in the liver (% FL), weight of fat (WFL) was divided by liver weight (WL) and then multiplied by 100%:

$$\%IF = (WIF/TW) \times 100\%$$

$$\%FL = (WFL/WL) \times 100\%$$

Unpaired t-tests were used to compare % IF and % FL in males and females.

Because females allocate energy differently at different sizes, it was necessary to separate males and females into different length classes (LC) to determine specific results within different stages of sexual maturity. The difference between the longest and shortest standard length in females was taken and divided by three to get three length classes (LC1, LC2 and LC3):

T (overall): 14-27.1 cm
LC1: 14-18.3 cm
LC2: 18.4-22.7 cm
LC3: 22.8-27.1 cm

Results

Data collected

Three outliers were removed because these individuals had clear abnormal values. All of them were males in LC1. %IF of 10.23 and 18.72 and %FL of 16.94 were

recorded. From here onward, "all" does not include these outliers in the data set (Table 1).

Table 1 Sample size (n) of studies. This information is split up into two sections: one with all individuals and one with just the individuals that had interstitial fat

		T (n)	LC1 (n)	LC2 (n)	LC3 (n)
All (%IF)	males:	90	20	38	26
	females:	71	18	30	23
All %FL)	males:	36	12	7	17
	females:	38	10	16	12
Just fat (%IF)	males:	89	25	38	26
	females:	57	18	18	21
Just fat (%FL)	males:	36	12	7	17
	females:	28	10	7	11

Data from all individuals

%IF in males is significantly higher than females in T, LC2 and LC3 (Table 2). Although the average %IF of females in LC1 is greater than males, the difference is not statistically significant. %FL in males is significantly higher than females in all classes. All males (36 out of 36) that had interstitial fat had also presented fat in their livers, whereas, not all females did (35 out of 38).

In total, 99% males had interstitial fat (n=90) and 80% females had interstitial fat (n=71). All males in LC1 had interstitial fat (n=26) and all females in LC1 had interstitial fat (n=18). In LC2 100% males had interstitial fat (n=38) and 60% females had interstitial fat (n=30). In LC3, 96% males had interstitial fat (n=26) and 90% females had interstitial fat (n=23).

Just comparing female lionfish in different length classes (Table 3) there is a stastical significance in %IF and % FL except when comparing females in LC1 and LC3.

Table 3 p-values among classes of female lionfish

		p
% IF	LC1 vs. LC2:	<0.001
	LC1 vs. LC3:	0.35
	LC2 vs. LC3:	0.02
% FL	LC1 vs. LC2:	<0.001
	LC1 vs. LC3:	0.10
	LC2 vs. LC3:	<0.001

Data from individuals only with interstitial fat

When comparing %IF between males and females only with interstitial fat (Fig. 3), in total, males have %IF 2.87±1.24 and females have %IF 2.53±1.44 with no statistical significance. There is no statistical significance between males and females in LC1 and LC3 either Males in LC2 have %IF 2.93±10 and females have %IF 2.16±0.98 (p=0.01).

When comparing %FL between males and females only with interstitial fat (Fig.4), in total, males have %FL

Table 2 Percent interstitial fat and percent fat in liver of all individuals. Difference between male and female tested for significance (Heteroscedastic T-test)

% Interstitial fat (%IF)					
	Males		Females		
	Average	S.D.	Average	S.D.	p
T:	2.83	1.26	2.03	1.64	5.76×10^{-4}
LC1:	2.4	1.44	2.84	1.15	0.29
LC2:	2.93	1.09	1.3	1.31	4.72×10^{-7}
LC3:	3.13	1.25	2.35	1.97	0.1
% Fat in liver (%FL)					
	Males		Females		
	Average	S.D.	Average	S.D.	p
T:	2.32	1.46	0.74	0.72	5.68×10^{-8}
LC1:	2.73	0.92	1.4	0.27	2.79×10^{-4}
LC2:	0.75	0.94	0.17	0.31	0.03
LC3:	2.69	1.5	0.94	0.8	1.06×10^{-3}

2.19±1.46 and females have %FL 1.00±0.66 ($p<0.001$). Males in LC1 have %FL 2.54±1.10 and females in LC1 have %FL 1.40±0.27 ($p=0.004$). Males and females in LC2 show no stastical significance. Males in LC3 have %FL 2.61±1.49 and females in LC3 have %FL 1.03±0.78 ($p=0.003$).

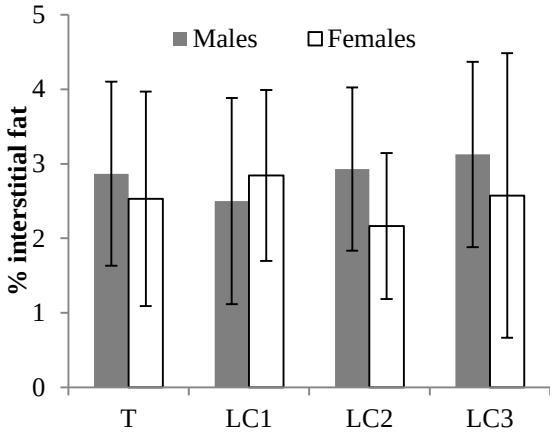

Fig. 3 Percent interstitial fat of male and female lionfish recorded with interstitial fat among different length classes. Individuals without interstitial fat were not included in this figure. For males vs. females in LC2, p=0.01. All other relationships are not statistically significant

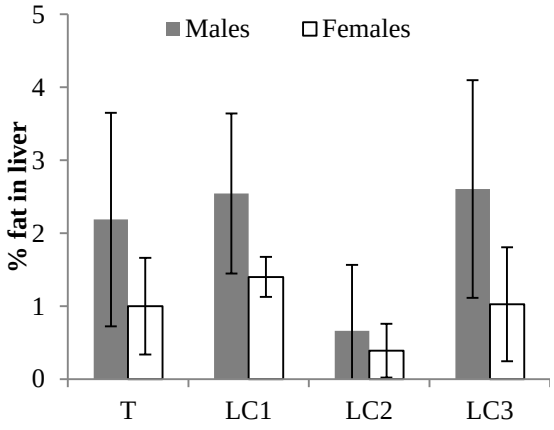

Fig. 4 Percent fat in livers of male and female lionfish recorded with interstitial fat among different length classes. Individuals without interstitial fat were not included in this figure. For males vs. females in T, $p = 1.6x10^{-4}$, for males vs. females in LC1, p = 0.004 and for males vs. females in LC3, p = 0.003

Discussion

A lot of the discussion presented will refer to Fig. 5. Please note that this figure is originally used to describe number of eggs produced in comparison to size. It has been modified substituting number of eggs produced with energy invested towards reproduction assuming that these go hand in hand. Three sections based on length classes have been made to fuel more questions and come up with possible answers.

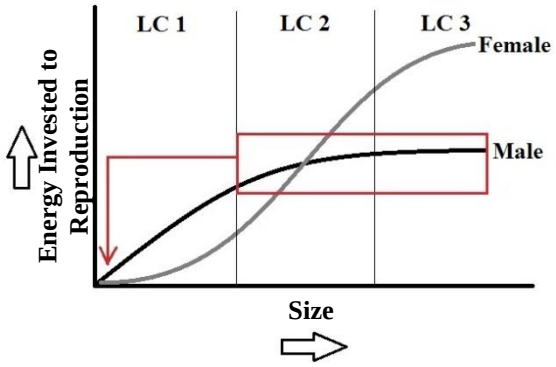

Fig. 5 Ghiselin's size advantage model (derived from Ghiselin 1969). The box highlighted around the line for males indicates a shift in this part of the theoretical model further explained in the text

%IF in males was statistically higher in T, LC2 and LC3, but not LC1. Males in LC1 had %IF of 2.40±1.44% while females topped over them at 2.84±1.15% however there was no statistical significance ($p=0.29$). Referring to Fig. 5, this could be because females in LC1 are allocating energy towards growth, not reproduction. Females in LC1 may not be allocating energy to reproduction because they might not be able to start sexually developing until a certain size but other factors could be at play such as age and/or genetic variability (Policansky 1983). A similar slope is seen for females in LC2 and males in LC1 yet only 60% females in LC2 have interstitial fat while 100% males in LC1 have interstitial fat. Because this is a theoretical model not based on hard data, data in this study suggests a shift in the line for males down and towards the left (Fig. 5). Males smaller than those in LC1 may actually be exhibiting this lower

percentage of individuals with interstitial fat however this was not looked at because it is difficult to determine sex in lionfish less than 15 cm in total length.

Although most females with interstitial fat presented fat in their livers, some of them did not. This may be because lionfish reproduce continuously while other reef piscivores reproduce seasonally (Costello et al. 2012). This leaves them less likely to accumulate an oversupply of lipids in their livers because they are constantly using more energy towards reproduction. However, when females reach LC3 the slope of the line in the Size Advantage model becomes less steep and a mirror image to the line for females in LC1. This could suggest that females in LC3 are allocating more energy towards fat storage because growth is slowing past a certain point. Table 4 shows that there is no significant difference between females in LC1 and LC3 for both %IF and %FL. Not to mention all females in LC1 and most females in LC3 (91.3%) had interstitial fat but only 60% females in LC2 had interstitial fat. An increase in the slope for females in LC2 indicates that they are allocating much more energy towards reproduction and growth, and they are therefore less likely to have interstitial fat.

This study compared individuals only with interstitial fat to see if males with interstitial fat are more obese than females with interstitial fat. Fig. 3 shows that there is only a statistical significance in %IF between males and females in LC2. However you can still see the general trend of males with interstitial fat having more interstitial fat than females with interstitial fat. Fig. 4 shows that there is a statistical significance between males and females in T, LC1 and LC3 for %FL. Although %IF is not as clear cut, these statistical significances in %FL (Fig. 4) are clear failures to disprove H_2 (males will be more obese than females).

In conclusion, based on the presence of interstitial fat and hepatic steatosis, lionfish in Bonaire are obese (H_1). All but one male (98.89%) had interstitial fat and most females (80.28%) had interstitial fat. Out of 36 males and 38 females, only three individuals (all of which were females in LC3) did not have fat in their livers. Further studies could go in a variety of ways. It would be interesting to see how these results would compare to lionfish in their native range. It would also be very interesting looking at lionfish livers microscopically, exploring the possibility of liver damage due to obesity. These results are an indirect indicator of how naive prey are and how crucial it is to keep lionfish populations down.

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REPORT

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Correlation analysis of garden and territory size of threespot damselfish, *Stegastes planifrons*

Abstract Various symbiotic relationships build and maintain coral reefs. Mutualistic relationships provide the organisms involved with an increased chance of survival and reproduction which prove important for the health and function of reef communities. The increasing presence of macroalgae is an indication of declining reef health. In order to maintain the growth of certain species of macroalgae, Threespot damselfish, *Stegastes planifrons,* cultivate and maintain algae gardens. If there is an abundance of algae in the gardens of *S. planifrons*, there is a limited opportunity for coral recruitment and growth; this makes them an important species in the ecosystem. Damselfish are very territorial and will defend their gardens by chasing and biting intruders. This study tested whether there is a particular sized territory surrounding the garden that correlates to the size of the garden itself. Attacks by *S. planifrons* in the gardens toward a laser pointer allowed the determination of garden and territory area. The area of the garden, the point where the attacks ended and the total surrounding territory of the damselfish were measured using a measuring tape. A positive trend between area of garden and area of territory was found indicating that both increased correspondingly. The algae gardens and territorial behavior of *S. planifrons* can be indicative of the current phase shift from a coral reef to a coral depauperate ecosystem. More algal cover is indicative of decreased coral cover and coral recruitment success. By understanding ecological dynamics, protection of coral reefs from a degrading phase shift can be implemented.

Keywords *Stegastes planifrons* • Threespot damselfish • Territory • Garden

Introduction

Coral reefs are built and maintained by various symbiotic relationships. Corals and Zooxanthellae depend on each other for energy; the dash goby *Ctenogobius saepepallens* and the sand snapping shrimp *Alpheus floridanus,* rely on each other for shelter and protection (Lyons 2013); *Polysiphonia* is a genus of algae that is only found in the gardens of damselfish; the two depend on each other for food and survival (Hata and Kato 2006). These mutualistic relationships are important for the health and function of the reef community because they provide each organism with an increased chance of survival and reproduction.

A phase shift of dominant benthic organisms from a reef with scleractinian corals to macroalgae is common with declining reef health (Mumby et al. 2007). The abundance of macroalgae increases the foraging opportunities of particular herbivores. The mass die off of the long-spined urchin *Diadema antillarum* in 1983-1984 caused the parrotfish community to become the dominant grazers.

While symbiotic relationships of many herbivores within the reef system are ecologically important, the Threespot

damselfish, *Stegastes planifrons*, is the focal species of this study. They cultivate and maintain algal gardens on and around corals (Aanen 2010). They maintain particular algal species in gardens by defending them from other herbivorous fishes, like parrotfish, and other individual damselfish (Souza et al. 2011). Because of this deterrence of herbivorous fish, there is an increased presence of turf algae and *Polysiphonia* in the gardens of damselfish. (Hata and Kato 2006) This presence impedes water flow and changes the substrate, from coral to algae, which decreases the possibility of coral larvae settlement. The survival of post-settlement larvae is less successful in areas with high algae biomass (Arnold et al. 2010), such as a damselfish garden.

Damselfish can be both beneficial and detrimental to the coral reef ecosystem. They are beneficial because their algae gardens can possibly provide food for herbivorous fishes, such as parrotfish, that attempt to graze on their gardens; if herbivorous fish have the opportunity to graze on this algae, it can help maintain the ecological structure of the reef community (Mumby et al. 2007). In an algae abundant reef, damselfish are detrimental because they remove coral tissue of slow growing scleractinian corals to create their algae gardens (Rotjan and Lewis 2008). These corals have slow growth rates when in direct contact with turf algae which can affect their long-term growth and survivorship in tropical reefs (Lirman 2001). Throughout Caribbean reefs, including Bonaire, the herbivorous fishes have the opportunity to exploit many algal resources in a coral reef declining in health (Hughes et al. 2007) because of the current phase shift to an algal-dominated ecosystem (Mumby et al. 2007). This makes Bonaire an ideal study site for damselfish garden and territoriality research.

This study is important because if *S. planifrons* are defending a large territory surrounding their algae gardens, then they are decreasing the settlement success of corals as the coral recruits have a lower success rate in a turf algae based substrate (Arnold et al. 2010). The protection of algae gardens by *S. planifrons* could also be promoting the current phase shift to the coral depauperate ecosystem because the recovery of coral populations may not be allowed by moderate increases in grazing (Mumby 2009).

The agonistic behavior of *S. planifrons* is demonstrated by means of biting and chasing (Di Paola et al. 2012). They will chase almost any intruder, including juveniles of their own species (Harrington 1993). Based on previous studies examined, this study hypothesizes that:

H_1:There is positive correlation between the size of the garden and the size of the surrounding territory is present

A positive correlation between these two variables can manifest three things: (1) that predatory herbivorous fishes such as parrotfish have a less likely chance of grazing on the particular algae species living within the gardens (Myrberg Jr. and Thresher 1974), (2) there is less space available for other damselfishes to possibly cultivate gardens of their own as the damselfish attacks garden intruders (Myrberg Jr. and Thresher 1974) and (3) less coral recruits will be present. This correlates with more algal cover and less coral recruitment success, which can be detrimental to the reef as corals cannot grow successfully in algae dense environments (Hughes et al. 2007).

Materials and methods

Study site

This study took place on the fringing reef on the leeward coast of Bonaire, Dutch Caribbean. Data was collected at the dive site Yellow Submarine (12º09’36.38’’N,

68º16’55.43’’W) (Fig. 1) at depths of 6-12 meters. This reef is separated from the shoreline by a large sandy area; the reef crest begins at approximately 5 meters in depth. The reef extends on a downward slope until about 27 meters in depth when sandy bottom starts and extends along the ocean floor.

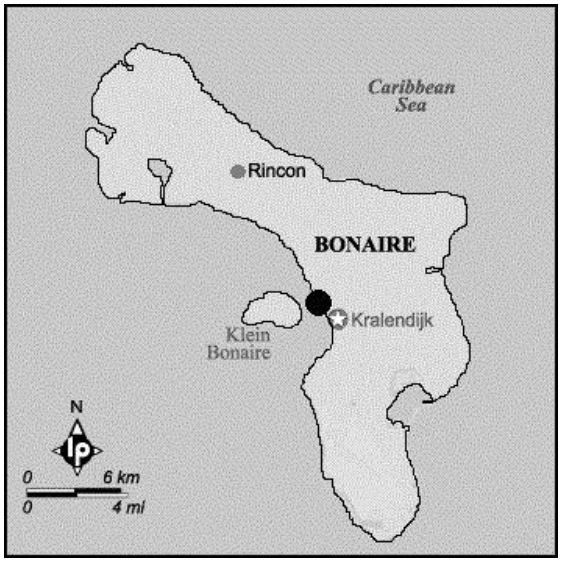

Fig. 1 Yellow Submarine dive site (12º09’36.38’’N, 68º16’55.43’’W) indicated by a black dot on the leeward coast of Bonaire, Dutch Caribbean (modified from greece-map.net)

Field research

To test the territoriality of *S. planifrons*, 10 gardens with a mean depth of 10 meters were selected by the garden distinctness. Distinctness was determined by a presence of a clear line separating different types of algae (Fig. 2). Attacks made by the subject *S. planifrons* were tested by hovering approximately one meter over the garden and shining an underwater laser pointer in various patterns into the garden until the *S. planifrons* began to attack it. The laser was moved slowly out of the garden at different corners until the damselfish ended the attack by retreating. The point of retreat was noted using natural landmarks. This process was repeated until the individual failed to react to the continuing presence of the laser. A measuring tape was used to measure the length and width of the garden and the length and width of the surrounding territory.

After the initial test with the laser, different colored weighted plastic fish replicates approximately 3 cm in length and 1 cm in height were presented to the garden from a distance of one foot away. The weighted fish replicates, attached to a 1m PVC pipe via fishing line also approximately1m in length, were slowly moved into the garden. The location of initial attack or retreat made by the individual was noted based on natural objects. *S. planifrons* in the case of this study only reacted to the fish replicates inside of the garden, so the fish replicates did not supply any viable data for this study.

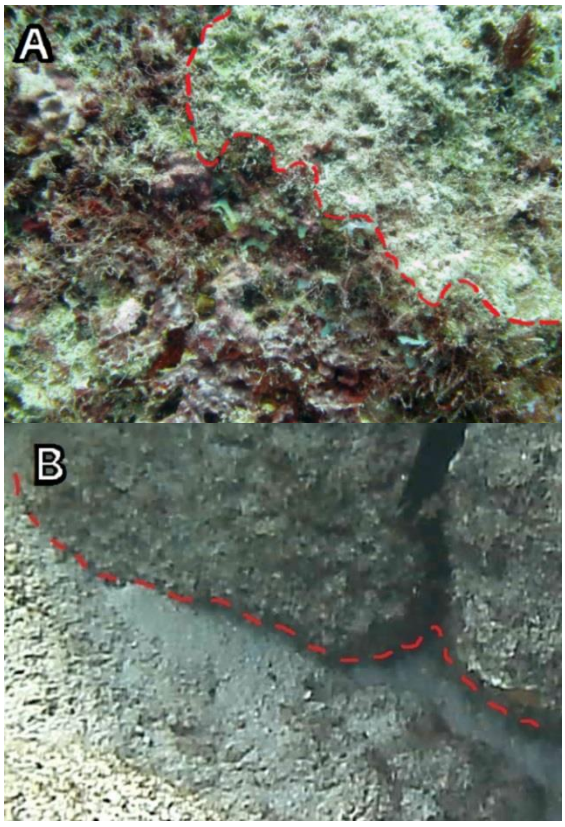

Fig. 2 Various algae garden edges of *Stegastes planifrons*. (A) The lower left corner is inside the garden and the upper right is outside of the garden separated by a dashed line. (B) The garden edge of *S. planifrons* is indicated by a dashed line. Above the line is the algae garden on old dead *Orbicella annularis*, below the line is the sandy bottom

Behavioral analysis

Attack behavior was identified as S. *planifrons* swimming patterns during the chasing of the laser or fish replicates. Retreat behavior was determined by the lack of swimming after the laser or fish replicates. Other specific behaviors regarding fin position were identified in the field.

Data analysis

The length and width measurements were used to calculate the area of the garden and the area of the territory. The depths of the gardens as well as the percent composition of the garden to territory were noted. This data was put into scatter plot graphs and analyzed with linear regression and correlation analysis (Pearson) between (1) area of garden and area of territory, (2) depth and area of garden, and (3) percent composition of garden area and territory area.

Results

Behavioral analysis

Attack behavior was identified by the individual facing the laser or fish replicates, widening of the pectoral fins, rising of the dorsal fin, and swimming towards the laser or fish replicates. Retreat behavior was determined by the facing away from the laser or fish replicates, shortening of the pectoral fins, lowering of the dorsal fin, and pausing and/or swimming away from the laser or fish replicates. However, as stated earlier, *S. planifrons* only reacted to the fish replicates while inside of their garden, not outside in the surrounding territory.

Data analysis

Ten algae gardens of *S. planifrons* were sampled in this experiment ranging in areas from 1332 cm^2 to 8800 cm^2; with an average garden area of 4209.6 cm^2. The gardens were observed on old, dead coral heads with their size limited based on the surrounding reef structures (i.e. sandy bottom, live coral, other damselfish, etc.) (Fig.3). The smallest territory was 10745 cm^2 and the largest was 23494 cm^2; the average territory area was 22261 cm^2. Territory was composed of the physical garden and the longest distance (used as the radius) traveled away from the garden by the individual in the attack. All subject damselfish reacted aggressively towards the laser pointer, but none of them acted towards the fish replicates aggressively outside of their garden; they only acted aggressively to them inside the garden, therefore none of this data from the fish replicates was used in the results of this research. A correlation analysis was performed using Pearson's correlation value (r). The correlation coefficient between area of garden (cm^2) and area of territory (cm^2) was 0.812, which illustrates a clear correlation. The goodness-of-fit was determined by linear regression (R^2=0.659) (Fig. 4). Garden size was divided by territory size to calculate the garden's percent occupation of the microhabitat of *Stegastes planifrons* (Fig. 5). The average percent occupation of the garden in the habitat was 19% (±6.6); the surrounding territory composed on average 81% of the habitat. This indicates that the average *S. planifrons* protects and defends a territory four times bigger than its garden. The correlation coefficient between depth and area of garden (cm^2) was also tested and determined to be -0.635 demonstrating a weak correlation. These two variables were also analyzed by linear regression (R^2=0.403) (Fig. 6).

Discussion

All gardens of the observed *S. planifrons* were limited based on the reef structures. Some gardens were observed on old, dead

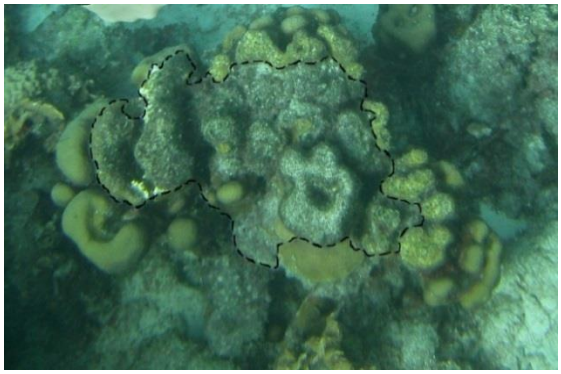

Fig. 3 Algae garden of a Threespot damselfish, *Stegastes planifrons*, demonstrated by a dashed line

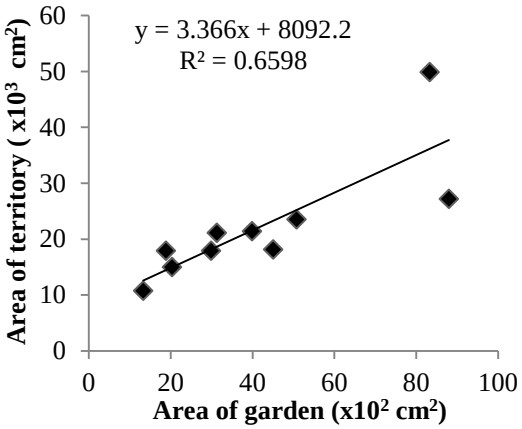

Fig. 4 Relationship between area (cm^2) of the algae garden and the area (cm^2) of the territory of the microhabitats of *Stegastes planifrons* on the fringing reef of Yellow Submarine dive site on the leeward coast of Bonaire, Dutch Caribbean (n=10)

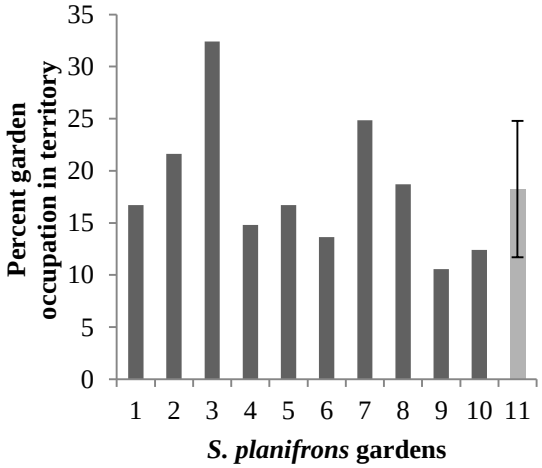

Fig. 5 Percent of the total territory occupied by the physical algae garden (n=10). Total average percent occupied demonstrated in last column (±SD)

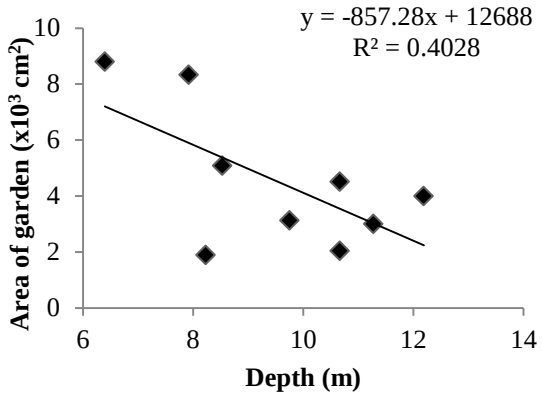

Fig. 6 Area (in cm^2) of the garden compared to the depth (m) of the garden of *Stegastes planifrons* (n=10)

coral heads that were surro unded by sandy bottom while others were surrounded by live coral. Because the gardens are limited on their available space, *S. planifrons* cannot extend their gardens without biting off live coral, so they protect their gardens and defend their territory more aggressively. Damselfish are well known to for attacking divers as they defend their gardens.

All observed *S. planifrons* in this study did not react to the fish replicates outside of their algae gardens. This could be due to the unrealistic nature of the fish replicates. Because the replicates had to be moved manually, *S. planifrons* did not appear to view the fish replicates as a threat demonstrates by the lack of aggressive behavior.

The results of this study support the hypothesis that there is a positive correlation between the area of the garden and the area of the territory. The results of linear regression supported this hypothesis with a 66% goodness-of-fit. This correlation implies that damselfish will defend its territory, not just its garden. Because of the deterrence created by the aggressive behavior of *S. planifrons*, fish, including herbivorous grazers, are unlikely to enter *S. planifrons'* territory without being attacked. Without the grazing by herbivorous fishes in the algae gardens, more algae is grown and less coral recruits are present (Myrberg Jr. and Thresher

1974). This is also supported by Hughes et al. (2007) who concluded that coral larvae cannot grow successfully in algae dense environments, such as a damselfish garden.

The percent occupation of the garden in the entire microhabitat of *S. planifrons* was on average 19%. The territorial behavior of damselfish monopolizes a habitat area irregular to its size. This is a limitation of the algae gardens size because a discrepancy is created between the total fish biomass and the available space on the reef for other fish to occupy. *S. planifrons* cannot create a garden in the territory of another because of the aggressive behavior of another individual *S. planifrons* protecting its territory. The other limitation is the surrounding substrate. A garden cannot be expanded on the surrounding live coral without the coral destruction caused by the damselfish. If live coral is present than the presence of algae is limited, and therefore cannot be cultivated by *S. planifrons*. The algae garden can also be limited due to the surrounding sandy bottom because algae cannot grow on that substrate (Rotjan and Lewis 2008).

The area of the garden and the depth of the garden were not strongly correlated with a correlation value of -0.635 and a goodness-of-fit of 40%. This could be due to the lack of large variance of depth. Depths in this experiment ranged from 6.4 to 12.2 meters. If depths had greater variance, then the results could be due to lack of algae and/or the lack of herbivorous fishes at greater depths. Algae are photosynthetic and need the sunlight in order to survive. With greater depths comes less sunlight; and therefore, less algae available for the damselfish to cultivate gardens and for herbivorous fishes to graze on.

The most important aspect that this study implies is the facilitation of the current phase shift from a coral reef to a coral depauperate ecosystem. This shift to an algal-based reef would retain less biodiversity of reef fish (Mumby 2009). As damselfish deter intruders, including herbivorous algae grazers, they are promoting the growth of certain algae species on the reef. This promotion decreases the opportunity for coral larvae recruitment, settlement, and growth, which causes declines in coral cover on the reefs of Bonaire and around the world (Arnold et al. 2010).

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REPORT

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Causative agent for dark spots in ocean surgeonfish (*Acanthurus tractus*)

Abstract Coral reef ecosystems provide a number of important ecological services, such as nurseries and protection from storms. This makes their health of vital importance for human populations. Past epidemics in the Caribbean involving high mortality of predominant species, such as long-spined sea urchins (*Diadema antillarum*) and elkhorn coral (*Acropora palmata*) have shown the potential of disease to fatally disrupt coral reef ecosystems already under stress. The high prevalence of an unknown disease in ocean surgeonfish (*Acanthurus tractus*) in the Caribbean, and its apparent ability to infect other fish, including parrotfish and other predominant grazers, is a source of concern since it affects a number of herbivorous fish that are integral to the health of the reefs. This disease is identified by the presence of black spots over the body and fins of infected fish. The number of spots can vary widely. Fin rot and lethargic behavior have been noted in fish with large numbers of spots. Bacterial cultures of swabs from healthy and dark spot epidermis, and necropsy of eight *A. tractus* specimens were used to attempt to identify the causative agent. This study found smaller bacterial numbers in the dark spot epidermis compared to healthy epidermis cultures, and the presence of encysting organisms embedded in the epidermis directly below black spots in body and fins of *A. tractus*. Additional encysting organisms were found deeper in the muscle tissue and did not produce a black spot. These encysting organisms are proposed to be digenean trematodes in the metacercariae life stage.

Keywords Disease • *Acanthurus tractus* • Parasite • Cyst

Introduction

The coral reef ecosystem provides a number of very important ecological services; its role as a protective barrier from storms and home to commercially important species, and its high productivity and biodiversity (Odum and Odum 1955; Barbier et al. 2011) make the health of these systems of great concern. Disease has the power to greatly affect the health of the coral reef community. White pox disease, which is caused by a bacteria originating in humans' colons, has eliminated great expanses of elkhorn coral (*Acropora palmata*), a major contributor to reef rugosity and habitat to many other species (Sutherland et al. 2010). The 1983-1984 mass mortality event of the long-spined sea urchin (*Diadema antillarum*) killed the majority of this once prevalent population throughout the western Atlantic (Lessios 1988) and initiated a shift in the reefs of Jamaica from predominantly coral to almost exclusively macroalgae (Hughes 1994). In addition to the disastrous effects of disease on reef communities; the prevalence of disease can be used as an indicator of coral reef health. For example, the presence of parasites with complicated life cycle interactions involving a number of hosts can help assess the integrity of an ecosystem's trophic structure (Marcogliese 2005) and can also be used as a bioindicator of anthropogenic pollution (Mackenzie 1999). In Caribbean reefs,

widespread diseases of reef building corals has been linked to pollutants that foster the proliferation of microbes and depress the immune system of the host (Hayes and Goreau 1998).

The population of *Acanthurus tractus* (ocean surgeonfish), formerly classified as *A. bahianus* (Bernal and Rocha 2011), in the reefs of Bonaire has been exhibiting symptoms of an unknown disease since the 1980s. A study from 2012 estimated that over 80% of the population exhibited characteristic black, diffused spots on their bodies and fins (Hoag 2012). Similar dark spots have been seen on the skin of a number of other fish species, such as parrotfish and bar jacks, but the level of "infection" is not as high or widespread as it is in *A. tractus* (Hoag 2012). The Hoag (2012) study found a correlation between depth and presence of disease in *A. tractus*, and it was hypothesized that the prevalence of disease was higher in shallower waters because the pathogen was more successful in warmer water. Ocean surgeonfish are highly mobile (Lawson et al. 1999), so individuals infected in shallower waters should be perfectly capable of foraging and being surveyed in deeper waters. The gradient in disease prevalence with depth must be caused by a factor different than a gradient in the presence of the pathogen itself.

The apparent low mortality of this disease makes its effects on the coral reef ecosystem health hard to discern. *D. antillarum* and *A. palmata* were previously widespread species. The ecological importance of these keystone species became apparent in their absence from the reef community (Lessios 1988; Hughes 1994; Sutherland et al. 2010). This disease has been reported in all three species of surgeonfish (*Acanthuridae*) and in some species of parrotfish (*Scaridae*) present in Bonaire (Hoag 2012), all predominant grazers in the Caribbean coral reef ecosystem (Lewis 1986). A decline in the health of the grazing fish population would favor an increase in macroalgae, which could compromise the health of the coral reef (Lewis 1986). Considering the very high prevalence of the unknown disease, identification of the pathogen may help understand its impact on both the population of *A. tractus* and the overall health of the reef (Hayes and Goreau 1998; Mackenzie 1999; Marcogliese 2005). This disease has value as a reef health indicator because it is easily visible and very widespread (Hoag 2012), and the prevalence of the disease in a population can be determined without the need to perform necropsy on the host. Linking the presence and prevalence of this disease to an environmental condition, such as pollution levels, would allow scientists and reserve managers to monitor the condition with visual surveys of infected reef fish. This type of survey does not require high levels of expertise or specialized equipment to be conducted, and can be used in no-take zones since it doesn't require the capture of diseased individuals, which would make it an easy to use indicator.

The purpose of this research is to attempt to identify the pathogen through external and internal anatomical examination and microbiological testings of skin samples of *A. tractus* specimens.

Materials and methods

Study site

Eight *A. tractus* were collected on the leeward side of the island of Bonaire, Dutch Caribbean (Fig. 1). Collection took place at the fringing reef 0.5 km south of the Something Special dive site (12º16'01"N, 68º28'19"W), between 5 and 8 m of depth.

Bacteriology

A bacterial pathogen responsible for the black spot lesions should only be present, or be much more numerous, in the

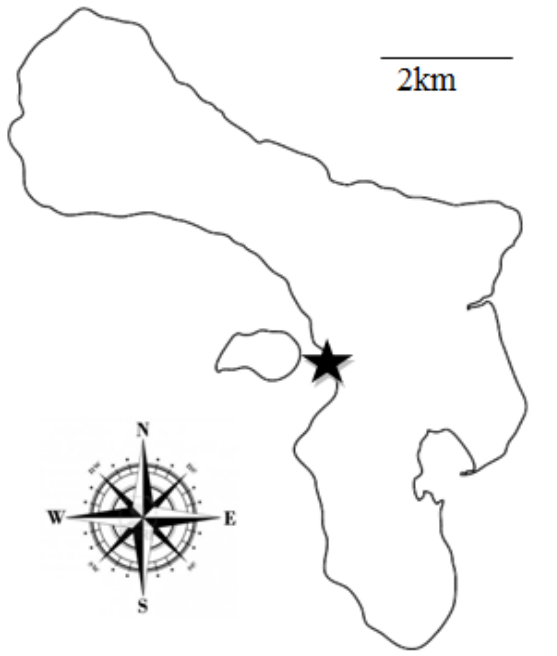

Fig. 1 Map of Bonaire, Dutch Caribbean. The *black star* represents the site where *Acanthurus tractus* specimens were collected by net in the shallow reef crest habitat

diseased tissue cultures. Culturing the bacteria present in the surface of healthy and dark spot tissue aids in the detection of differences in bacterial presence between the two types of tissue. *Escherichia coli* and Enterococci are common indicators of the presence of human contamination. Culturing specifically for these organisms can allow to asess the involvement of this factor in the presence of dark spots.

Using sterile loops the fish epidermis was swabbed in areas directly atop a dark spot lesion and in adjacent healthy areas. These loops were used to inoculate Tryptic soy agar medium and Columbia™ nutrient-rich blood agar medium for bacterial culture. The soy agar medium was chosen for its ability to grow a broad range of marine bacteria, and the blood agar medium for its ability to grow pathogenic bacteria that will not grow on other general media. One plate of each medium inoculated with each type of tissue was cultured at 26ºC (room temperature) and another at 37ºC (Noga 2010). The number of colony forming units (cfus) on each plate were counted and described after 48h of incubation. Additionally, skin scrapes from spotted areas in one fish were collected with a sterile loop and placed into two sterile vessels with 100 ml of milliQ water. IDEXX Colilert-18™ or Enterolert™ substrate pellets were added, and each sample was incubated for 24h at the temperature indicated by the IDEXX methodology, in order to detect presence of *E.coli* and enterococci.

External examination

The specimens were euthanized immediately before examination and photographed. The fork length, standard length, total length and weight of each specimen were recorded. Number and size of dark spots on the body surface was recorded and each side of the fish was photographed. Using a scalpel, samples of spotted and apparently healthy skin, fins and muscle tissue were excised and examined under dissection and light microscopes. Gills, a common site of parasite infestations (Noga 2010), were examined for abnormalities in coloration, gross lesions and presence of visible parasites.

Internal examination

The specimens were placed into lateral recumbency, left side down. Four incisions were made using surgical scissors to expose the internal organs. One from the anal vent, extended anteriorly through the pelvic girdle and to the base of the operculum. Another two following the shape of the operculum and from the anal vent upwards following the shape of the body cavity until the swim bladder became visible. A fourth incision was used to join the latter two, and a scalpel was used to detach the peritoneal lining from the internal organs, removing the detached tissue to expose the viscera. Care was taken not to puncture any internal organs.

The interior condition of each fish was examined and photographed, and cuts were made proximal to the esophagus and distal to the gastrointestinal tract to remove and examine the organs. The liver was weighed, and the liver, stomach, intestines, gall bladder and heart were examined for gross abnormalities. The muscular stomach was cut open and stomach contents were examined (Noga 2010).

Results

Bacteriology

Sixteen plates were inoculated with bacterial swabs taken from *A. tractus* epidermal tissue. Eight from healthy tissue and eight from tissue overlying dark spots. The number of cfus growing per plate after a period of 48h ranged from zero to a number too great to effectively differentiate (Table 1). Overall, every plate inoculated with spot skin tissue grew a number of colonies equal or smaller than the same medium, same temperature plate inoculated with healthy skin tissue. Two plates, the soy agar healthy tissue inoculations at 25ºC and 37ºC, liquefied, rendering a count of cfus not possible.

The result for the test for enterococci was negative. The test for *E. coli* showed two cfus of coliforms but it was negative for *E. coli*.

External examination

The number of dark spots visible on each fish ranged from zero to 33. Examination of normal epidermal tissue and epidermal tissue with dark spots, both in the body and fins revealed the presence of pigmentation, but no superficial lesions (Fig. 2). A small spherical tissue growth was found in the center of most spots. These small growths were particularly prominent if present on the soft skin of the fins, but were also present fused with fin rays. The micrographs in Fig 2 show the concentrated pigmentation on a pectoral fin clip with dark spot (Fig. 2a) and a healthy fin ray from the same *A. tractus* specimen (Fig. 2b). Examination of a cyst embedded in immediate proximity to a fin

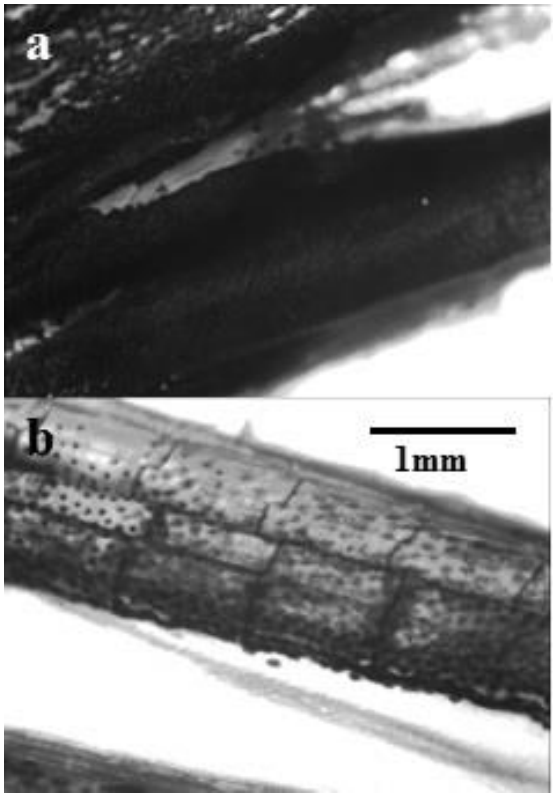

Fig. 2 Micrographs of the pectoral fin of *Acanthurus tractus* with **a.** dark spot present and **b.** a healthy area

Table 1 Comparison of bacteriological growth in samples from spotted and healthy *A. tractus* epidermis. Blood and soy agar plates were inoculated and incubated at 25ºC and 37ºC for 48 hours. - indicates compromised cultures. * indicates cultures that grew cfu's too thickly to be accurately counted

Type of Tissue	Number of colony forming units (cfu)			
	Blood Agar 25°C	Blood Agar 37°C	Soy Agar 25°C	Soy Agar 37°C
Fish 1-Healthy swab	1	0	8	5
Fish 1-Spot swab	0	0	2	5
Fish 2-Healthy swab	17	20	-	-
Fish 2-Spot swab	14	16	*	*

ray revealed structural damage to the cartilage of the ray where the cyst was embedded (Fig. 3).

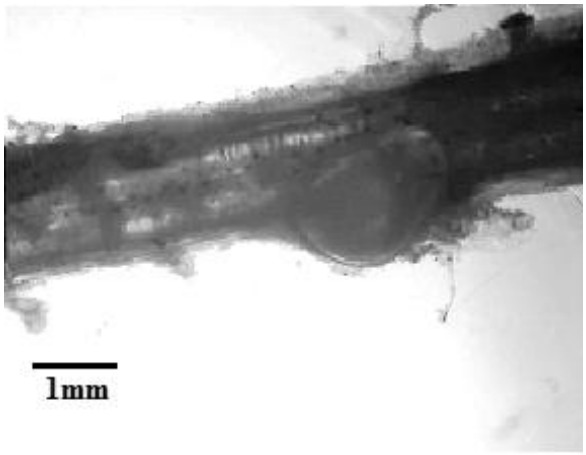

Fig. 3 Micrograph of a cyst embedded in a soft ray from an *Acanthurus tractus* dorsal fin. Note that part of the cartilage is missing from the ray where it has been replaced by the cyst

Examination of the tissue underneath a pigmented spot on the flank of fish specimen number 4 revealed a small, white, slightly dome shaped cyst of 2.0 mm (height) and 2.3 mm (width), embedded in the superficial layer of the skin directly underneath the center of the spot. Cysts were found embedded beneath the epidermis in every dark spot examined in the body and fins of the fish.

Two blocks of tissue of approximately 1x1x0.5 cm containing a black spot were removed from the body of two surgeonfish. On the first block, one cyst-like organism was found embedded in the skin centered under the dark spot, and eight additional cysts were located deeper in the muscle tissue. The second black spot also contained a cyst embedded below the dark spot and three additional cysts on the underlying muscle tissue. A micrograph of an excised cyst (Fig. 4) shows the clear, spherical membrane surrounding the organism.

Movement was detected within most cysts. One cyst was excised and an intact organism was extracted. This organism was 2.7 mm long and exhibited bilateral symmetry (Fig. 5). The shape of the body was flat and elongated, slightly wider at one end, and had two paired whorls of tissue at the opposite end. The width of the body was 0.8 mm at the thicker end and 0.6 mm at the thinnest, central part. The whorls had a maximum width of 2.3 mm.

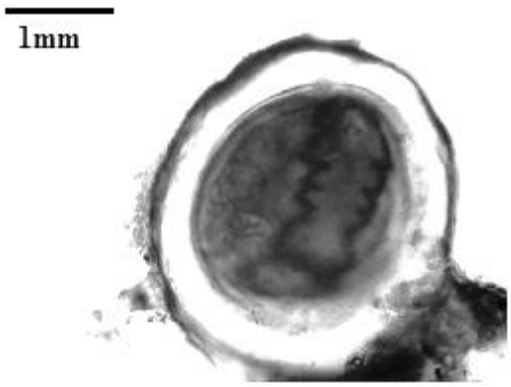

Fig. 4 Micrograph of a cyst removed from the tissue of a specimen of *Acanthurus tractus*. The clear capsule surrounding the organism is 0.2 to 0.3 mm thick. The diameter of the cyst is 2.0 mm (width) by 2.3 mm (length)

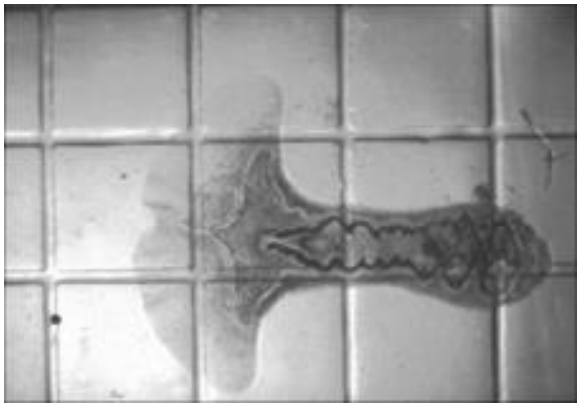

Fig. 5 Encysting organism removed from its capsule. The organism is placed over a grid with each square being 1 mm x 1 mm

Internal examination

Gross examination of the internal organs of the eight specimens revealed no abnormalities. Stomach contents included filamentous algae, small amounts of sand and on three separate specimens, an *A. tractus* tooth. No additional parasites were recorded anywhere on any of the fish.

Discussion

This study determined the causative agent for dark spots in *A. tractus* to be a parasite, tentatively identified as a Digenean trematode in the metacyrcariae life stage. Heavy infestation can damage the fins of the fish, but no other severe adverse effects were noted. Bacterial composition between dark spot and healthy epidermis areas differs, as there were more bacteria present in healthy epidermis.

Every valid analog pair of culture plates (same medium, same incubation temperature, same fish, different type of tissue) yielded more cfus from the swab of healthy tissue than from the swab of the dark spot epidermis. This suggests that the presence of the encysting organisms modifies the bacterial populations in the epidermis of the fish. No colony morphology appeared exclusively in bacterial cultures from dark spot areas of the epidermis, which suggests that the dark spots were not caused by any of the cultured bacteria.

Encysting organisms were found embedded in the epidermis under every dark spot examined, both on the body and fins of *A. tractus*. This result suggests that these encysting organisms are the causative agent for the dark spots. In body spots, pigment concentrations often have a lighter area where a cyst is embedded in the skin tissue, but beyond the space the encysting organism occupies there was no evidence of damage to the surrounding muscle tissue. In fin spots, pigmentation was also concentrated, and organisms encysted in close proximity to cartilaginous fin rays were observed to be capable of causing structural damage, as they were encysted directly into the cartilage. Hoag (2012) reported that specimens with heavy infestation exhibited damage to the fins. The observed damage to the fin rays produced by the cysts suggests this symptom is likely directly linked to the presence of the encysting organisms and not caused by secondary infection.

No other life stages of the parasites found were located anywhere on the fish, and none of the dark spots were ulcerated, which would suggest that *A. tractus* is a host to a specific life stage, and that parasites do not exit through the skin. Morphology suggests a digenean trematode (fluke). Flukes reproduce twice in their life cycle, as adults and as larvae, and generally have complicated life-cycles that involve more than one host species. The observed organisms are most consistent with the metacercariae life stage of a fluke. Metacercariae cysts usually occur in a second intermediate host. The host is then consumed by a predator, and if this is the appropriate final host, the cysts get digested and the adult flukes are released into the organs of the host (Williams and Bunkley-Williams 1996).

The possible taxonomic classification of this organism is only proposed to the subclass Digenea level. Taxonomic identification requires a high level of expertise in parasitology. Identification to the genus or species level may lead to identification of the local host species on the reef in Bonaire. If it is indeed a metacercariae cyst, the final host may include any large carnivore capable of ingesting *A. tractus* present within the range of the disease.

If a correlation between the number of cysts visible as dark spots and the total number of cysts can be established, visual surveys would give a more accurate idea of the level of infection of *A. tractus*. Similar dark spots have been reported in other species of fish (Hoag 2012). Examination of the muscle tissue and dark spot epidermis of other species would confirm if these pigmentations are caused by the same parasite. In order to establish the life cycle of this parasite, examination of the organs of predators in the Caribbean coral reef ecosystem could help identify the intended final host. Establishing the complete life cycle of this parasite would

be very useful in order to determine the reason for its abundance in the metacercariae stage and to assess its abundance in other hosts, which in turn could help determine the effect of this organism in the coral reef ecosystem health.

Overall, a better understanding of the life cycle and abundance of the black spot-causing fluke could not only provide understanding of its impact on the ecosystem, but could give it relevance as an indicator of the coral reef conditions that favor its proliferation. Its visibility in the skin of the fish makes it ideal for visual survey techniques, which can be freely applied in any coral reef, including no-take zones.

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REPORT

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Caribbean parrotfish foraging: An interspecific comparison of algal preferences

Abstract In recent decades, reduced grazing pressure caused by a die-off of *Diadema antillarum* and the overexploitation of herbivorous fishes have facilitated a phase shift from coral to algal dominated reefs. Thus, conservation of herbivorous fishes has become increasingly important on coral reefs. In the Caribbean reefs, parrotfish are the dominant herbivores. Studies have been conducted on parrotfish grazing, but there is a lack of knowledge about specific algal preferences. This study examined differences in bite frequencies on algal types and algal preferences of the most common parrotfish species of Bonaire, Dutch Caribbean. Mean bite frequencies (bites 30 min^{-1}) and preferences were determined by offering algal plates with *Padina* sp., *Ulva* sp., *Sargassum* sp., and turf algae to parrotfish on the coral reef flat. During field observations, data was collected on the number of bites taken and algal type grazed by each individual parrotfish. Parrotfish as a group, and individual species (*Sparisoma rubripinne*, *Scarus viride*, and *Sparisoma aurofrenatum*), demonstrated significant differences in mean bite frequencies on algal types offered. There were also significant differences in mean bite frequencies among the three parrotfish species. All species of parrotfish, collectively and individually, demonstrated preferences for *Padina* sp. and avoidances for all other algal types offered. Determining which algal types parrotfish graze, and how grazing differs among parrotfish species is ecologically important. The results provide an understanding of how the selective pressures of specific herbivores may help regulate harmful macroalgae, and suggest the importance of maintaining the diversity of herbivorous fishes on the reef.

Keywords Parrotfish • Foraging • Algal preferences

Introduction

Coral reefs are economically and ecologically important ecosystems that provide a basis for tourism, fisheries (Hughes 1994), and extraordinary biodiversity (Lewis 1986; Bruggemann et al. 1994). The health of Caribbean coral reefs has declined in the past few decades, leading to a phase shift from coral dominated reefs to those overgrown by fleshy macroalgae (Hughes 1994; Smith et al. 2006; Dixon and Hay 2012), threatening the survival of coral reefs in the region. The shift is attributed partly to the massive disease induced die-off of the grazing urchin *Diadema antillarum* in the early 1980s (Lessios et al. 1984; Hughes 1994; Kuffner et al. 2006; Mumby et al. 2006; van Woesik and Jordan-Garza 2011). In the absence of *D. antillarum*, the importance of herbivorous fishes in preventing algae from outcompeting coral (Rotjan and Lewis 2006), facilitating re-colonization, recruitment, and growth of coral, and enhancing reef resiliency (Kuffner et al. 2006; Hoey and Bellwood 2008; Dixon and Hay 2012) has increased

proportionately. Therefore, historical and current overfishing of dominant grazers, such as parrotfish (Lewis 1985, 1986; Mumby et al. 2006; Hoey and Bellwood 2008), jeopardizes the biological diversity and survival of coral reefs (Bellwood et al. 2004; Mumby et al. 2006; van Woesik and Jordan-Garza 2011; Dixon and Hay 2012).

Grazing by parrotfish is an important pathway of converting primary productivity to higher trophic levels in the coral reef community (Mumby et al. 2006). Previous studies on feeding behaviors of parrotfish have described grazing methods, rates, and general algal preferences (Lewis 1985; McAfee and Morgan 1996; Hoey and Bellwood 2008; Cardoso et al. 2009). Parrotfish species demonstrate a difference in bite frequency on different algal types possibly based on their categorization into either scraping or excavating foraging groups; scrapers tend to take frequent superficial bites, while excavators take fewer, more forceful bites, often biting into the substrate (Cardoso et al. 2009; Francini-Filho et al. 2010). Parrotfish feed on turf algae and macroalgae such as *Padina* sp., *Sargassum* sp., and *Turbinaria* sp. (Lewis 1985). Since it is clear that parrotfish are somewhat selective feeders (Lewis 1985; Bruggemann et al. 1994), parrotfish may show a preference for types of algae that have a high nutritional value, or are more palatable. Turf algae provides the highest amount of protein (113.9 mg g^{-1} ash-free dry weight (AFDW) whereas macroalgae and turf algae have the highest energetic value (18.7 ± 1.5 kJ g^{-1} AFDW and 19.1 ± 2.0 kJ g^{-1} AFDW, respectively) (Bruggemann et al. 1994). In terms of taxonomic differences, green algae is more energy-rich than brown algae (Bruggemann et al. 1994). Differences in feeding structures of parrotfish, and in algal properties could lead to a difference in preference.

The current study focused on testing feeding patterns on a more specific range of algal types, which were examined individually, rather than as functional groups. The main objectives of the study were to determine (1) if parrotfish in general exhibit differences in mean bite frequencies among types of algae (2) if certain parrotfish species exhibit differences in mean bite frequencies among types of algae, and (3) if there is a variation in algal preference among species of parrotfish. Field experiments were used to test the following hypotheses:

H_1: Bite frequencies of parrotfish as a group, and separated by species, will differ among algal types offered in a field experiment in the coral reef habitat

H_2: Bite frequencies on algal types will differ among parrotfish species

H_3: Parrotfish, as a group, and specific species, will show preferences and avoidances of algal types

It is important to determine if species of parrotfish graze specific algae because the algae that are less preferred may overgrow and outcompete coral for space, becoming increasingly problematic to the survival of the reef (Lewis 1986). Since certain species of parrotfish may select particular algal types, conservation of parrotfish diversity may have additional value to the coral reef community.

Materials and methods

Study site and species

All observations and data collection were conducted along the leeward, western coast of Bonaire, Dutch Caribbean (Fig. 1). The study site is characterized by a fringing reef, beginning at approximately 5 m depth. Parrotfish are abundant and according to surveys reported by Reef Environmental Education Foundation (REEF 2013), five parrotfish species have sighting frequencies >70% at the study site: stoplight (*Scarus viride*), princess

(*Scarus taeniopterus*), queen (*Scarus vetula*), striped (*Scarus isierti*), and redband (*Sparisoma aurofrenatum*). All five species are protogynous hermaphrodites, having an initial phase (IP) as a smaller female, and a terminal phase (TP) as a larger male. Foraging rates do not vary significantly with body size or between IP and TP parrotfish (Cardoso et al. 2009), and diet does not vary significantly between IP and TP parrotfish (Bruggemann et al. 1994). Therefore, individuals were not categorized by length or phase within species for this research.

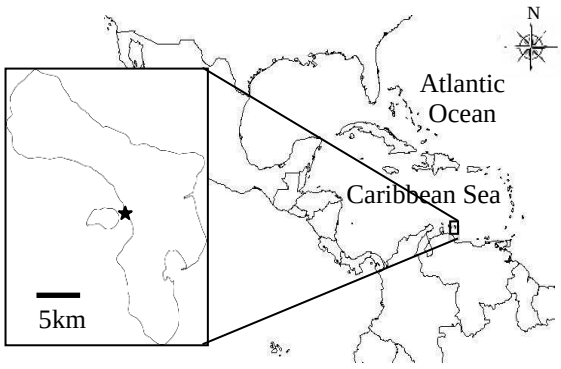

Fig. 1 Map of Bonaire, Dutch Caribbean (12°9'36.47"N, 68°16'55.16"W). The *black star* indicates the study site

Preparation of algal plates for field experiments

Algae was collected from shallow intertidal zones at the study site, and transported back to the lab. In the lab, 1.02 g of high acyl gellan gum powder was mixed into 250 ml of distilled water, boiled, and used as the basis for the algal plates offered in the field preference experiments (adapted from Henrikson and Pawlik 2009). Gellan gum can be submerged in seawater without significant degradation to the gel for up to six weeks (Henrikson and Pawlik 1995). Petri dishes with a 10 cm diameter were filled with 50 ml of gel solution and were cooled slightly at room temperature before adding algae. As gels solidified, 25 g (wet weight) of algae were weighed and embedded into the gel in an upright position (Fig. 2). *Padina* sp., *Ulva* sp., *Sargassum* sp., turf algae, and a control (gel only) were prepared and stored at 4°C until deployment onto the reef the following day.

Weights were attached with an epoxy to the bottom of each petri dish to anchor algal plates on the substrate when placed at the study site. Because the gels were slightly positively buoyant, two intersecting rubber bands were wrapped around each petri dish to keep the gels from floating out. Algal plates were replaced at the start of each trial.

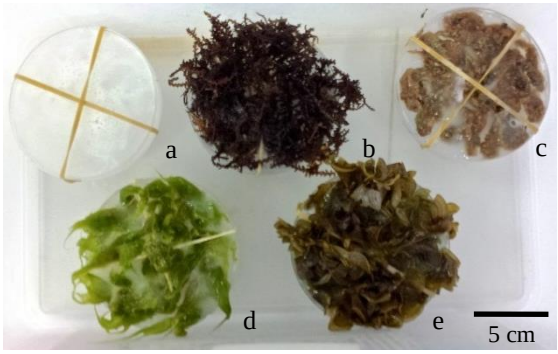

Fig. 2 Algal treatments made with gellan gum and 25 g of each algal type: **a** control (gel with no algae) **b** *Sargassum* sp. **c** turf algae **d** *Ulva* sp. **e** *Padina* sp.

Feeding observations

Fifteen trials were conducted from 19 October 2013 to 2 November 2013. For each trial, five algal plates (*Padina* sp., *Ulva* sp., *Sargassum* sp., turf algae, and a control) were placed in a patch of rubble at a depth of 1.5 m on the reef flat using SCUBA. The rubble habitat was chosen because parrotfish do not feed on bare sandy areas and, although parrotfish graze on the reef crest and slope, grazing time is highest in shallow areas where most parrotfish are non-territorial (Bruggemann et al. 1994). Algal plates were lined up in a row spaced 20 cm apart and the order of the algal plates was randomized for each trial. A video camera was set up on a PVC stand 30 cm above the substrate and recordings were made at 10:00 h, 11:00 h, 12:30 h, and 14:00 h, since feeding does

not differ significantly between mornings and afternoons (McAfee and Morgan 1996). Videos were then downloaded from the camera in the lab and analyzed. The number of bites taken by each individual parrotfish and the type of algae grazed were documented. Because individuals were not tagged, there is a possibility that an individual may have been sampled more than once.

Data analysis

Algal preferences

Mean bite frequencies by all parrotfish on the five types of algal plates were compared using a one-way analysis of variance (ANOVA). Significant differences in mean bite frequencies between pairs of algal types were then identified using t-tests ($\alpha = 0.05$).

Only parrotfish species for which more than a total of 10 individuals were observed grazing on an algal plate were considered for species-specific analysis of grazing preferences. Mean bite frequencies of yellowtail parrotfish (*Sparisoma rubripinne*), *S. viride*, and *S. aurofrenatum* were compared using a two-way analysis of variance (ANOVA) to determine (1) if there were differences among parrotfish species and (2) if there were differences among types of algal plates. Significant differences between pairs of algae within each parrotfish species and between pairs of parrotfish species for each algal type that was grazed were then identified using t-tests ($\alpha = 0.05$).

Electivity

Parrotfish grazing preference for algal types were calculated using Ivlev's electivity index (E_i), as applied in a study by Rotjan and Lewis (2006):

$$E_i = \frac{r_i - n_i}{r_i + n_i}$$

for which r_i is the proportion of parrotfish bites per minute taken on the *i*th algal type, and n_i is the proportion of abundance of the *i*th algal type. The electivity index was calculated first using data from all parrotfish species, then individually for each species of parrotfish. Values of electivity range from -1.0 to +1.0, for which a negative value expresses avoidance and a positive value expresses a preference; an electivity value of zero indicates an algal type was grazed in proportion to its abundance (Rotjan and Lewis 2006).

Results

Fifteen field trials were conducted for a total of 7.5 h of observation of feeding by parrotfish on experimental algal plates placed on the coral reef. The mean (± SD) bite frequency by parrotfish (n=104) on the five algal plates presented (*Padina* sp., *Ulva* sp., *Sargassum* sp., turf algae, and the control) was highest on *Padina* sp. (8.78 ± 12.11 bites 30 min^{-1}) and was lowest on turf algae (0.07 ± 0.686 bites 30 min^{-1}). The highest bite frequency of *S. rubripinne* (n=60), *S. viride* (n=15), and *S. aurofrenatum* (n=21) was on *Padina* sp. (9.42 ± 12.07 bites 30 min^{-1}, 4.13 ± 3.68 bites 30 min^{-1}, and 12.62 ± 15.17 bites 30 min^{-1}, respectively) and lowest on turf algae (zero, zero, and 0.333 ± 1.528 bites 30 min^{-1}, respectively).

Algal preferences

There were significant differences in bite frequencies of parrotfish as group among the algal plates offered (One-way ANOVA; $p<0.001$; Fig. 3). The mean bite frequency on *Padina* sp. was significantly greater than on *Ulva* sp. ($p<0.001$), *Sargassum* sp. ($p<0.001$), turf algae ($p<0.001$), and the control ($p<0.001$). The next highest mean bite frequency was on *Ulva* sp., which was significantly greater than on *Sargassum* sp. ($p=0.026$), turf

algae (p=0.005), and the control (p=0.003). The mean bite frequency on *Sargassum* sp. was significantly greater than on the control (p=0.011) the mean bite frequency on turf algae (p=0.068). There were no significant differences between mean bite frequencies on turf algae and the control (p=0.319).

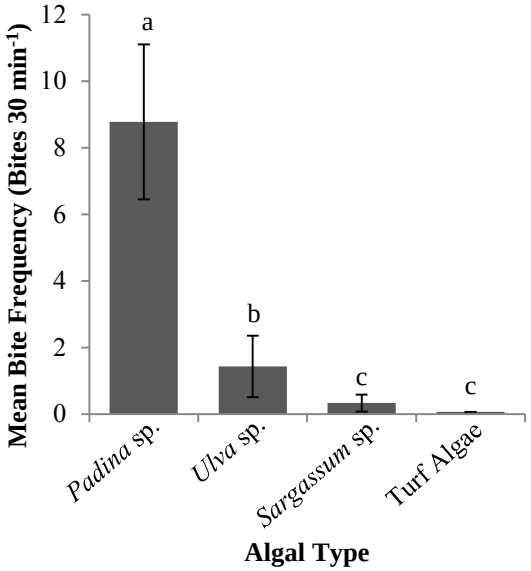

Fig. 3 Comparison of bite frequency on algal plates during 30 min field trials of parrotfish observed in field trials (n=104). Error bars indicate 95% confidence intervals. *Letters* above *bars* indicate significant groupings determined by t-tests. There was no grazing on the control plates

Species of parrotfish showed significant differences in mean bite frequencies on algal types in the field experiment (Two-way ANOVA; p<0.001; Fig. 4). *S. rubripinne* had a significantly greater mean bite frequency on *Padina* sp. than on *Ulva* sp. (p<0.001), *Sargassum* sp. (p<0.001), turf algae (p<0.001), and the control (p<0.001). The mean bite frequency on *Ulva* sp. was significantly higher than on *Sargassum* sp. (p=0.016), turf algae (p=0.012), and the control (p=0.012). There were no significant differences between mean bite frequencies on *Sargassum* sp., turf algae, and the control. *S. viride* and *S. aurofrenatum* each had a significantly greater mean bite frequency on *Padina* sp. than on *Ulva* sp. (p=0.045 and p=0.002, respectively), *Sargassum* sp. (p=0.045 and p=0.002, respectively), turf algae (p=0.045 and p=0.001, respectively), and the control (p=0.045 and p=0.001, respectively). There were no significant differences between mean bite frequencies on *Ulva* sp., *Sargassum* sp., turf algae, or the control.

There were significant differences in mean bite frequencies on algal types among the three parrotfish species tested (Two-way ANOVA p<0.001; Fig. 4). Two-sample t-tests were used to determine where differences occurred between each parrotfish species. *S. rubripinne* and *S. aurofrenatum* did not significantly differ from each other in mean bite frequencies on any algal type. *S. rubripinne* and *S. aurofrenatum* each had significantly greater mean bite frequencies than *S. viride* on *Padina* sp. (p=0.033 and p=0.046, respectively). *S. rubripinne* had a significantly higher mean bite frequency than *S. viride* on *Ulva* sp. (p=0.021), but the mean bite frequency on *Ulva* sp. by *S.*

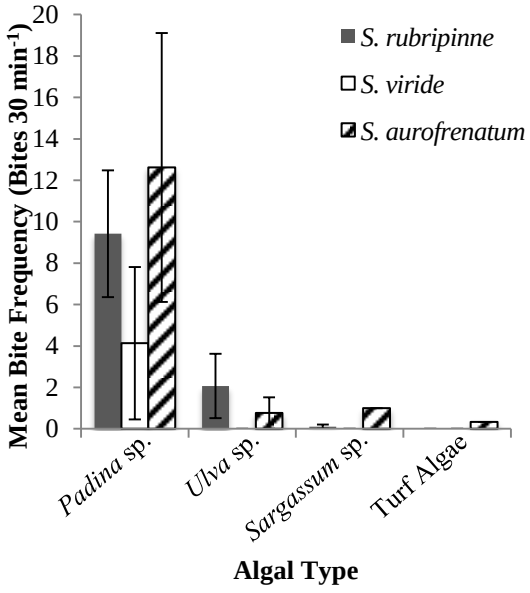

Fig. 4 Comparison of bite frequency during 30 min field trials of *S. rubripinne* (n=60), *S. viride* (n=15), and *S. aurofrenatum* (n=21) on algal plates. Error bars indicate 95% confidence intervals. There was no grazing on the control plates

Table 1 Ivlev's electivity index (E_i) for parrotfish in a field experiment that offered 4 types of algae. Parrotfish did not feed on the control. Number in parenthesis indicates n

Species	*Padina* sp.	*Ulva* sp.	*Sargassum* sp.	Turf Algae	Control
All Species (104)	0.611	-0.194	-0.726	-0.939	-1.00
S. rubripinne (60)	0.605	-0.057	-0.917	-1.000	-1.00
S. viride (15)	0.667	-1.000	-1.000	-1.000	-1.00
S. aurofrenatum (21)	0.622	-0.589	-0.493	-0.797	-1.00

aurofrenatum and *S. viride* did not differ significantly (p=0.087). There were no significant differences among parrotfish species in mean bite frequencies on *Sargassum* sp., turf algae, and the control.

Electivity

Electivity indices were calculated, using Ivlev's index (E_i), to determine preferences or avoidances of algal types (Table 1). When all species of parrotfish were grouped together, a preference was shown for *Padina* sp., whereas avoidances were shown for all
other types of algae offered. Similarly, when species were compared separately, *S. rubripinne*, *S. viride*, and *S. aurofrenatum* showed preferences toward *Padina* sp. and avoidances for all other types of algae.

Discussion

Bite frequencies of parrotfish, as a group and individual species, were highest on *Padina* sp. and lower on the other types of algae, which supports H_1 and H_2. Parrotfish, as a group and individual species, demonstrated grazing preferences and avoidances for certain types of algae in the coral reef habitat, which supports H_3.

The preference for *Padina* sp. was shown by each of the parrotfish species, however there were slight differences in the degrees of avoidance of the remaining algal types. The categorization of parrotfish species as scrapers or excavators could explain the differences in mean bite frequencies among parrotfish species (Cardoso et al. 2009). The jaw morphology and force exerted during each bite by excavating species results in lower mean bite frequencies (Francini-Filho et al. 2010). Mean bite frequency did not differ between the scraping species *S. rubripinne* and *S. aurofrenatum*, while the excavating species *S. viride* had significantly lower mean bite frequency than the two scrapers on some types of algae.

The lightly calcified morphology of *Padina* sp. could protect the alga from grazing by most herbivores, but the strong jaw and fused dental plates of parrotfish (Lewis 1985; Mantyka and Bellwood 2007) allow for consumption of calcified algae (Lewis 1985; Mantyka and Bellwood 2007). *Padina* sp. was highly susceptible to parrotfish grazing in this experiment, whereas other herbivores with more delicate jaw structures, such as members of the surgeonfish (Acanthuridae) family (Lewis 1985; Mantyka and Bellwood 2007), may be deterred by the calcification of such algae.

Although turf algae is more nutritious than other algal types (Francini-Filho et al. 2010), only one individual grazed the turf algae plates during the field trials. Turf algae may be less accessible to herbivores than macroalgae because it is densely packed, filamentous, and finely branched, making it difficult for some herbivores to bite without specialized jaws (Fricke et al. 2011). However, most fish are able to scrape and excavate turf algae. A more likely scenario is that turf algae, which is the dominant benthic substrate on the reef (Sandin et al. 2008), may not have been attractive because it is available in great abundance in areas surrounding the location of the trials. Further studies conducted in areas where turf algae is naturally sparse could determine if the

presence of nearby turf algae affected the observed grazing preferences.

This study was limited by the quantity of algal types tested; a broader understanding of algal preferences can be gained through the inclusion of additional types of algae.

The results presented provide an understanding of how selective parrotfish can be and since parrotfish are important to the grazing community on coral reefs, it is important to understand how diet breadth might affect algal community structure. Thus, it is important to conserve the diversity of herbivorous fishes on coral reefs, as the selective pressures of specific herbivores may be keeping macroalgae densities in check.

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REPORT

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Effect of competition on dark spot syndrome in *Stephanocoenia intersepta*

Abstract Corals are frequently in competition with other benthic organisms, as space on the coral reef is highly sought after. With increased coral cover loss, and a possible coral-algal phase shift on reefs, competition between corals and other benthic organisms may become increasingly common. Competition between coral and other organisms, such as algae, other corals, and sponges may be a stressor for corals and possibly lead to increased disease prevalence and severity. Dark spot syndrome (DSS) is a highly prevalent disease in Bonaire and is found in the coral species *Stephanocoenia intersepta* and *Siderastrea siderea*. Some studies show that competition in corals increases their susceptibility to disease. This study investigates the correlation between coral competition and disease severity of dark spot syndrome in *S. intersepta*. One reason for this is that energy is allocated for competition rather than immune function and thus the coral's ability to fight off disease is lowered. No significant correlation was found between the amount of competition (measured by the percent edge of the coral in competition) and the level of disease (measured by the percent of the coral with DSS). The mean disease level of all coral colonies is 2.56 **(**±0.34**)**. There was also no correlation found between depth and severity of disease. Although no correlation was found, longer and more intensive studies are suggested to better understand the effect of competition on dark spot syndrome.

Keywords Coral competition • Coral disease • Dark spot syndrome

Introduction

Coral reefs ecosystems are in decline worldwide (Hughes 1994). It is estimated that 20% of coral reefs have died and an additional 50% are threatened (Barott et al. 2009). Many factors such as nutrient pollution, overfishing, mass bleaching due to rising sea temperatures (Hughes et al. 2003), and disease (Sutherland et al. 2004) are leading to a decline in coral cover (Hughes 1994). Reefs are undergoing a coral-algal phase shift, where the reef changes from a coral dominated reef to an algae dominated reef (Smith et al. 2006). This phase-shift has led to more interactions between corals and other benthic organisms such as macroalgae and sponges. Along with increased algal cover, there has been increased disease prevalence (Sutherland et al. 2004), which reduces live coral cover and thus contributes to the coral-algal phase shift (Bythell and Sheppard 1993).

Dark spot syndrome (DSS), a common disease found in scleractinian corals, was first identified on Colombian reefs in the early 1990's (Solano et al. 1993). Since then it has spread throughout the Caribbean and has become one of the most prevalent coral diseases (Porter et al. 2011). While this syndrome has become extremely common it remains unclear whether the syndrome is the same disease infecting different coral species or if it is actually multiple diseases with a similar

appearance (Porter et al. 20011). DSS may not be a disease but rather a stress response in either the coral host or zooxanthellae (Borger 2005). Due to the confusion over this syndrome and lack of identification of an etiological agent, it will be referred to as dark spot syndrome (DSS) in this paper though it is also referred to as dark spot disease (Gochfeld et al. 2006). DSS appears on corals as small to large dark spots that may grow over time. The dark spots often expand into a ring around dead coral and are sometimes observed with a depression of the coral surface (Gil and Garzon-Ferreira 2001) (Fig. 1). DSS mainly affects the species *Orbicella annularis*, *Siderastrea siderea*, and *Stephanocoenia intersepta* (Cervino et al. 2001).

Fig. 1 A large colony of *Stephanocoenia intersepta* with a high level of dark spot syndrome. Note the surrounding corals in competition. Picture taken at N 12°09'36.3", W 68°16'55.2", Bonaire by Sarah Girouard

Stress is an important factor influencing coral diseases. There has been evidence that resistance to disease and pathogen virulence are impacted by stress (Gochfeld et al. 2006). Stress reduces the chemical defenses and thus the corals ability to fend off disease (Gochfeld et al. 2006). Sessile organisms such as corals are constantly in competition with other benthic organisms due to limited space on the reef (Van Veghal et al. 1996). Competition in corals increases stress and has been shown to lower immune function and health of the corals, leading to a greater susceptibility to disease (Ritson-Williams et al. 2009). The previously mentioned reports lead to the prediction that when there are high levels of competition on coral colonies, there will be higher prevalence and severity of DSS on the colony. The first hypothesis is:

H_1: The severity of dark spot syndrome is positively correlated with the level of the corals competitive interactions with other benthic organisms on the reef

The second hypothesis is based off temperature related stress on corals. In shallower waters the temperatures are usually lower than deeper waters. With decreasing depth the waters get warmer. Based on the idea that increased temperatures put corals under stress the second hypothesis is:

H_2: The mean disease level is will decrease with increasing depth

The results of this study help contribute to further understanding of the effect of competitive interactions on DSS in *S. intersepta*.

Materials and methods

Study site

Data collection was carried out during the month of October along a 1 km section of the fringing reef in Bonaire, Dutch Caribbean between (N 12°09'36.3", W 68°16'55.2") (Fig. 2) and the dive site, Something Special (N 12°09'40.1", W 68°16'59.7"). This location was selected based on high prevalence of dark spot syndrome determined during preliminary research and ease of accessibility for diving. Reefs in this area are

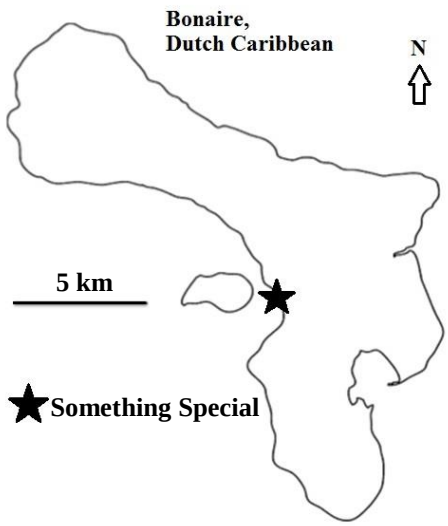

Fig. 2 Map of Bonaire, Dutch Caribbean showing the location of the present study, which took place at the dive site Something Special and 1 km to the south between 5 and 20 m depth

representative of most of the reefs along the central, west coast of Bonaire.

Data collection

Initially all corals and diseases were surveyed however, the focus of this study soon changed due to the high prevalence of DSS in *S. intersepta* specifically. Using SCUBA, surveys were conducted following a U-shaped search pattern swimming for 10 min at each of the following depths from deep to shallow: 18 m, 15 m, 12 m, and 9 m. Starting at a depth of 18 m, divers would swim for 10 min in one direction, then ascend 3 m and swim for 10 min in the opposite direction. This was done for depths of 18 m, 15 m, 12 m, and 9 m. During each 10-min swim, one diver would locate any corals with DSS and record the coral species, the percent edge of the coral in competition, and the percent of the coral that is diseased. The percent of the colony affected by DSS was estimated visually. The percent competition was determined *in situ* by visually estimating the percent of the edge of the coral in competition. Competition is defined as any direct contact between coral and another benthic organism. These organisms include other corals, macroalgae, turf algae, cyanobacteria, anemones, and sponges. The other diver would photograph the diseased coral colony and its competitors to document the condition of each coral. These pictures were used as qualitative data for the study and a reference for each data point.

Data analysis

A correlation analysis was used to determine if there is a correlation between the amount of competition of a coral and the severity of disease on that coral. The level of competitive interaction of each coral was estimated in the field using intervals of 1-10%, 11-20%, 21-30%, 31-40%, 41-50%, 51-60%, 61-70%, 71-80%, 81-90%, and 91-100%. The percent of the coral that was diseased was grouped using the following scale: 1 = 1-19%, 2 = 20-39%, 3 = 40-59%, 4 = 60-79%, 5 = 80-100% (Fig. 3). Additionally, the relationship between coral colony depth and disease was analyzed using correlation of mean disease level versus depth.

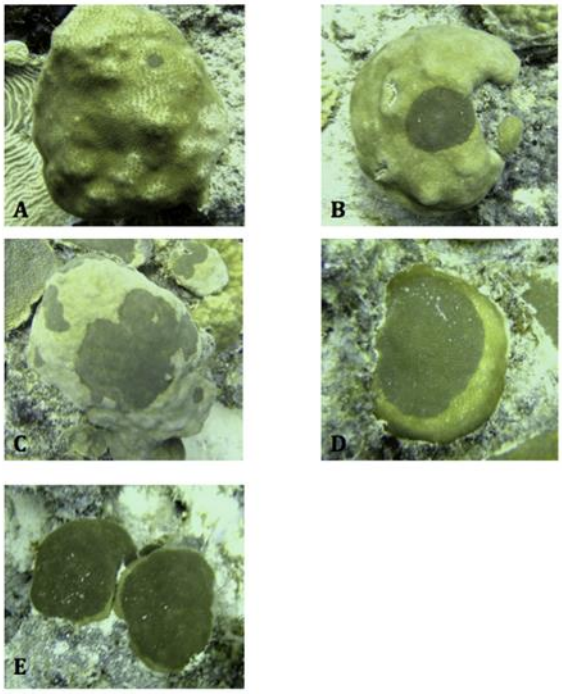

Fig. 3 A Disease level 1: 1-19%. **B** Level 2: 20-39%. **C** Level 3: 40-59%. **D** Level 4: 60-79%. **E** Level 5: 80-100%. Photographs Sarah Girouard

Results

Over 8 days of sampling (240 min), DSS was only found in two coral species: *S. intersepta* (183 colonies) and *S. siderea* (19 colonies). Since there were only 19 diseased colonies of *S. siderea* they were excluded from the analysis. Competitive interactions, including macroalgae (usually *Dictyota spp.*), coral-coral competition, coral-sponge competition and some boring anemones were observed competing with corals.

The mean disease level of all coral colonies is 2.56 **(**±0.34**)**. There was no significant relationship between depth and mean disease level (Fig. 4). Field observations showed that most *S. intersepta* colonies had DSS. The majority of the observed corals were found at a depth of 12 m or greater. While only a small percent of corals were observed at 9 m (Fig. 5).

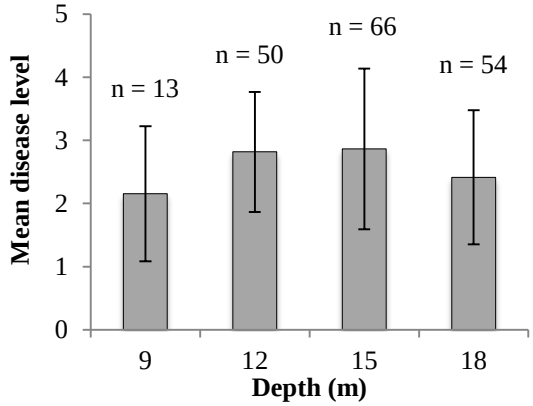

Fig. 4 Comparison of mean disease level (±SD) of dark spot syndrome at each depth interval

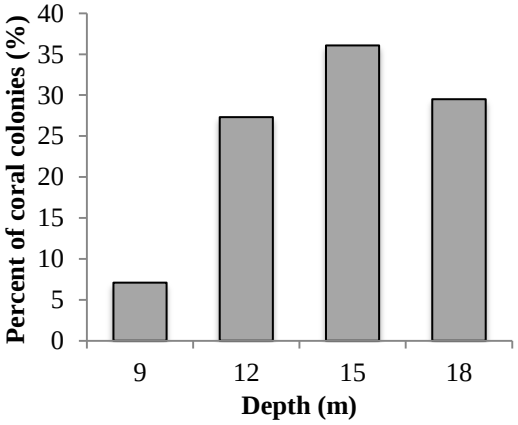

Fig. 5 Percent of total coral colonies observed at each depth

A weak positive correlation was found between percent edge of the coral in competition and level of DSS (r = 0.29, n = 183) (Fig. 6a-b). Figs. 6a and 6b show the same data except Fig. 6b is modified to show points that are overlapping in Fig. 6a. In Bonaire, DSS was observed almost solely in *S. intersepta* with some observations of *S. siderea* and no observations of DSS in *O. annularis*.

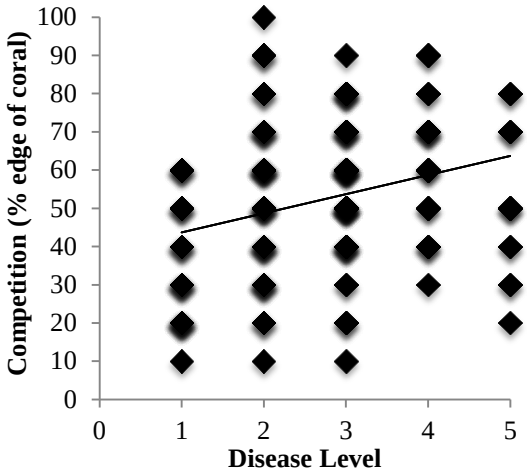

Fig. 6a Percent edge of the coral in competition with respect to disease level (1-5)

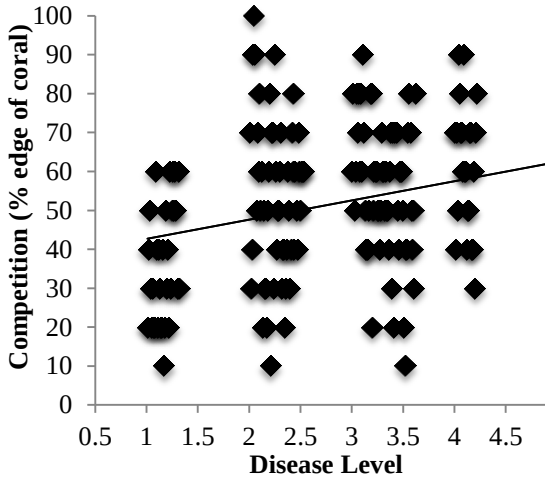

Fig. 6b Percent edge of coral in competition with respect to disease level. Note disease level is displayed with numbers corresponding to disease level and the decimal used to show overlapping data points

Discussion

There is a weak positive correlation between level of disease and amount of coral competition. Though it is possible that coral competition has no effect on the severity of DSS in *S. intersepta,* further study is needed to determine the effect of competition on DSS. Almost all coral colonies observed were in some form of competition, which suggests that competition probably varies in degree of stress on the coral. It is possible that rather than having an effect on disease severity competition may have an effect on coral disease prevalence and may lead to increased rate of infection but not increased severity. Often competition was observed on places other than the edge of the coral and many corals had boring sponges growing in and around them. A more accurate method may need to be employed to re-examine the effects of competition on DSS.

The original hypothesis is based on energy allocation for competition being a stressor for corals. This increased stress was thought to contribute to disease (Ritson-Williams et al. 2009). Competition uses energy that is critical to proper immune function and resilience of healthy corals, which may contribute to disease infection (Ritson-Williams et al. 2009). In addition, some macroalgae in competition with corals are vectors for disease (Nugues et al. 2004). This does not seem to be the case here as a large portion of the competition observed was with macroalgae, mostly *Dictyota* spp.

An important question is how much stress does competition put on corals, if at all? Some studies have found that competition does not affect the growth of corals and thus have concluded that it requires a low energy investment (Connell et al. 2004, Lapid and Chadwick 2006). Another study, however, showed coral tissue mortality and decreased growth rates when in competition with macroalgae (Lirman 2001). These contradicting findings and the results of this study show the need for further study of coral competitive interactions.

In this study, each data point was taken at a single point in time rather than repeated observations of the same corals over time. The best way to study coral competition and its effect on coral disease would be to find and mark both healthy and diseased coral and follow the corals over an extended period of time. A long-term study would show how competition, disease progression, and lethality changes. The severity of DSS has been shown to vary over time in the coral colony (Gil-Agudelo and Garzon-Ferreira 2001).

The results of this study are important in contributing to the understanding of DSS and coral competition. There has yet to be an identified infectious agent of DSS but judging by the clumped distribution of the disease it is likely a pathological agent. Though this disease only affects *S. intersepta* and *S. siderea* in Bonaire it is known to affect *O. annularis* much more in other parts of the Caribbean, showing the importance of further studies on DSS. In addition competition is still understudied and further research on the competitive interactions of corals is important in understanding how corals deal with stress and other organisms.

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