PHYSIS

Journal of Marine Science

Volume XV
Spring 2014
CIEE Research Station

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Physis
Journal of Marine Science

CIEE Research Station
Tropical Marine Ecology and Conservation Program
Volume XV, Spring 2014

φύσις

Physis (φύσις) comes from the Greek word for nature and describes the natural progression of living things without interference from outside forces. *Nomos* (νόμος) represents laws and customs, encompassing the anthropogenic influences that are the natural opposite of *physis*. While *physis* represents the ability of nature to stand resilient in the face of change, *nomos* represents the pressure humanity has on nature and its destructive repercussions.

Nature has an unprecedented ability to restore balance. Examples of this natural harmony surround us. Apex predators exhibit top down control on prey in their particular environment; an increase in the population of barracuda results in a decrease in the population of prey, while an upwelling that stimulates the growth and abundance of macroalgae will strengthen the population of herbivores. These cycles feed and balance each other. Recently, mainly as a result of our industrial society, this balance has been thrown off-track. Fujita tells us "our desire to tame the environment stemmed from a need for self preservation on a wild and dangerous frontier but very quickly transformed into greed and disregard for the natural resources on which we have build our empires".

The heart of our semester on Bonaire had a central theme of exploring the mechanisms of the marine environment, conservation tactics, and environmental harmony. *Physis* remains a focal point of these studies. With that said, it becomes clear why our journal is titled *Physis.* We explored all aspects of the environment surrounding Bonaire. From social studies to marine environments, *Physis* applies this knowledge and challenges us to think critically in terms of connectivity between ecosystems and the environment's natural ways of operating.

"Every creature is better alive than dead, men and moose and pine trees, and he who understands it aright will rather preserve its life than destroy it." -Henry David Thoreau

We present Volume XV of *PHYSIS: Journal of Marine Science* as a step towards understanding life in the marine environment. Only when humanity recognizes the intrinsic value of nature can we make strides to not only protect but rejuvenate the natural world.

Cheers,
Julia Middleton & Sean O'Neill
CIEE Class of Spring 2014

Forward

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 16^{th} and 17^{st} of April, 2014 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 Colin Howe, Lucien Untersteggaber and Stephanie Villalobos. Astrid de Jager was the Dive Safety Officer and provided 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 individual goals.

Dr. Rita Peachey



Faculty

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

Dr. Enrique Arboleda is the Coral Reef Ecology Faculty for CIEE and co-teaches Independent Research 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. 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.

Staff

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. Amy currently provides accounting and administrative support for staff and students at CIEE She serves as the student resident hall manager.

Astrid de Jager is the Dive Safety Officer. She came to Bonaire in 2009 and has been working in 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.

Molly Gleason is the lab technician for CIEE. She graduated with a M.S. in Biology from University of California: San Diego after several years of research at a marine biology laboratory at Scripps. For her Master's research, she studied the affects of ocean acidification on survival, shell composition and settlement behavior of invertebrate larvae. She is involved in research at CIEE studying the nutrient and bacterial levels of the coral reefs

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

Casey Benkwitt is the Volunteer Outreach Coordinator and Research Associate for CIEE. She received her B.A. from Bowdoin College in Environmental Studies and Sociology with a minor in Biology. Casey is currently in the fourth year of her PhD in Zoology from Oregon State University. Her research focuses on the population dynamics and ecological effects of invasive lionfish in the Caribbean.

Interns

Colin Howe is the Conservation and Outreach Intern at CIEE. He graduated from Old Dominion University in 2012 with a B.S. in Biology, concentration in Marine Science and is a PADI Rescue Diver. Before coming to CIEE he worked at Reef Environmental Education Foundation (REEF) teaching fish identification techniques and assisting with lionfish research. Before that he studied coral reef ecology and conservation in Belize and in the Florida Keys.

Lucien Untersteggaber is the Coral Reef Ecology and Dive Safty Intern at CIEE Bonaire. He graduated at the University of Vienna 2012 (*Mag. rer.nat.*) with a specialisation in Marine Biology/Coral Reef Ecology. After graduation and publishing in the "*Marine Biology Journal*" he traveled to Egypt to work at an Environmental Center and became a PADI dive master. Currently he is applying at several Universities for a PhD. position. Lucien volunteered in Spring 2014 and is planning to come back to Bonaire in Summer 2014 to continue working on the long term research projects of CIEE.

Stephanie Villalobos is the Coral Reef Ecology and Lab Safety intern at CIEE. She graduated from the University of South Florida in Tampa with a B.S. in Biology with Marine Concentration and a B.A. in Music Studies with a focus on Flute Performance. She has participated in a range of research topics, from marine natural products to benthic mapping and coral spawning. She was recently accepted into the graduate program at the University of North Carolina at Wilmington and will begin research and school in the fall. She hopes to focus on the chemical ecology of coral reefs and the shifts in reef ecosystem composition.

Students

Alice Vejins is a sophomore at the University of Colorado Boulder, where she is an Ecology and Evolutionary Biology major. She loves diving and exploring the globe and hopes to do her divemaster internship in Thailand after graduating.

Belle Perez is a sophomore at Case Western Reserve University in Cleveland, Ohio. She is majoring with a B.S in Biology and minors in Chemistry and Astronomy. In her free time, Belle likes to catch frogs, study human anatomy, and play ultimate frisbee. Belle hopes to attend medical school after graduating.

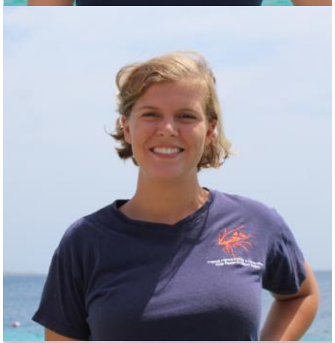
Ben Gulmon is a junior at Seattle University in Seattle,Washington where he is majoring in Marine & Conservation Biology with minors in Chemistry and Creative Writing. On being asked about future plans, he has responded “If I could get paid to SCUBA dive for the rest of my life, I would be happy camper.’

Brooke Davis is a sophomore at Colorado College where she is majoring in Biology with a concentration in Ecology. Her other passions include documentary films, photography, traveling, horseback riding, hiking, and technical theatre.

Elena Johannsen is pursuing a BS in Biology with a minor in religion at Denison University. In addition to marine biology, she loves singing in her a cappella group and doing musical theatre. After college she hopes to go to graduate school for marine conservation and possibly even continue her research on ocean acidification!

Students

Jenny Mathe is a junior at SUNY College of Environmental Science and Forestry where she is majoring in Conservation Biology with a minor in Applied Statistics. She hopes to attend graduate school for marine biology.



Julia Middleton is a junior at Colby College in Waterville, Maine, where she is a double major in Biology with a concentration in Ecology & Evolution and Chemistry. She hopes to attend graduate school for marine chemistry and ecological modelling and hopes to continue coral reef research in the future.

Nicole Kleinas is a junior at Ohio University in Athens, Ohio where she majors in Biological Sciences with a concentration in Ecology. After graduating, she plans to get her dive master and attend graduate school for Animal Behavior.

Nicole Sikowitz is a junior at Roger Williams University in Rhode Island, where she is a double major in Marine Biology and Environmental Science. She hopes to attend graduate school in marine conservation research after graduating.

Sarah Bruemmer is a senior at Arizona State University in Tempe, Arizona where she is a double major in Conservation Biology and Anthropology. She loves manatees, yoga, and traveling the world. She hopes to join the Peace Corps after graduating.

Students

Sarah Fleming is a junior at Denison University in Granville, Ohio where she is a double major in Biology and Environmental Studies. In the future, she hopes to attend graduate school for conservation biology and to continue working on coral reefs.

Sean O'Neill is a junior at the University of Maryland, Baltimore County, where he is majoring in Environmental Science with a concentration in Conservation ecology. He loves animals and hopes to become a wildlife biologist one day, either on land or in the sea.

Shannon Wood is a junior at Susquehanna University, Pennsylvania, where she is majoring in Ecology and minoring in Earth and Environmental Science. She enjoys playing basketball and field hockey in her spare time and hopes to attend graduate school for marine biology in the future.

Taylor Robinson is a junior at Seattle University in Seattle, Washington where she is majoring in Marine and Conservation Biology. She enjoys discovering new areas of her city and studying the Italian language, which she hopes to one day become fluent in.

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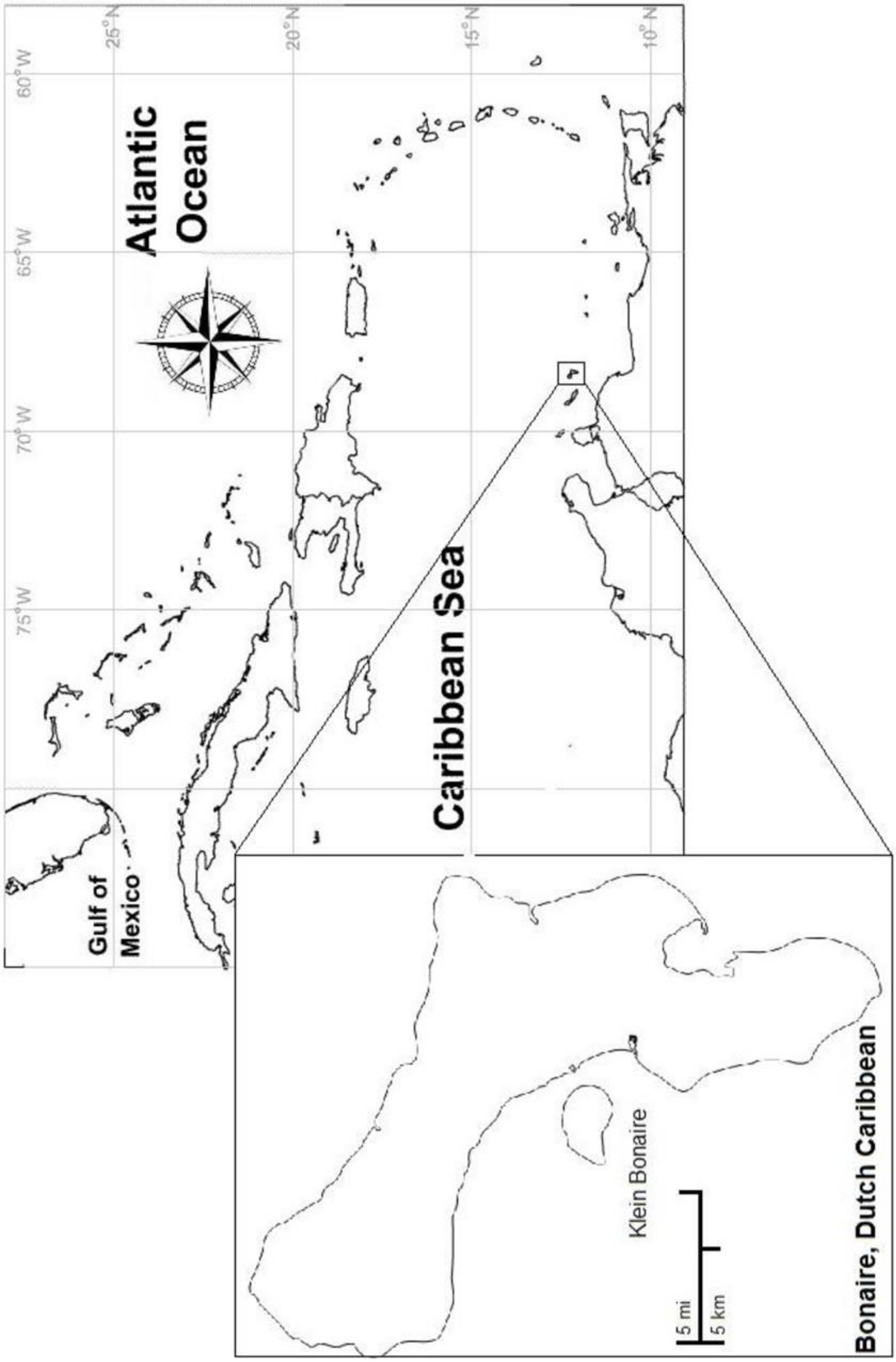
Atlantic Ocean
Gulf of Mexico
Caribbean Sea
Bonaire, Dutch Caribbean
Klein Bonaire
5 mi
5 km
25° N
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15° N
10° N
60° W
65° W
70° W
75° W

Physis (Spring 2014) 15:1-8

REPORT

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Impacts of cleaner shrimp, *Ancylomenes pedersoni*, density at cleaning stations on cleaner and client behavior

Abstract Mutualisms and symbiotic relationships are common in the marine environment. Relationships between cleaner species, their hosts, and their client species are prime examples of these types of relationships. Cleaner shrimp, which are typically found in association with sea anemones, exhibit mutualistic behavior through the removal and consumption of parasites, injured tissue and various other particles from their client fish. The shrimp may inhabit their host anemone alone, or in groups ranging up to more than ten individuals. This study focused on the cleaner shrimp species *Ancylomenes pedersoni* and examined the relationship between the number of shrimp present at cleaning stations and the number of client fish visiting that station. The relationship between the number of shrimp present and the size of the host anemone was also investigated; the data collected did not support any significant relationships between the variables tested. All data was collected through observational studies and video analysis of specimens in the field. Because cleaner species are crucial to the heath of their clients and therefore to the overall heath of the reef, enhanced understanding of the behavior of *A. pedersoni* will contribute to better conservation of the species and consequently their client fish.

Keywords *Ancylomenes pedersoni* • cleaner shrimp • cleaning mututalism

Introduction

Symbiotic and mutualistic relationships are common in the marine environment (Roughgarden 1975). Cleaners, their hosts, and their client species are prevalent among these types of relationships. Cleaner species participate in symbiotic relationships through the removal and ingestion of parasites, injured or diseased tissue, and other particles from their client fish species (Losey 1972; Mahnken 1972). Relationships like these may be facultative or obligatory depending on the cleaner species (Côté 2000). Though cleaning interactions are typically beneficial to both the cleaner and client, cleaners will occasionally "cheat" their client by removing bits of flesh rather than parasites or unwanted particles, which usually causes the client to jerk away (Côté 2000; Mahnken 1972).

The presence of cleaning stations has numerous positive impacts on coral-reef ecosystems and has been shown to be correlated with the distribution of coral-reef fishes (Côté 2000; Mahnken 1972). Beyond removing ectoparasites that negative effects on their hosts, cleaner species have been shown to promote an increased abundance and diversity of their clients (Côté 2000; Limbaugh 1961). In an experimental removal of cleaners from reefs in the Bahamas, Limbaugh (1961) reported emigrations of non-territorial fish species, as well as an increase in wounds and diseases found on fish that remained in the removal area. It is clear that cleaner species are an essential component to maintaining the health of coral-reef organisms.

Among the various species of cleaner organisms are cleaner shrimp that depend primarily on cleaning for their food; the shrimp will often alter their behavior to increase their total cleaning capacity (Mahnken 1972). As obligate cleaners, *A. pedersoni* obtain more than 85% of their food from their client interactions (Côté 2000).

Generally, marine shrimp on coral reefs are found only living in close association with various species of anemones (Criales 1984). Host specificity, however, is variable between shrimp and host species and may change among populations and locations (Mascaro et al. 2012). Shrimp living on anemones receive the benefit of protection by the anemones' stinging nematocysts, but benefits to the anemone remain relatively unknown (Smith 1977; Mahnken 1972).

Shrimp of the genus *Periclimenes* are among the cleaner species found commonly in the Caribbean (Mascaro et al. 2012; Wicksten 1995). This study, based in the southern Caribbean Sea on the island Bonaire, focused specifically on the shrimp *Ancylomenes pedersoni* (previously known as *Periclimenes pedersoni*). *Ancylomenes pedersoni* are distributed from North Carolina, south along the east coast of the United States, throughout the Bahamas, West Indies, Dutch Caribbean and Belize (Mascaro et al. 2012). In a study conducted by Wicksten (1995), *A. pedersoni* were found to be the most abundant and active cleaning species on Bonaire. Though *A. pedersoni* have not been found to exhibit extreme host specificity, the shrimp are most commonly found in association with the anemone species *Bartholomea annulata*, commonly known as corkscrew anemones (Mascaro et al. 2012; Silbiger and Childress 2008; Mahnken 1972).

Ancylomenes pedersoni actively search for advantageous positions to attract clients when establishing cleaning stations and typically remain on their host anemone for long periods of time (Mascaro et al. 2012; Mahnken 1972). During the initial anemone acclimatization period, the shrimp develop protection from the anemone's nematocysts by acquiring mucus from the host (Silbiger and Childress 2008). *Ancylomenes pedersoni* have been found inhabiting a single host alone, and in numbers ranging up to more than ten individuals. Studies have reported territorial and aggressive behavior between conspecific *A. pedersoni*, however the number of client species visiting cleaning stations has been observed to increase with the number of shrimp located at a given station (Mascaro et al. 2012; Mahnken 1972). The increase in clients and therefore feeding opportunities implies that it is beneficial for the shrimp to live in association with each other, and Mascaro et al. (2012) suggest that despite aggressive behavior between shrimp, previous conspecific inhabitants may actually encourage individuals to associate with an anemone to attract more client species.

Although many observations have been made on the grouping patterns and resulting client frequency of *A. pedersoni*, few concrete studies have been conducted regarding these patterns. The goal of this study was to answer the questions (1) how does the number of *A. pedersoni* living on an anemone affect number of client fish visiting that anemone? and (2) what is the relationship between the number of *A. pedersoni* on a cleaning station and the size of the associated host anemone?

H_1: Higher densities of *A. pedersoni* at a cleaning station will result in greater numbers of clients visiting the station

H_2: The number of *A. pedersoni* present at a cleaning station will increase with the size of the host anemone

Materials and methods

Study site

This study was conducted on Bonaire, a small island (~290km^2) located roughly 80 km north of Venezuela and 50 km east of Curacao in the southern Caribbean Sea. Famous in the SCUBA diver community for beautiful dive sites and fish abundance, the island is encompassed by fringing coral reefs. The sandy bottom is replaced by reef habitat approximately 50 m from the shoreline 10 m below the surface. Research dives were done along the waterfront of the island's capital, Kralendijk, located on the western and leeward side of the island. Data was collected between dive sites 'Yellow Sub' (12°09'36.5"N 68°16'55.2"W) and 'Kas di Arte' (12°09'21.4"N 68°16'45.3"W; Fig. 1).

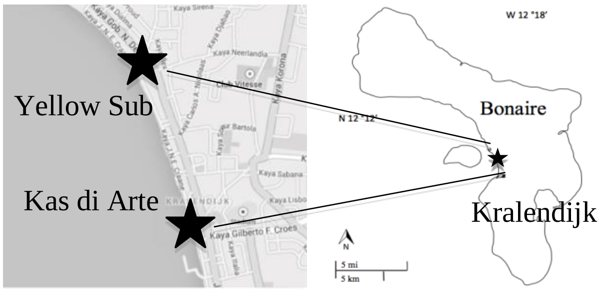


Fig. 1 Map of the Kralendijk waterfront from dive site 'Yellow Sub' to dive site 'Kas di Arte' (Google Maps)

Cleaning station location

Field research was conducted on SCUBA. At each dive site, random swims were performed to locate cleaning stations on *Bartholomea annulata* anemones containing varying numbers of *Ancylomenes pedersoni* to be used throughout the duration of the study. These swims were conducted at depths ranging from 5 m to 12 m, as cleaning stations are found most abundantly at these depths near the reef crest and sandy bottom (Mahnken 1972). Seven cleaning stations were used in the study; each cleaning station was measured (cm^3) and the number of shrimp present was recorded. Cleaning stations were marked with labeled air filled recycled plastic soda bottles tied with string to nearby substrate.

Field observations and video analysis

The marked cleaning stations were observed three times a week around 09:00 hrs, because ectoparasite load has been found to increase overnight resulting in greater activity at cleaning stations earlier in the day (Côté 2000). Rather than direct sampling, which could affect client and cleaner behavior, videos were recorded of each cleaning station. Two GoPro Hero 3^{+} ™ cameras were mounted on stands assembled from three-pound dive weights, PVC pipe and zip ties for this study (Fig. 2). The cameras were positioned as close to the cleaning stations as possible without harming the surrounding environment. Each cleaning station was recorded from two angles to better capture shrimp and client activity. Cleaning stations were recorded for 20 minutes at a time and the first five minutes of video was discarded to account for an acclimatization period after the installation disturbance.

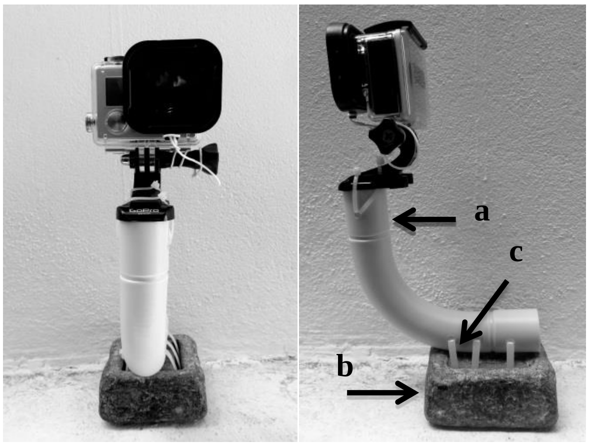


Fig. 2 GoPro Hero 3^{+} ™ mounted on PVC pipe a. and 3lb dive weight b. All parts are secured with c. zip ties

Videos were analyzed and data was recorded on (1) number of *A. pedersoni* present, (2) number and species of clients visiting the station, (3) number of cleaning attempts made by clients, (4) number of successful cleanings, (5) number of shrimp on the client during cleaning, (6) number of times cheating occurred, and (7) amount of time each client spent getting cleaned.

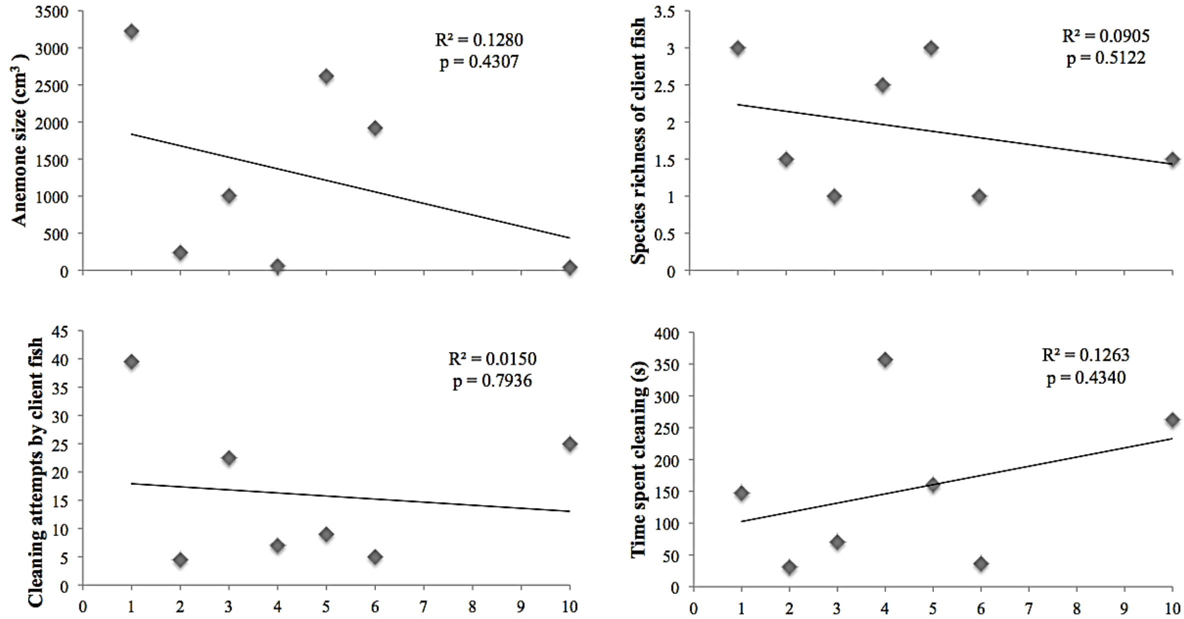
Data analysis

Linear regressions were run to determine the statistical significance of the relationships between the number of *A. pedersoni* on a cleaning station and (1) the number of visiting clients, (2) number of cleaning attempts by clients, (3) number of shrimp on the client during cleaning, (4) number of times cheating occurred, (5) the amount of time each client spent getting cleaned, and (6) the size of the host anemone.

Results

No significant results were found between the number of shrimp present at each cleaning station and the size of the host anemone ($F=0.734$, $p=0.431$; Fig. 3a), the species richness of client fish ($F=0.497$, $p=0.511$; Fig. 3b), the number of cleaning attempts made by clients ($F=0.076$, $p=0.794$; Fig. 3c), and the time spent cleaning by the shrimp ($F=0.723$, $p=0.434$; Fig. 3d). A negative trend exists between the size of the host anemone and the number of shrimp at each cleaning station, though no significant relationship was found. A positive trend also exists between the number of shrimp at each cleaning station and the time spent cleaning by the shrimp.

Client species varied between cleaning stations as displayed in Table 1. While *Chromis multilineata* were present at five out of seven stations, other species such as *Cephalopholis cruentatas* were found at only one.



All x-axes indicate number of *Ancylomenes pedersoni* present at cleaning stations

Fig. 3 Linear relationship between the number of *Ancylomenes pedersoni* present at the cleaning stations (n=7) and a. the anemone size,b. species richness of client fish,c. cleaning attempts made by client fish (per 15 min interval), and d. time spent cleaning by the shrimp (per 15 min interval)

Table 1 Average number of cleaning attempts made by client fish (per 15 mins) at cleaning stations with 1, 2, 3, 4, 5, 6, and 10 shrimp

	Cleaning attempts						
# Shrimp	1	2	3	4	5	6	10
Client Species							
Chromis multilineata	33.5	0.0	22.5	0.0	1.0	5.0	20.0
Canthigaster rostrata	1.5	1.5	0.0	3.0	4.0	0.0	0.5
Stegastes partitus	0.0	3.0	0.0	0.0	1.5	0.0	0.0
Haemulon flavolineatum	4.5	0.0	0.0	0.0	0.0	0.0	0.0
Pomacanthus paru	0.0	0.0	0.0	0.5	2.0	0.0	0.0
Mulloidichthys martinicus	0.0	0.0	0.0	0.0	0.0	0.0	4.5
Cephalopholis cruentata	0.0	0.0	0.0	3.0	0.0	0.0	0.0
Scarus iseri	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Sparisoma aurofrenatum	0.0	0.0	0.0	0.5	0.0	0.0	0.0

Discussion

This study suggests that the number of *Ancylomenes pedersoni* present at cleaning stations may have little impact on the variables tested. The results did not support the hypothesis that higher densities of *A. pedersoni* at a cleaning station would result in greater numbers of clients visiting the station or the hypothesis that the number of shrimp present would increase with anemone size. The number of *A. pedersoni* was not correlated with the size of anemone and they were often found in larger numbers on smaller anemones.

Although few studies have examined the reproductive patterns of *A. pedersoni*, such behaviors may influence distribution of the shrimp. In a series of laboratory choice experiments conducted by Mascaro et al. (2012), *A. pedersoni* acclimated to anemones where conspecifics were already present more often than to uninhabited anemones, though there were no significant differences in acclimation frequencies. It was speculated that the presence of conspecifics on host anemones may promote association with that anemone during larval settlement and reproduction (Mascaro et al. 2012). It is possible that this pattern could explain the occurrence of large numbers of *A. pedersoni* on small anemones in this study.

Multiple observations have also been made of apparent hierarchical arrangements between conspecifics inhabiting the same host anemone (Mascaro et al. 2012; Guo et al. 1996; Mahnken 1972). When found in groups, *A. pedersoni* were frequently observed arranging themselves by size, with the largest individuals on or near the oral disk and smaller individuals distributed around the edges of the anemone and on the nearby sand or given substrate (Mascaro et al. 2012; Guo et al. 1996; Mahnken 1972). The shrimp are believed to actively search and compete for such advantageous positions within an anemone cleaning station (Mascaro et al. 2012; Mahnken 1972). The potential existence of hierarchical arrangements between the shrimp on a single host anemone may also contribute to the large number of *A. pedersoni* found on small anemones. If shrimp can arrange themselves preferentially within a host, there may be less incentive to associate with a larger or uninhabited host.

There was no correlation between the species richness of client fish or the number of cleaning attempts made by clients and the number of *A. pedersoni* present at cleaning stations. In a study conducted by Bshary and Schäffer (2002) some client fish were found to deliberately choose between cleaning stations. The ability to choose cleaning stations is directly related to the client species' home range; clients with large home ranges have the ability to choose between cleaners, while clients with small home ranges do not. The results of their study revealed that cleaner wrasses actively prioritize cleaning clients with large home ranges over those that do not have the ability to choose between stations. This was attributed to the fact that clients with larger home ranges actively used their choice options and changed cleaning stations if they were ignored, eliminating the food source for the cleaner (Bshary and Schäffer 2002). Though no studies have examined these patterns in cleaner shrimp, it is possible that *A. pedersoni* and their clients exhibit the same behaviors.

If the patterns observed by Bshary and Schäffer (2002) are similar in *A. pedersoni* and their clients, this may explain the lack of correlation between the number of shrimp present and the species richness of client fish and the number of cleaning attempts made by clients. If clients with large home ranges are able to activity choose certain cleaning stations, and often return to the same one if service was good, then it is likely that the quality of cleaning service rather than the number of shrimp would influence species richness and cleaning attempts. Further research should be conducted to investigate the extent to which clients are able to remember cleaning stations.

The lack of significance of the relationship between the number of *A. pedersoni* present at the cleaning stations and the time the shrimp spent cleaning might be attributed to the differences in time the shrimp spent cleaning certain species of client fish. Though the statistical significance was not examined, observations from this study suggest that the time *A. pedersoni* spend cleaning varies greatly between client species. While *Chromis multilineata* were rarely cleaned for longer than ten seconds, *Cephalopholis cruentata* were observed being cleaned for more than four minutes. As client species richness was highly variable between stations regardless of shrimp density, it is not surprising that the time *A. pedersoni* spent cleaning and the number of shrimp did not exhibit a significant linear relationship.

In order to better understand the symbiotic relationships involving *A. pedersoni*, further studies that examine a greater number of cleaning stations for an extended period of time should be conducted. While this study did not yield any significant results, it is crucial to continue investigating the behavioral patterns of *A. pedersoni* and their clients because of their vast influence on the overall health of coral reef environments.

Acknowledgements Thank you to my advisor, Dr. Patrick Lyons, and my intern advisor, Lucien Untersteggaber, M.Sc., for being great mentors and incredibly helpful throughout every stage of my study. Also, thank you to my research partner Julia Middleton, as well as Stephanie Villalobos and Shannon Wood for assisting on research dives. Finally, I would like to thank all of the CIEE staff and my home university for making this whole project possible.

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Physis (Spring 2014) 15: 9-14

REPORT

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Species dependent aggression in bicolor damselfish during reproductive and non-reproductive cycles

Abstract Bicolor damselfish, *Stegastes partitus*, are well known for their aggressive nature. They are known to attack almost any species threatening their food, territory or spawn, often regardless of the size of the intruder. Studies show that three spot damselfish are even able to identify certain species based on the threat they present by displaying different aggressive behaviors. However, little is known on how, and if, these aggressive displays are species dependent in bicolors. The purpose of this study was to test for species dependent aggression in bicolor damselfish during reproductive season to determine if this behavior relates to increased egg protection during reproductive season. Four damselfish individuals were analyzed over the course of five weeks during one reproductive and one non-reproductive cycle using underwater video camcorders. *S. partitus* was shown to increase frequency of aggression toward egg threatening intruders while guarding eggs. Conversely, they were shown to decrease in frequency of aggression towards intruders threatening their food resources. Lastly, the frequency of intrusion for egg-eating intruders was not shown to significantly increase while bicolor damselfish eggs were present.

Keywords Bicolor damselfish • Aggression • Territoriality

Introduction

Interspecific aggression has long been studied as an important biological behavior. Studies in as early as the 1960's show interspecific aggression to be a normally programmed behavior in many organisms such as birds, mammals, and fish (Thresher 1976.) Damselfish in particular have proven to be model organisms for aggression studies because of their extreme territoriality and effective defensive behaviors (Deloach et al. 1999).

The aggressive behaviors of damselfish are well studied and documented (Thresher 1976). Damselfish aggression, particularly in *Stegastes planifrons* (threespot damselfish), varied as a function of the time of year and species of intruder (Myrberg 1972). In a different study, Thresher (1976) found that these damselfish displayed different behaviors toward different species, but nearly identical behaviors toward "cousin" species. For example, threespot damselfish were shown to behave toward all parrotfish the same way rather than distinguishing rainbow parrotfish (*Scarus guacalama)* from greenblotch parrotfish (*Sparisoma automarium)*.

Attacks varied based on the "threat" the intruder presented to the damselfish (Losey 1982). Often, the intruding species only presented a threat to one resource, food, territory, or eggs (Thresher 1976). Cleveland (1999) found that these aggressive behaviors were not the result of calculated energy cost to attack different species, but that energetic costs in aggression appeared to be minimal on all levels (with the assumption the damselfish were not eaten). This finding is important because it shows aggressive behavior does not come at a cost to biological fitness.

Stegastes partitus, bicolor damselfish, can be seen in every tropical part of the Western Atlantic (Myrberg 1972). This small, pervasive damselfish can create territories on practically every available hard structure including coral, sponges, conch shells, and rocky outcrops. Individuals are found at depths from 6-24 m, making *S. partitus* a good subject to find, collect, and study (Deloach et al. 1999).

Myrberg (1972) found that social structure in bicolor damselfish colonies was arranged so that the smallest male damselfish within the colony were the most frequent targets of aggression. The alpha male would direct intruder chases toward these damselfish, making the lesser individuals easy to spot and observe. Myrberg (1972) showed that in *S. partitus*, aggression differed toward wrasses compared to other species, for example, surgeonfish.

In contrast to the Thresher (1976) study where threespot damselfish were used, bicolor damselfish were shown to be not as focused on defending their algal farms (Myrberg 1972) as they were on the water space near their shelterholes. This is explained by the bicolor's diet; bicolors feast mainly on copepods, pelagic tunicates, and larvae from the water column (Deloach et al. 1999) no more than 1.5 meters above the shelterhole (Thresher 1976). The portions of the water column containing copepods, tunicates, and larvae are guarded by bicolors, and are thus part of their territory (Myrberg 1972).

Myrberg (1972) revealed that male bicolor damselfish, while aggressive year round, displayed increased aggression during reproductive season. Knapp et al. (1991) discovered that males and females defended permanent feeding territories, which also doubled as shelters for egg clutches during reproductive season. Reproductive seasons start a few days before the full moon and end a few days after, with each spawning period occurring for a one hour period at dawn. Egg clutches remain in the shelterhole, defended by male bicolors, for a period of three to five days before the larvae swim into the water column and eventually settle into the sand. The age of the egg clutches could be identified by color; one-day-old clutches are dull yellow, two-day-old clutches are pink, three-day-old clutches are dark purple. Clutches hatch after no longer than five days (Knapp et al. 1991).

While it is known which species of intruder provoke aggression in male bicolor damselfish, it is unknown to what extent this aggression increases during reproductive season. Furthermore, data is lacking on how behavioral aggression may change toward certain species during reproductive seasons.

This study aimed to identify aggressive interspecific interaction during reproductive and non-reproductive seasons and attempted to show an association between reproduction times and aggression toward specific fish groups based on the threat (Losey 1982; Thresher 1976). Insights into the multi-functionality of territoriality in bicolor damselfish may show territoriality is not one homogenous behavior toward all fish, but dependent on how residents prioritize between their egg clutches, algal farms, and source of food within the water column.

H_1: During reproductive cycles, brooding male individuals of *S. partitus* will exhibit more aggression toward intruders that threaten the livelihood of egg clutches (Egg-eating fish) compared to aggression toward the same species during non-reproductive cycles

H_2: During reproductive cycles, *S. partitus* will exhibit lower aggression toward intruders who compete for territorial space or food

H_3: During reproductive cycles, there will be higher rates of interspecific interaction between *S. partitus* and intruders who threaten egg clutches

Materials and methods

Study site

The bicolor damselfish in this study defended territories year round on a fringing reef located off of the ‘Kas di Arte’ (12° 09' 21.4"N, 68° 16' 45.3"W) in Kralendijk, Bonaire, Dutch Caribbean (Fig. 1). The site chosen was right on the reef crest, with small patch reefs and coral rubble at a depth of 6-8 m.

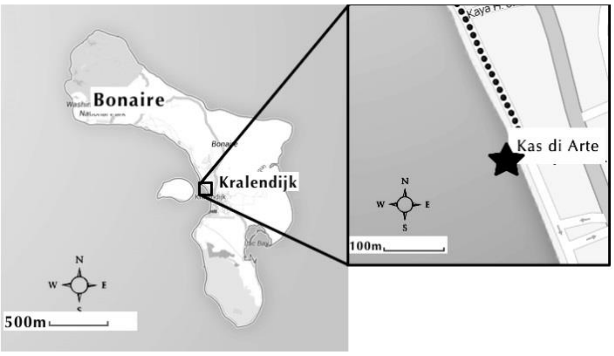

Fig. 1 Dive site Kas di Arte(12°09'21.4"N 68°16'45.3"W) in Kralendijk, Bonaire, Dutch Caribbean

Study subjects

The study began at the halfway point of mating season, directly between the full and new moon. Four male bicolor damselfish were identified for this study. Males were identified by the black mask pattern displayed during courting periods (Deloach et al. 1999).

The territories were primed by affixing a small piece of sanded PVC pipe onto hard (dead) substrate within damselfish territory (Hixon et al. 1982). The PVC was affixed using zip ties or dive weights. PVC pipes were chemically inert and not shown to change fish behavior (Hixon et al. 1982). Bicolor damselfish are known to move their activities to artificial territories within 1-2 days after installation (Thresher 1976). By using artificial territories, the diver was able to ascertain whether or not the damselfish was guarding a clutch of eggs.

Data collection

Residents were recorded by video camera (SONY HDR-XR550V) twice a week for a period of five weeks; individuals were recorded for a total of 15 min per recording.

The camera was secured inside a Stingray Underwater Case aimed at the individual of interest by diver adjustments in manual tilt and control. The diver exited the area to remove confounding variables in the fish behavior and returned to collect the recording equipment after. Videos were taken at 10:00 to 12:30 hrs and 14:00 to 16:30 hrs. The five weeks of data collection coincided with a reproductive cycle and non-reproductive cycle so as to provide comparative interspecific data.

Data analysis

Data was analyzed using video software Picture Motion Browser (PMB) Version 5.2 for video playback. Data collection began at the 10 min mark of each video, under the assumption that normal activities of the fish resumed after the presence of the diver and camera had been normalized. Aggressive behavior actions were counted per fish and were characterized by a forward lunge and/or a nip toward the intruder (Myrberg 1972). Chases from the shelterhole were recorded as a separate behavior. The total number of interactions was also counted per fish. Interactions include any intrusion within damselfish territory around PVC pipe.

The data were compiled by intruding fish into groups by family and genus when higher classification was necessary to distinguish groups of fish. Data were grouped based on the cycle collected: reproductive versus non-reproductive season and a paired t-test was performed.

Results

Relationship between frequency of attack and reproductive season

The most frequently attacked species were Pomacentridae - *Stegastes (*bicolor damselfish), Labridae (yellow head and blue head wrasse)

Table 1 The frequency of attack of bicolor damselfish based on species and presence of eggs. Bolded entries denote significant differences in frequency of attack when comparing presence of eggs to absence of eggs

Intruding species	Eggs Absent±SEM	n	Eggs Present±SEM	n	P-value
Labridae	**0.545±0.022**	**78**	**0.898±0.079**	**50**	**0.035**
Pomacentridae-*Chromis*	**0.596±0.137**	**146**	**0.125±0.094**	**12**	**0.041**
Pomacentridae-*Stegastes*	0.645±0.084	67	0.657±0.236	16	0.983
Gobiidae	0.105±0.105	22	0.25±0.25	2	0.665
Scaridae	0.256±0.116	12	0.083±0.083	4	0.376
Acanthuridae	0.05±0.05	5	0±0	0	0.391
Chaetodontidae	0±0	2	0.083±0.083	2	0.391
Tetraodontidae	0.062±0.062	8	0±0	1	0.391
Ostraclidae	0.05±0.05	1	0.25±0.25	1	0.391
Serranidae-*Serranus*	0.031±0.031	4	0±0	0	0.391
Serranidae-*Cephalopholis*	0.025±0.025	2	0±0	2	0.391

and Pomacentridae - *Chromis* (brown and blue chromis; Table 1). Labridae experienced a significant increase in attack frequency when eggs were present in damselfish shelterholes (Table 1). Conversely, Pomacentridae - *Chromis* experienced a significant decrease in attack frequency when eggs were present in damselfish shelterholes (Table 1; Fig. 2). Attack frequencies toward other species did not show significant differences over the course of the reproductive and non-reproductive seasons.

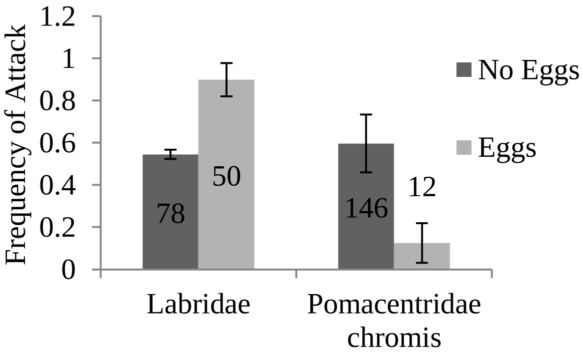

Fig. 2 The frequency of bicolor damselfish attack on *Labridae* (wrasses) and Pomacentridae-*Chromis* (brown and blue chromis) grouped by presence of eggs. Numbers on the columns denote number of total encounters per species. Error bars represent the standard deviation of the mean

Intrusion rate during reproductive season

The most frequent intruders were Labridae and Pomacentridae - *Chromis*. Frequency of encounters of any species did not show any statistically significant difference (Table 2). An increase of encounters appeared in Labridae in the presence of eggs (Table 2). A decreasing trend was seen in Pomacentridae-Chromis (Table 2; Fig. 3).

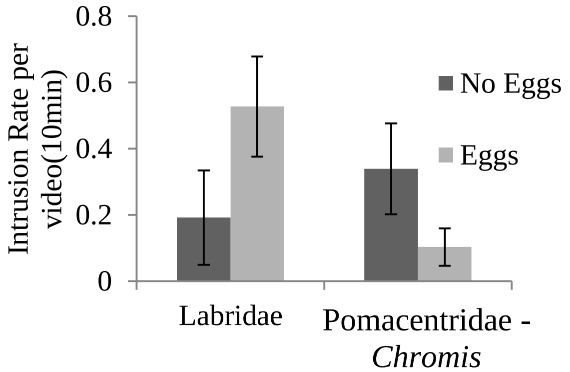

Fig. 3 Intrusion rate of *Labridae* (wrasse) and Pomacentridae-*Chromis* (brown and blue chromis) on damselfish shelter hole grouped by presence of eggs. Error bars represent standard deviation of the mean

Discussion

This study revealed bicolor damselfish aggression is species specific and varies with reproductive season. There was a significant increase in aggression toward Labridae (yellow head and blue head wrasse) during reproductive season, and a significant decrease toward Pomacentridae - *Chromis* (brown

Table 2 Intrusion frequency of different species on bicolor damselfish shelter hole. Bolded entries denote a visible trend in changes in intrusion frequency; however the data was not shown to be statistically significant

Intrusion Frequency	Eggs Absent±SOM	Eggs Present±SOM	P-value
Labridae	**0.191±0.142**	**0.527±0.151**	**0.121**
Pomacentridae-*Chromis*	**0.338±0.137**	**0.102±0.056**	**0.227**
Pomacentridae-*Stegastes*	0.187±0.032	0.213±0.083	0.812
Gobiidae	0.102±0.059	0.018±0.016	0.328
Scaridae	0.027±0.011	0.031±0.027	0.932
Acanthuridae	0.013±0.006	0±0	0.200
Chaetodontidae	0.036±0.031	0±0.013	0.278
Tetraodontidae	0±0	0±0.006	0.391
Ostraclidae	0.002±0.002	0±0.074	0.391
Serranidae-*Serranus*	0.010±0.010	0±0	0.391
Serranidae*Cephalopholis*	0.254±0.248	0±0	0.382

chromis). The study showed a corresponding trend in frequency of encounter; Labridae increased while Pomacentridae - *Chromis* decreased.

Competition takes many forms, as does territoriality. Species that interact with damselfish often resent only one threat. Wrasses are exclusively egg-eaters when interacting with bicolors, while *Chromis* are known to eat above the damselfish shelterhole, picking off their main food source (Thresher 1976.) It is suggested that aggression is driven by threat, and that bicolor damselfish are able to perceive the threat presented per species.

Furthermore, bicolors seemed to have “priority” in what they choose to protect. During reproductive season, they may “rank” protection of egg clutches much higher than protection of food in the water column. Thresher (1976) indicates similar findings, however, this study used distance at which attack occurred rather than frequency of attack.

Intrusion rates were highest for Labridae and Pomacentridae - *Chromis*. While *Chromis* species tended to hover in large groups (50-70 individuals) over the damselfish holes, the wrasses tended to travel across the reef crest in small groups, (four to ten) seemingly travelling in and out of the damselfish territory (personal observation). Myrberg (1972) bicolor study indicates that the majority of chases were also directed at wrasses. An increase in wrasse intrusion rates during reproductive season may indicate that wrasses can sense when damselfish are guarding eggs, which may attribute to the higher frequency of attack. The decrease of total *chromis* encounters is unknown, as food availability in the water column should hypothetically not change during reproductive season.

It is important to note that while there were obvious trends in Labridae and Pomacentridae - *Chromis* total encounters, these results were not significant. For all four bicolor individuals studied, there were only two to three instances where recordings were done in the presence of eggs. A study that includes more samples with eggs is necessary to produce significant results.

There was one instance during reproductive season when no damselfish individuals were protecting eggs. Bicolor damselfish are known to lay dormant at night, leaving shelterholes unattended (Myrberg 1972). Fish such as surgeonfish or parrotfish are known to nestle in damselfish holes at night, which may effectively kill eggs. However, it is more likely that a single predator found the easily noticeable and accessible PVC pipes and consumed the eggs. Clutches are laid almost every day during reproductive season; bicolors may protect more than multiple clutches at a time (Knapp et al. 1995) making it unlikely that shelterholes would be without eggs for even one day during reproductive season.

The methodology was limited by time, as divers had to stay at a depth of 6-7m with the data collection cameras for up to an hour at a time. Furthermore, the cameras had limited visual space around the shelterhole and only interactions within the scope of vision were collected. This proved to be a problem when individuals under observation would swim out of view in order to attack or forage for food in the water column.

This study might be furthered by recording the amount of time each damselfish individual spent in the immediate area of its shelterhole. Additionally, more sample individuals should be recorded, possibly on areas of the reef other than the reef crest, where other fish species might intrude on shelterholes. Follow up data could give complete picture of species specific damselfish aggression, as well as the function of territorial behavior.

It was previously thought that bicolor damselfish displayed a blanket increase in aggression toward all fish intruders during reproductive season (Myrberg 1972). Based on this new data, it is suggested that bicolor damselfish aggression increases at different levels toward fish intruders, or not at all during reproductive season. Bicolor damselfish aggression may be multifunctional, a term suggested in an earlier study done on three spot damselfish (Thresher 1976), where territoriality might serve different functions (i.e, to protect eggs or to guard food and resources) rather than serve as a single, homogenously driven behavioral display. Based on the findings of this study, it is suggested that bicolor damselfish also display aggression on a temporal timescale, possibly controlled by lunar cycles.

The multifunctional territoriality and temporally controlled aggression of bicolor damselfish is a novel finding. Further studies may reveal similar behavioral patterns in other reef fishes, and thus can be applied to understanding interspecific and conspecific interactions between fishes at a greater level of detail.

Acknowledgements Thank you to my advisor Dr. Patrick Lyons and my intern advisor Lucien Untersteggaber M. Sc. for their constant input and editing of my project. I would also like to acknowledge my dive partners, Sean O'Neill, Nicole Kleinas, Julia Middleton, and Stephanie Villalobos for overseeing my data collection, taking pictures of the experimental set up, and pointing out cool interactions between fish on the reef while we waited for the cameras to complete recording. Thank you to Sarah Fleming for her doll sized hands and preparing the PVC. Many thanks to Angel Perez and Andrew Maroncelli for editing my drafts throughout this entire writing process. Lastly, I would like to thank the staff and students at CIEE Bonaire and Case Western Reserve University for the opportunity to conduct this research.

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Physis (Spring 2014) 15:15-20

REPORT

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A study of succession in algal communities on coral reefs

Abstract The transition in the Caribbean Sea from coral dominated reefs to algal dominated reefs poses a serious risk to the current existing community. Current research suggests that algal communities will follow a predictable pattern of growth and succession based on the environmental conditions of the community. Depth of the coral head hosting the community and location on the coral head may have a role in succession. This study was conducted on a fringing reef on the leeward side of the island of Bonaire, DC in March of 2014. Algal communities were sampled at two locations on coral heads: just below the livecoral/dead coral interface and between 10 and 30 cm below the first sample. Analysis of genera richness and mass percent of algal divisions were not found to be correlated with depth for all algal divisions. However, richness of Rhodophyta genera was shown to be different between sampling locations on the same coral head and mass percent of Chlorophyta genera was shown to be negatively correlated with distance between sampling locations. The lack of variation found among many of the samples suggests that depth and sampling location on a coral head are largely unimportant in determining the make-up of an algal community, except the aforementioned relationships. Within the framework of creating a predictive model for algal succession, depth and community location on the coral head are components, but more work is needed. The creation of a predictive model will let reef managers forecast future threats and mitigate potential catastrophes.

Keywords Algae • Succession • Community

Introduction

Formerly coral dominated ecosystems in the Tropical Western Atlantic and Caribbean Sea are shifting to algal dominated systems (Hughes 1994). Removal of large herbivorous fish, vulnerabilities in critical stages of life histories of coral species, and storm damage are all contributing factors in triggering a shift, although the relative significance of each of these factors is not well understood (Hughes 1994; Hughes and Tanner 2000; Hughes et.al 2007). In most cases macroalgae species are not capable of settling on living coral tissue, though there are some macroalgal species that, once established, can produce negative allelochemicals to kill the surrounding corals (Diaz-Pulido and McCook 2004, Bonaldo and Hay 2014). Filamentous turf algae species and some encrusting algae species have been shown to overgrow healthy coral (Cetz-Navarro et al. 2013; Eckrich and Engel 2013). Macroalgae can interfere with corals by taking up the free space that coral planula larva need settlement and can even provide a false substrate for settlement on that has been shown to ultimately increase recruit mortality (Jackson 1977; Nugues and Szmant 2006).

Algae across many taxa have shown sophisticated recruitment location selection mechanisms. Deviations from this normal succession pattern are only noticed if there exists an accurate baseline to which future studies can be compared (Hellawell 1991). Little research has been conducted to examine the specifics of algal progression, but the research that has been done points to a rudimentary timeline of successional growth (Diaz-Pulido and McCook 2002). In small polyp stony coral, namely *Porites* spp, dead coral is dominated by turf algae within the first 30 months after polyp mortality, and then shifts to either crustose coralline algae or fleshy macroalgae as soon as 15 to 20 months respectively. Algal communities were initially dominated by diatoms but rapidly shifted to branched filamentous algae. However, the algal communities did not appear at any point during the study to be on convergent trajectories (Diaz-Pulido and McCook 2002). One explanation for this is that the coral heads used to host the study were not in similar enough environments to produce similar communities. If these environmental characteristics to direct algal community development can be identified, the mechanistic functions of algal progression can be uncovered. Therefore:

H_1: For a given set of environmental conditions, algal communities of similar ages will be similar in composition in terms of genera richness and percent mass by algal division.

H_2: The deterministic environmental conditions include and are not necessarily limited to: depth of coral head and location on the coral head.

Materials and methods

Study site

This study was conducted off the coast of Kralendijk, Bonaire DC (12°09'36.5"N 68°16'55.2"W). The current local name for the dive site is "Yellow Submarine" (Fig 1). It is a fringing reef with approximately 17% scleractinian coral coverage and 45% macroalgal coverage with a combined coverage across all groups at approximately 75% (Sommer et al. 2010). Average herbivorous fish biomass density was previously calculated and found to be approximately 25 g m^{-2} (Sandin 2008). Concentrations of nitrate in the seawater were recently measured andfound to be 4±1.44 µmol L^{-1} and concentrations of phosphorous in the seawater were found to be below the detectable limit of 1 µmol L^{-1}(Sikowitz pers. comm.). Samples (n=18) were collected between March 12th and March 19th, 2014 at approximately 1300 local time. Coral heads were sampled between depths of 8.2 m and 16.2 m were selected for high levels of macroalgal growth and low levels of non-algal organisms.

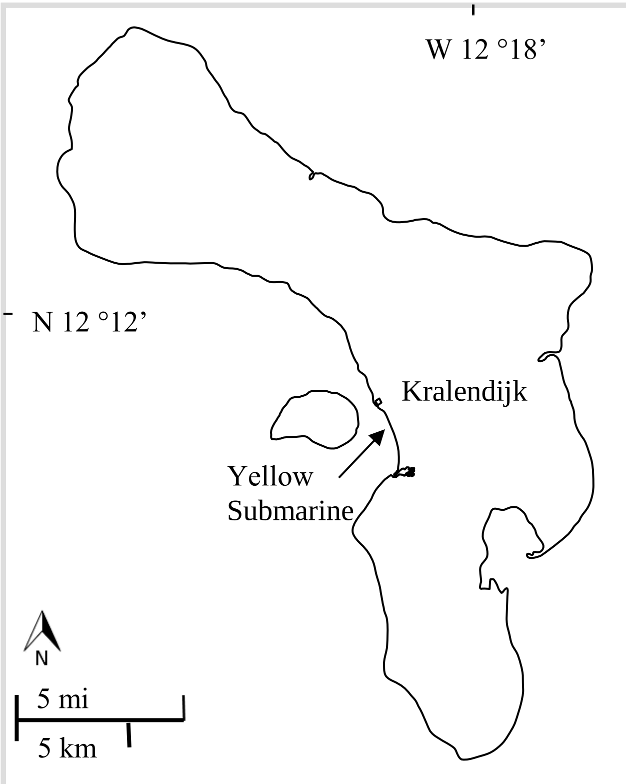

Fig.1 Map of Bonaire with the dive site, 'Yellow Submarine' (12°09'36.5"N 68°16'55.2"W), marked with the arrow

Sampling methodology

Coral heads and potential sampling locations on coral heads that lacked algal communities were not sampled. The depth of the coral head was recorded as well as the vertical distance between the two sampling locations on the coral head. The 'high' algal samples were collected within 5 cm of the living tissue and the 'low' sample was between 10 and 30 cm down the coral head from the 'high' sample point. Algal samples were collected by using a 2.54-cm blunt edge putty knife to scrape an approximately 4-cm^2 section of algal community from the substrate. The samples were then mechanically divided by visual inspection through a microscope with tweezers and identified to the genus level with a microscope and *Caribbean Reef Plants* (Littler and Littler 2000). Wet weight of each genus in each sample was recorded and discarded.

Statistical analysis

Genera richness and mass percent was compared to location on coral head using paired, two-tailed *t*-test in Microsoft Excel 2013 RT ™. Genera richness and the percent contribution of each genera to total wet weight were analyzed for correlation with depth of the coral head in Microsoft Excel 2013 Genera richness and percent contribution of each genera to total wet weight were analyzed for correlation with the distance between sampling points in Microsoft Excel 2013.

Results

Summary

Analysis of algae samples produced 20 distinct genera with an average of 6.3 distinct genera per sample (n=36). Each sample was composed of an average of 3.71 of Rhodophyta genera, 1.11 Phaeophyta algae genera, and 1.48 Chlorophyta algae genera (Fig. 2).

Genera richness

Genera richness was not significantly different between the higher and lower sampling points (Fig. 2**;** Table 1). Genera richness of the upper sampling point correlated with depth for Chlorophyta genera (Fig. 2; Table 2) in which it was negatively correlated. Rhodophyta and Phaeophyta genera richness showed no such trends. Genera richness of the lower sample point did not correlate with depth for any of the algae types (Table 2). The absolute difference in genera richness as compared to the distance between the two sampling points did not show any significant correlations (Table 2).

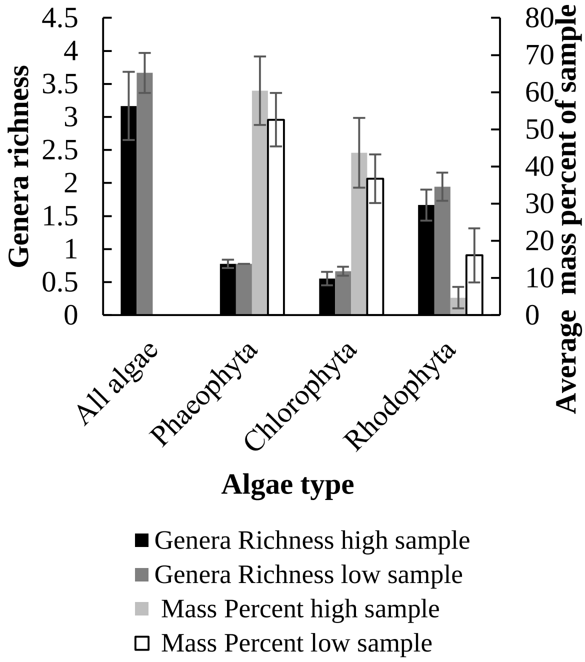

Fig. 2 The genera richness and the average mass percent of the sample as a function of the different types of algae. Error bars are standard error of the mean. Genera richness is total number of genera counted for each type of algae. Mass percent is the percent contribution of each genera to total wet weight

Table 1 *p*-values for 2-tailed, paired *t*-tests. An asterisk (*) denotes a statistically significant result. These tests compare the average genera richness and mass percent at the higher sampling point to the lower sampling point to determine if they are statistically significantly different

Genera richness of high vs. low	***p*-value**
Rhodophyta	0.311
Phaeophyta	1
Chlorophyta	0.579
Mass percent of high vs. low	
Rhodophyta	0.046*
Phaeophyta	0.751
Chlorophyta	0.771

Mass percent

For this paper, mass percent is defined as the contribution of each division to total wet

Table 2 R^2correlation values for linear correlations. A alpha (α) denotes a statistically significant result. The sign next to the number indicates the direction of the correlation. These tests calculate the correlation coefficient of the average genera richness and mass percent at the higher sampling point and the lower sampling point to the range of depths sampled, as well as the absolute change in genera richness and mass percent as it correlates to the distance between sampling points

		R^2
Genera richness vs. depth at the high sample point		
	Rhodophyta	0.1311
	Phaeophyta	0.0696
	Chlorophyta	0.6737α (-)
Genera richness vs. depth at the low sample point		
	Rhodophyta	0.0248
	Phaeophyta	0.0000
	Chlorophyta	0.0280
Mass percent vs. depth at the high sample point		
	Rhodophyta	0.0704
	Phaeophyta	0.0925
	Chlorophyta	0.0071
Mass percent vs. depth at the low sample point		
	Rhodophyta	0.0262
	Phaeophyta	0.0404
	Chlorophyta	$8.0000*10^{-6}$
Absolute difference in genera richness vs. distance		
	Rhodophyta	0.0109
	Phaeophyta	0.1429
	Chlorophyta	0.0357
Absolute difference in mass percent vs. distance		
	Rhodophyta	0.0033
	Phaeophyta	0.5045α (-)
	Chlorophyta	0.0942

weight of the sample. The difference in the average mass percent of the high and low samples points on each coral head were statistically insignificant for Phaeophyta genera and Chlorophyta genera but the difference in the average mass percent of the high and low samples on each coral head was significant for Rhodophyta genera (Fig. 2; Table 1). The mass percent of the three divisions do not correlate with depth at the high or low sample point (Fig. 2; Table 2). However, the absolute change in the difference in mass percent of Phaeophyta genera correlates negatively with increasing distance between sample locations (Fig. 2; Table 2).

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Discussion

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The lack of variation found among many of the samples shows that depth and sampling location are minorly important in determining the composition of algal communities. This can be explained by the relatively narrow range of depths sampled. The high sampling points showed abundance Chlorophyta and Phaeophyta genera, which suggests that they are better adapted to utilize an increased of available light and outcompete the rest of the genera found. This conclusion appears to be the opposite of observed trends in terrestrial grassland ecosystems as well in *Diadema* controled marine algal communities where natural increases in resource availability, such as increased sunlight or increased nutrient levels, tends to promote diversity as more and more niches no longer compete as fiercely for resources (Olff and Ritchie 1998; Sammarco 1982). However, this does not take in to account changes in herbivory pressures which have their own complex relationship with depth and location on coral head (Hay 1981; Hay 1984; Hixon and Brostoff 1996). The low sampling points had more genera diversity, and significantly greater percent mass of Rhodophyta genera, suggesting that lower available light promotes diversity by not allowing a single genus outcompete others.

A majority of the algae samples followed the general plan of one or more genera growing around another structurally more robust genus. *Dictyota* spp. was observed to be providing structure to *Cladophora* spp. and epiphytic Rhodophyta genera and *Jania* spp. was seen providing a calcareous skeleton for *Cladophora* spp to grow around. In these instances *Dictyota* spp. and *Jania* spp. increase the available free space within a given unit of available substrate and allow for a more complex and diverse community to develop.

This trend supports the hypothesis that for a given set of environmental conditions, the algal community will be essentially the same. In this study, environmental conditions such as nutrient and pollution levels, fish populations, and factors like light availability were the same for all samples as they were taken from a single dive site within the span of one week. The effect of depth, which appears to play some role in community dynamics, may be mitigated by the relatively narrow range of depths sampled. Of the two hypotheses, they were only supported for certain divisions. For the given set of environmental conditions, the algal communities sampled were very similar in their genera richness and percent mass by algal division. The algal communities do not show widespread significant differences based on depth or sampling location from the data collected. However, there were some cases where a specific taxa of algae correlated with changes in these factors. In terms of a predictive model for algal succession, this is a piece of the puzzle, but more work needs to be done to create any sort of functional model.

The uses of such a model can be helpful in stopping the algal phase shift by giving ecosystem managers the ability to forecast algal communities and predict which seemingly innocuous reef habitat may be one the verge of catastrophe. It can also be used to help assess reef health by indicating abnormal algal progression sequences and the point of divergence can be quickly pinpointed and fixed. It is a tool that can help restore reefs and act as an early warning system.

Acknowledgements I would like to thank Dr. Patrick Lyons and Lucien Untersteggaber, M.Sc. for their assistance in designing my study and advising me along the way. I would like to thank Nicole Sikowitz for her assistance in the field with sample collection and Molly Gleason M.S. for her assistance in the lab with algae identification. Finally, I would like to thank CIEE Bonaire and Seattle University for providing me with the opportunity to conduct this independent research project.

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Physis (Spring 2014) 15:21-29

REPORT

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Anti-predator responses of herbivorous ocean surgeonfish, *Acanthurus bahianus*, to invasive lionfish, *Pterois volitans*

Abstract Since their introduction into the western Atlantic, lionfish (*Pterois volitans*) have become a major threat to the coral-reef ecosystems. Lionfish have proven to be formidable predators and it has been demonstrated that they have to potential to reduce recruitment of native fishes and even contribute to phase shifts. It is known that lionfish are capable of hunting and catching many species of native fishes, however little is known about the anti- predator responses of those prey fish. This study examined the anti-predator responses of herbivorous ocean surgeonfish to lionfish. The ocean surgeonfish were visually exposed to a lionfish and their resulting behaviors were analyzed to determine if the fish exhibited an anti-predator response to an invasive predator. The naïve prey hypothesis predicts that prey will not react to predators if they do not share an evolutionary history with those predators, therefore it was predicted that juvenile ocean surgeonfish would not exhibit anti-predator behaviors in response to lionfish. The results of this study were inconclusive due to the absence of a positive control, but they indicate that ocean surgeonfish do not exhibit an anti-predator response to lionfish. If this study were to be successfully repeated with a positive control, the results may help to enhance our understanding of the ecological effects lionfish are having on the coral-reef ecosystem and will help us to mitigate those effects.

Keywords Lionfish • Anti-predator response • Ocean surgeonfish

Introduction

Over the last century, international trade has increased exponentially, allowing exotic species to travel from continent to continent, either accidentally or purposefully. Although many exotic species fail to establish and spread, some go on to become very detrimental invasive species. Even one invasive species has the potential to change whole ecosystems by altering the physical environment or biotic community, both of which can lead to trophic cascades. The thousands of invasive species that exist in the Unted States alone have been estimated to cost over $120 billion in damage and control expenses (Pimentel et al. 2005). One of the most severe invasions to date is the lionfish (*Pterois volitans*) invasion of the western Atlantic (Albins and Hixon 2011).

Lionfish were likely introduced through an accidental release of aquarium specimens in southern Florida in the 1980s (Johnston and Purkis 2011) and have become the first invasive marine fish to successfully establish themselves throughout the western Atlantic (Schofield 2009). Since their initial release, *P. volitans* have thrived and expanded their range from Virginia, USA to Venezuela (Morris and Whitfield 2009). Assuming sea surface temperature is the only limiting factor, lionfish have the potential to continue to expand down the South American coast as far as Uruguay (Kimball et. al. 2004).

Researchers have yet to find an effective method of lionfish control apart from manually culling the population using hand nets and SCUBA. Moray eels and groupers have occasionally been observed eating lionfish

(Maljković 2008; Jud 2011), however recent studies have shown no correlation between grouper density and lionfish density (Hackerott 2013). Lionfish also have no known susceptibility to disease or parasites, eliminating the possibility of parasites serving as a natural population control. With the current knowledge and removal techniques there is no possibility of completely eradicating the lionfish population, necessitating research on the effects of lionfish on coral-reef ecosystems (Albins and Hixon 2011).

Since their introduction, lionfish have become a major threat to coral-reef ecosystems due to their significant consumption of reef fishes. A recent study by Cure et al. (2012) found that lionfish attempted to catch a great diversity of prey in their native range, but were successful only with a few species. Conversely, in their invasive range the lionfish were very successful in catching a much larger variety of species. Albins and Hixon (2008) found that in the western Atlantic, lionfish are capable of eating over 40 species, including important herbivores like the juvenile parrotfish and surgeonfish. In their native range, there are parrotfish present, but lionfish have not been observed eating them (Cure et al. 2012). Additionally, compared to native predators like the grouper, lionfish consume a greater variety and quantity of fishes, reducing recruitment of prey significantly (Albins 2010).

The reasons for invasive lionfish success and its immense effects on the coral-reef ecosystem are not fully understood, but it is most likely due to four major factors: superior growth rate, high fecundity, no significant predation in invasive or native range, and no evolution with native fishes (Côte 2013). The least is known about the latter reason and more research is necessary in order to understand the full extent of prey naïveté.

The naïve prey hypothesis posits that if a native prey species shares no evolutionary history with an invasive predator, that prey species will have ineffective or no anti-predator behavior (Sih et al. 2012). If a native prey species utilizes specific cues to detect predation and if the predator is novel, the prey will not recognize the predator as a potential threat. It is also possible that the prey utilizes more general cues to detect predation and the prey may demonstrate anti-predator behaviors, though they may not be as effective. These scenarios would result in the invasive predator consuming the native fish. The other possibility is that, despite lacking evolutionary history with a predator, native prey does exhibit effective anti-predator responses. This would result in the predator having non-consumptive effects on the prey because the prey item will expend extra energy hiding or fleeing and will gain less energy feeding.

Magurran (1990) conducted a comparative study of anti-predator behaviors with lab-bred juvenile and wild-caught adult minnows. This study discovered the wild-caught and lab-bred minnow species that were previously exposed to predators reduced their feeding time when exposed to a predator model. The species that had not been previously exposed to the predator fed for longer and were less wary of the predator model. These findings support the naïve prey hypothesis, in that the species that shared no coevolution, did not respond appropriately to the novel predator. Lab-bred individuals showed the same response, therefore the study demonstrates that the anti-predator response is not learned by experience in this particular species. However, it is possible, that other species are able to learn to avoid predators after being exposed to them one or more times.

Anti-predator responses and non-consumptive effects of lionfish on native fishes have yet to be studied extensively. Albins (2010) hypothesized that because native Caribbean fishes did not evolve with lionfish, they have not developed resistance to the lionfish's toxic spines. He also hypothesized that the lionfish's cryptic coloration, which is unfamiliar to native fishes, allows lionfish to blend into the background or appear to be a tuft of seaweed. This camouflage would aid their hunting strategy, which is to slowly corner a fish using their large pectoral fins.

There has been one recent study of anti-predator responses of native fishes to lionfish, upon which this study is based. Marsh-Hunkin et al (2013) visually exposed two different goby species to lionfish, Nassau groupers (*Epinephelus striatus*), and French grunts (*Haemulon flavolineatum*). Although many of the results of this study were inconclusive, most likely due to their small sample size, they did find that both species of gobies had decreased movements and increased numbers of bobs in response to the grouper compared to the lionfish and controls. A study of the effects of lionfish on native herbivores like the ocean surgeonfish (*Acanthurus bahianus*) may help us to better understand the overarching effects lionfish are having on coral-reef ecosystems.

Ocean surgeonfish are one of the most abundant and widespread herbivorous coral-reef fish in the Caribbean (Robertson et. al. 2005). They are found throughout the tropical northwest Atlantic ranging from Bermuda to Brazil (Rocha et. al. 2002). Like all parrotfish species (Scaridae), their diet primarily consists of macroalgae, making them important in regards to controlling the macroalgae population. These fish are therefore vital to the Caribbean coral-reef ecosystem and a reduction in their abundance would likely have extremely detrimental effects on coral reef health, particularly in terms of the phase shifts from coral dominated systems to algal dominated systems (Lirman 2001).

This study assessed the anti-predator responses of ocean surgeonfish to lionfish. Juvenile ocean surgeonfish were exposed to lionfish and feeding and hiding habits were observed. It was hypothesized that:

H_1: If the ocean surgeonfish are naïve to lionfish, then the ocean surgeonfish would take a similar number of bites and spend a similar amount of time hiding with both the lionfish and a non-predator or no fish present.

H_2: If the ocean surgeonfish have adapted to the lionfish or if they can in recognize the lionfish as a predator, then the ocean surgeonfish would attempt an anti-predator response. This would be as evidenced the ocean surgeonfish taking fewer bites and spending more time hiding in the presence of the lionfish than in the presence of a non-predator or without the presence of another fish.

Materials and methods

Study site & species collection

This study was conducted at CIEE laboratory in Bonaire, NE (Kaya Gobernador Debrot 21, Kralendijk). Ten juvenile ocean surgeonfish (*Acanthurus bahianus*) (~4 cm TL) and one French grunt (*Haemulon flavolineatum*) (~20 cm TL) were caught using hand nets in the shallows (0-1 m depth) at the dive site 'Yellow Submarine' (Fig. 1). Additionally, one lionfish

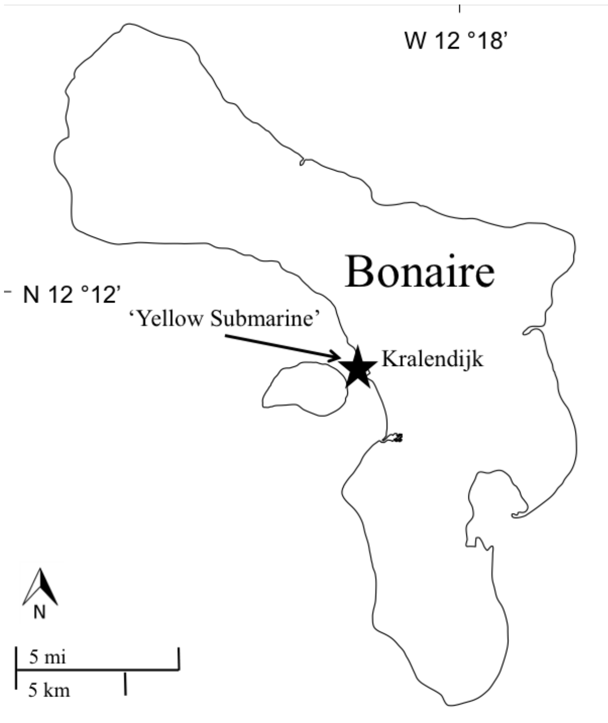

Fig. 1 *Black star* indicates fish collection site: 'Yellow Submarine', Kralendijk, Bonaire, Dutch Caribbean (12°09'36.5"N 68°16'55.2"W) (Google Maps)

(*Pterois volitans)* was caught using SCUBA at a ~18 m depth (~20 cm TL). At shallow depths (0-2 m) the dive site Yellow Submarine consist primarily of coral rubble covered in algae. Starting at ~10 m depth a fringing reef begins and extends to ~35 m. In the shallows both juvenile ocean surgeonfish and French grunts are abundant and consistently come in contact with each other. Lionfish are sometimes observed in the shallows, however they are not as common and it is likely that ocean surgeonfish used in this study had never been exposed to lionfish previously.

French grunt were kept in 0.6 X 0.45 X 0.45 m trial tanks. The ocean surgeonfish were fed a variety of algae collected from the reef, the lionfish was fed live baitfish (2-4 cm, species unknown), and the French grunt was fed freeze-dried bloodworms and mysis shrimp (San Francisco Bay Brand). The experimental trials were conducted in four dark-sided tanks that were divided into two compartments by a clear barrier (Fig. 2). One compartment of the tank contained the lionfish, French grunt, or no fish. The other compartment contained two ocean surgeonfish with algae covered rocks and a shelter where they could feed and hide.

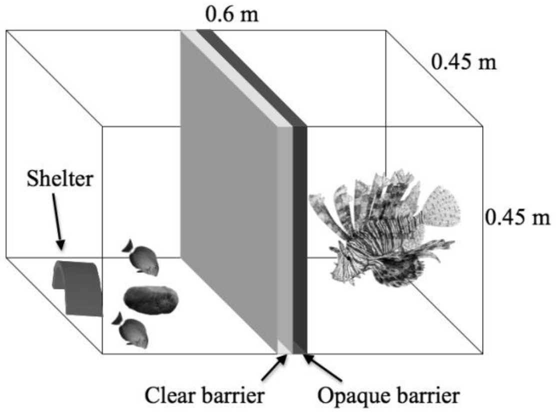

Fig. 2 Diagram of experimental setup. Two *Acanthurus bahianus* on one side of the barrier with treatment (no fish, *Pterois volitans*, or *Haemulon flavolineatum*) on the other side. The clear barrier is stationary and the solid barrier is removed after the 10 min acclimation period

Trials were conducted between the hours of 11:00 and 17:00 hrs and each trial was filmed by a camera (GoPro 3 or GoPro 3+) mounted on the clear barrier separating the two compartments of the tank (Fig. 2). Two ocean surgeonfish were placed in the tank for each trial. Originally the intention was to use one fish, however the ocean surgeonfish failed to acclimate when they were alone (personal

Staged predator-prey trials

A single lionfish, French grunt (non-piscivorous control), and no fish (control) served as three treatments. All ocean surgeonfish were kept in 0.45 X 0.20 X 0.30 m aquaria throughout the study. The lionfish and observation). Initially, the other side of the tank was hidden from the fish by a opaque barrier. The fish were then allowed to acclimate to the new environment for 10 minutes. The barrier was then removed so the ocean surgeonfish could see the fish on the other side. The ocean surgeonfish's behavior was filmed for five minutes after visual exposure to the other side of the tank.

The five-minute response videos from each trial were watched in the video viewing software 'VLC Player' by a single viewer. For every minute elapsed, the viewer counted the number of bites taken by each ocean surgeonfish (n=6). Additionally, the viewer determined percentage of time spent hiding for each minute elapsed for both fish combined (n=3) due to dominance of the shelter by only one of the fish.

Data analysis

For each of the two behaviors (feeding and hiding), a one-way ANOVA was performed using Microsoft Excel 2010 to examine the difference in response of the ocean surgeonfish to the lionfish as opposed to the controls (H_0: $\mu_{lionfish} = \mu_{French\ grunt} = \mu_{control}$, where μ=mean time feeding/hiding). A regression was also performed to examine the change in feeding and hiding over time.

Results

Rate of feeding

Ocean surgeonfish did not demonstrate a statistically significant ($p>0.05$, Table 1) difference in feeding amongst the three

Table 1 ANOVA table for the differences in average number of bites per minute amongst the no fish present, *Haemulon flavolineatum*, and *Pterois volitans* treatments

Sources	SS	df	MS	F	P value
Between Groups	61.00	2	30.50	2.47	0.13
Within Groups	148.09	12	12.34		
Total	209.09	14	14.94		

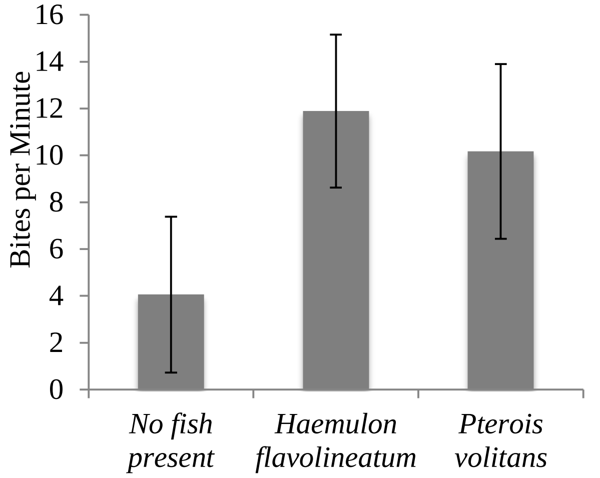

Fig. 3 The average number of bites taken by *Acanthurus bahianus* (n=6) per minute in the presence of no fish, *H. flavolineatum*, and *P. volitans*. Error bars indicate standard deviation

treatments; lionfish, French grunt, and no fish (Fig. 3). A linear regression revealed that initially, the feeding rate of the ocean surgeonfish in all treatments was low because the lifting of the barrier startled the fish. However as time passed, the number of bites taken every minute increased by different rates for the different treatments. In the presence of a French grunt or lionfish the number of bites taken every minute increased by a greater rate in contrast to when there were no other fish present (Fig. 4).

Time spent hiding in shelter

Similar to the feeding results, ocean surgeonfish did not demonstrate a statistically significant ($p>0.05$, Table 2) difference in time spent hiding among the three treatments—presence of lionfish, French grunt, or no fish (Fig. 5). The rate of change of time spent hiding also differed between the empty tank and the French grunt/lionfish treatment. Initially, the percent of time the ocean surgeonfish spent hiding was high, however, if the ocean surgeonfish were in the presence of the French grunt or lionfish, the percent of time they spent hiding increased at a relatively high

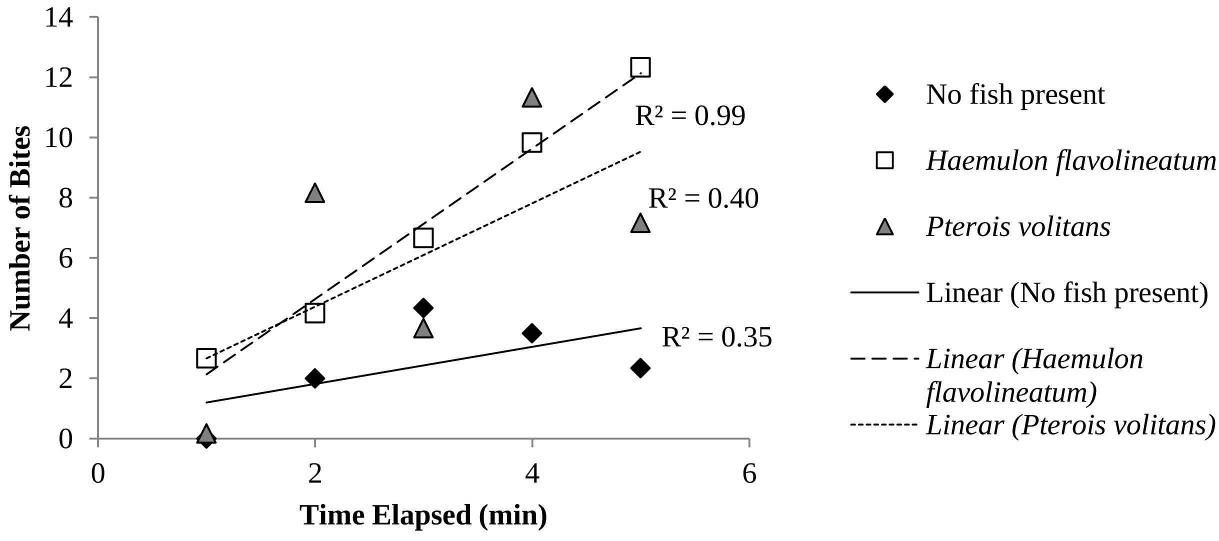

Fig. 4 At time zero an opaque barrier was lifted to reveal the *Acanthurus bahianusto* the treatment (*no fish present, H. flavolineatum, or P. volitans*). The total number of bites taken every minute was recorded and graphed. Linear best fit lines were generated

Table 2 ANOVA table for the differences in average percent of time spent in the shelter amongst the empty tank, *Haemulon flavolineatum*, and *Pterois volitans* treatments

Sources	SS	df	MS	F	P value
Between Groups	0.16	2	0.08	2.39	0.17
Within Groups	0.20	6	0.03		
Total	0.36	8	0.05		

rate, whereas if they were alone, the percent of time the ocean surgeonfish hid only decreased slightly (Fig. 6).

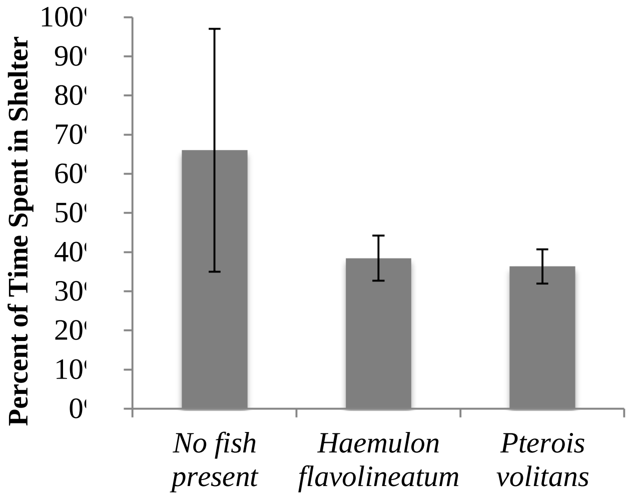

Fig. 5 The combined percent of time both *Acanthurus bahianus*(n=3) spent in the shelter in the presence of no fish, *H flavolineatum*, and*P. volitans*.Error bars represent standard deviation

Discussion

The results showed that there was no statistical difference in ocean surgeonfish response between the lionfish, French grunt, and no fish treatments, therefore H_1 was supported. The ocean surgeonfish did not act any differently around a non-predator than they did around a very dangerous predator. These result are consistent with the results of the study done by March-Hunkin et al (YEAR) on goby anti-predator responses to lionfish.

Although not statistically significant, there was an unexpected trend in the data. In the feeding trials the ocean surgeonfish fed more in the presence of another fish (lionfish or French grunt) than they did when they were alone in the tank (Fig. 3). The same trend was observed in the hiding trials when the fish hid a higher percent of the time when they were alone than when they were in the presence of the lionfish or French grunt (Fig. 5). Additionally, both linear regressions revealed that the ocean surgeonfish recovered from the disturbance of the screen lifting faster in the presence of other fish than when they were alone (Fig. 4, Fig. 6). This trend is most likely explained by ocean surgeonfish's apparent preference for feeding in groups. These trials were originally to be done with one ocean surgeonfish, however when a fish was put in the test aquaria alone, the fish did not come out of the shelter even after an acclimation period of 20 minutes.

Additionally, when catching the ocean surgeonfish, it was observed that they were almost always in a school of two to 20 individuals and often attempted to school with other species as well, indicating that they have a tendency to act more naturally in groups. Although French grunts and lionfish are larger than the ocean surgeonfish, it is possible that the presence of these fish made the ocean surgeonfish act more naturally, causing them to feed more and hide less.

An alternate explanation for this unexpected trend is the possibility that boldness is selected for in ocean surgeonfish. A 2004 study of three-spined sticklebacks (*Gasterosteus aculeatus*) found that bold individuals (those that hide less in the presence of a predator) outcompeted shy individuals and had faster growth rates in the lab. However, in the wild, bold individuals had a greater chance of predation, allowing shyness to persist (Ward et. al. 2004). If there is a similar pattern in ocean surgeonfish, this could explain why most ocean surgeonfish did not reduce their feeding rate in the presence of the lionfish and it may explain the considerable variation in results. However, this theory does not explain why the fish had reduced feeding rates when there were no other fish in the tank.

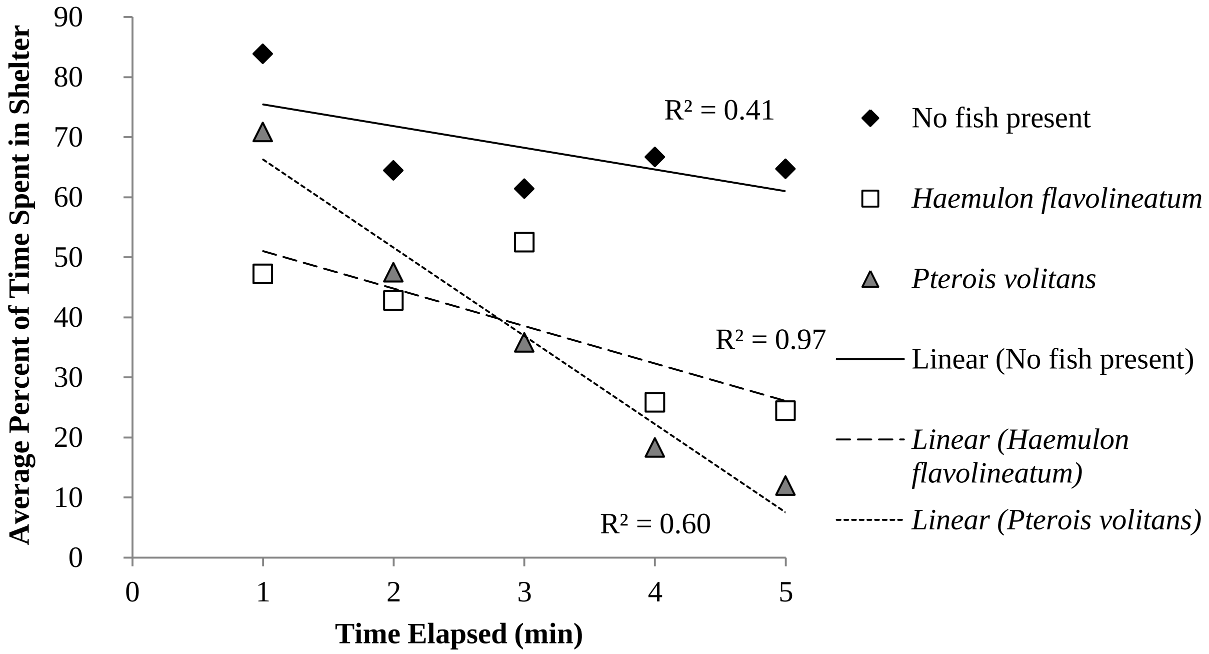

Fig. 6 At time zero an opaque barrier was lifted to reveal the *Acanthurus bahianus*to the treatment (*no fish present, H. flavolineatum, or P. volitans*). The percent of time spent hiding for every minute elapsed was recorded and graphed. Linear best fit lines were generated

Originally, this study was supposed to include a native predator treatment of a coney grouper (*Cephalopholis fulva*), but the trials could not be performed. Without this positive control, it is unknown whether juvenile ocean surgeonfish outside of their natural habitat exhibit behavioral changes in response to any native predatory fish. If a coney grouper were used for a set of trials and if the ocean surgeonfish did reduce their feeding time and increase their hiding time in response to the coney grouper, then these results could be strengthened.

During the trails, it was observed that the lionfish recognized the ocean surgeonfish as prey and if the barrier had not been present, the lionfish would have likely attempted to consume the ocean surgeonfish, yet the ocean surgeonfish did not alter their behavior. This indicates that juvenile ocean surgeonfish do not have the ability to recognize lionfish as a predator. Once again, this response is likely due the lionfish and ocean surgeonfish sharing no evolutionary history. Historically, ocean surgeonfish were likely selected for the ability to exhibit anti-predator responses only to possible predators, since lionfish were not historically present, there was no selection for individuals whom evaded lionfish. Therefore, most ocean surgeonfish may not recognize the lionfish as a threat. It is likely that other juvenile coral reef fishes will be equally unable to identify lionfish as a predator. Juvenile fish's inability to recognize lionfish as a predator likely explains why lionfish are able to so readily reduces recruitment.

Even if some juvenile fishes cannot innately recognize lionfish of predators, there may still be a possibility that fishes can learn to recognize lionfish as predators. To test this, this same experiment could be conducted but with ocean surgeonfish that had been previously exposed to a lionfish killing either a conspecific or a non-conspecific. Lönnstedt and McCormick (2013) conducted a study of damselfish (*Chromis viridis)* anti-predator responses to P. volitans with experienced and unexperienced individuals and found no statistically significant difference in anti-predator response behaviors. Results of an experiment like this may indicate whether ocean surgeonfish are capable of learning to react to a novel predator. These studies could be done with several species of parrotfish and could be highly beneficial because parrotfish are another important herbivore on reefs in preventing phase shifts on reefs.

Herbivores consumed by lionfish are crucial to the coral-reef ecosystem. Herbivores eat macroalgae which takes up space on suitable substrate for larval coral settlement and thes preventing coral from establishing new colonies. Without the appropriate amount of herbivores to eat the macroalgae, coral reefs may become algal-dominated (Lirman 2001). Increased algal-coral competition could be devastating to many species because coral is a keystone species that provides a habitat for thousands of species (Mumby et al. 2006). Additionally, millions of people worldwide utilize coral reefs for their beauty and for their abundance of fishes; estimates show that over 500 million people depend on coral reefs in some way (Wilkinson 2008).

A correlation between the lionfish invasion and phase shifts has already been observed. Lesser et al. (2011) investigated the effects of the lionfish invasion on mesophotic reefs in the Bahamas. The study collected data on at depths ranging from 30 m to 92 m, before and after an invasion of lionfish and found a significant difference in percent cover of coral, algae, and sponges at certain depths. They found that at 46 m and 61 m depth the percent cover of algae significantly increased (27% to 94% at 46 m) from pre-invasion (2003) to post-invasion (2009). The coral cover showed the opposite pattern, decreasing drastically (16% to <2% at 46 m) from pre-invasion to post-invasion. The researchers also tested factors other than lionfish that could have contributed to the phase shift and found no evidence that disease, nutrients, overfishing, light availability, or hurricanes were responsible, indicating that lionfish invasions are influencing the phase shifts found throughout the Caribbean.

Understanding the predator-prey dynamics of lionfish and herbivores will help us to understand not only why lionfish are so successful, but may also help us to fully grasp the scope of the impacts that lionfish are having and will continue to have on coral-reef ecosystems.

Acknowledgements Thank you to my two advisors Dr. Patrick Lyons and Lucien Untersteggaber, M.Sc. Additionally, I would like to thank all those who dove with me including Sarah Fleming, Jenny Mathe, Julia Middleton, Colin Howe. Finally, thank you to the CIEE Bonaire Research Lab and the rest of its staff: Dr. Rita Peachey, Dr. Enrique Arboleda, Molly Gleason, M.Sc, Stephanie Villalobos, and Amy Wilde.

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Physis (Spring 2014) 15:30-35

REPORT

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Effects of elevated CO_2 levels on *Coryphopterus glaucofraenum* response to injured conspecific chemical cues

Abstract Carbon dioxide levels in the ocean are predicted to double by the end of the century, making the marine environment more acidic than it is today. This study aimed to analyze whether increasing acidity affects anti-predator survival behavior of the bridled goby, *Coryphopterus glaucofraenum*. A group of 10 adult gobies were treated with elevated CO_2 levels, simulating predicted conditions by the year 2100, and another group of 10 were treated in present-day levels. Each group was exposed to the chemical cue of an injured conspecific, a predation chemical alarm signal, and the behavioral responses of each individual were recorded. The two groups were compared according to average time spent under shelter, number of feeding attempts, and amount of time spent motionless after exposure to cue. Overall, this experiment supported the hypothesis that gobies treated in acidified water would fail to fully exhibit such predator avoidance behaviors; gobies treated in elevated CO_2 levels spent less time motionless after exposure to predation chemical cue. This study attempts to make important observations about the effect of environmental factors on fish behavior as well as far-reaching implications for the future survival of fish species and the stability of marine ecosystems as a whole.

Keywords Ocean acidification • Anti-predator response • Carbon dioxide

Introduction

Many new findings are surfacing on the effects that elevated carbon emissions are having on the Earth, centralized around climate change and acidification of the ocean. The Intergovernmental Panel on Climate Change (IPCC) predicts that the oceans are going to become drastically more acidic by the end of the century, with a pH value of 0.2-0.4 less than it is today (Rhein et al. 2013). This drastic change in chemical composition of the oceans may have radical effects on important marine ecosystems.

Nearly one third of anthropogenic carbon emissions are absorbed directly into the ocean (Rhein et al. 2013). Once taken up by the aquatic environment, the dissolved CO_2 forms a weak acid, H_2CO_3, which dissociates into HCO_3^- ions and H^+ ions (Fabry et al. 2008). This causes a decrease in available CO_3^{2-} ions and decreases the water's $CaCO_3$ saturation state, which can hinder the ability of corals to grow their calcium carbonate skeletons, decreasing the resilience of the reefs (Anthony et al. 2011; Rhein et al. 2013). This makes algal phase shifts more likely and could change the entire trophic structure of the reef, jeopardizing the function of coral as an anchor for biological architecture and diversity (Anthony et al. 2008). In addition, the increase in H^+ ions gradually lowers the pH of the seawater, which can considerably change the biotic and abiotic composition of the environment. Ocean acidification can also affect the survival of non-calcifying organisms, such as fish, as evidenced by studies on fish anti-predator behavior.

In the reef ecosystem, adult fish adopt behaviors to avoid predators, survive, and reproduce, the only way to ensure continuation of their species. Fish species often respond to the odor of an injured conspecific, a member of the same species, in the same way they would respond to a predator. This olfactory cue of an injured conspecific acts as a chemical alarm signal that there is a predator nearby that should be avoided (Larson and McCormick 2004). A previous study by Brown and Smith (1998) has shown that, when exposed to the skin extract of a conspecific, fish exhibited decreased area use and increased time under cover. In the absence of an actual predator, this experiment exclusively tests fish ability to avoid predation-related chemical cues. Studies have shown that increased ocean acidity reduces the use of anti-predator behavior by fish, as high CO_2 levels interfere with neurotransmitter function involved in anti-predator responses to olfactory cues (Nilsson et al. 2012). Previous studies have found that test fish exposed to elevated CO_2 levels failed to exhibit advantageous anti-predatory behaviors (Ferrari et al. 2012). Other findings demonstrate that adult fish previously exhibiting the proper survival behaviors are cognitively impaired after treatment in elevated CO_2 levels (Nilsson et al. 2012), with an extreme result showing treated fish actually being attracted to injured conspecific cue rather than avoiding it (Dixson et al. 2010).

This study aimed to measure whether elevated CO_2 levels affect anti-predator behavioral responses in the bridled goby, *Coryphopterus glaucofraenum*, a small reef-associated fish that lives on the sandy bottom. Common displays of anti-predator behavior tested in previous experiments are increased time spent near the bottom of test aquaria and time under a shelter (Wisenden and Sargent 2010). A study of goby response to predator cues defines "freezing", a period of staying motionless after detection of danger, as a survival response typical of *C. glaucofraenum* (Marsh-Hunkin et al. 2013). Tests in laboratory conditions operated under the assumption that gobies exposed to the chemical cue of an injured conspecific will respond as though a predator is near and exhibit survival responses (i.e. hiding under shelter and staying motionless) and tested the following hypothesis:

H_1: Gobies treated in elevated CO_2 levels exhibit less anti-predator behavior than gobies in control levels and do not effectively avoid the olfactory chemical cues of an injured conspecific.

Materials and methods

Study site and organism

This study was conducted at the CIEE Bonaire Research Lab at Kaya Gobernador N. Debrot 26, Kralendijk, Bonaire. Test subjects were collected on SCUBA in the sandy shallows at approximately two meters in front of Yellow Submarine Dive Site (12°09'36.5"N 68°16'55.2"W). Bridled gobies, *Coryphopterus glaucofraenum,* were selected as the test species due to their abundance as a common prey item targeted by reef predators such as groupers and snappers. The gobies were approximately 2.5-6 cm long with a site density of ~5 individuals per square meter. At the study site, *C. glaucofraenum* were observed to feed on benthic invertebrates and use dead pieces of coral as refuge from predators. Adult individuals (n = 22) were collected using hands nets and a 1:9 dilution of clove oil and ethanol, an effective anesthetic determined by personal observations, and brought to the lab to be tested.

Treatment phase

Gobies were split into two plastic aquaria with fresh seawater and sand, with 12 gobies in the control group (10 for control and two extra for later procurement of chemical cue) and 10 in the experimental group. A standard aerator was put within each aquarium and gobies were fed live brine shrimp, *Artemia franciscana*, three times daily. The brine shrimp were hatched from eggs in an aerated chamber in the lab. A CO_2 tank with attached regulator valve was used to gently bubble the treatment tub three times a day, each time until a Hanna Instruments pH meter read a value of 0.2 lower than the control tub (~7.5), to simulate hypothesized acidity by the end of the century (~7.2). The two fish groups were treated in their respective conditions for four days without change, except to maintain the 0.2 difference in acidity by bubbling CO_2 and testing with a pH meter three times daily.

The fish were treated for four consecutive days, as this time span has been shown to be long enough to yield results yet operated within the limits of available resources (Ferrari et al. 2012). The two extra gobies in the control tank were anesthetized by bubbling CO_2 into a small beaker with the fish. For the control group testing phase, these two gobies were put in a petri dish, decapitated, and ~20 incisions were made along the entirety of each body to ensure enough chemical cue was obtained. The bodies were then rinsed with 10 mL of seawater, as a similar study on *Asterropteryx semipunctatus* used 15 mL (Larson and McCormick 2004), it was determined that a lesser volume of 10 mL would be more likely ensure a high enough concentration of chemical cue from these small specimens to observe a response when administered. The tissue was removed, leaving a 10 mL solution of chemical cue in the petri dish. For the treatment group, two individuals from the control group were euthanized and prepared in the same way after being tested.

Experimental phase

The same method was performed first for the control group, then the experimental group. Ten gobies were moved from the treatment tank to a clear, 10-gallon aquarium containing a 2-cm-deep sand substrate, fresh seawater (with normal CO_2 levels), and two coral shelters. After the acclimation period of 10 min, the chemical cue solution was administered into the tank and the behavior of all 10 gobies was recorded with a Sony Handycam HDR-SR7 video camera held ~0.5 m above the tank for five min. In order to test willingness to move after chemical cue, 10 mL of seawater containing brine shrimp was poured into the tank one min after addition of the chemical cue. The purpose of the feeding was a way to observe whether the gobies were avoiding the predator cue by decreasing activity levels, thereby not attempting to feed on the brine shrimp. The gobies were recorded and observed to determine whether they utilized the shelter or exhibited decreased activity levels when given the opportunity to feed. Gobies hidden from view for entire trial were assumed to have made no feeding attempts.

Data analysis

The control and treatment groups of gobies were compared by mean time spent under shelter, mean number of feeding attempts, and mean amount of time spent motionless after addition of chemical cue. Video analyses were performed on Picture Motion Browser (PMB) software on each group in which the behavior of each individual was recorded. Gobies were numbered 1-10 in each group and observed. The number of displays for each behavior were then used to calculate an average for the group. A two-way, unpaired t-test was performed between the averages of the two groups for each of the three behavioral responses.

Results

No significant difference in mean time spent under shelter ($t_{1,18}$=2.10, p=0.808; Fig. 1) nor in mean number of feeding attempts ($t_{1,16}$=2.12, p=0.718; Fig. 2) between the control and treatment groups was found.

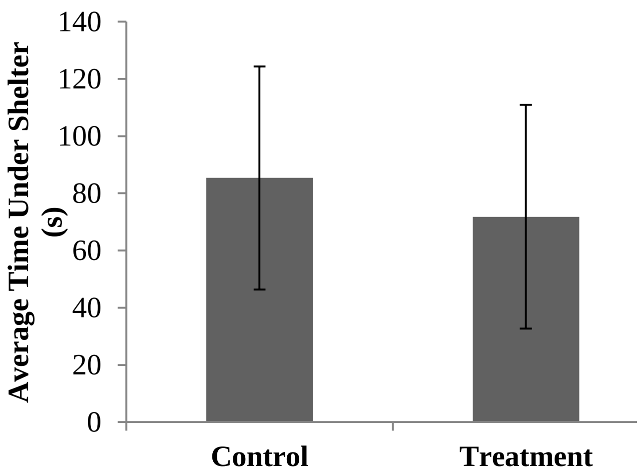

Fig. 1 Comparison of the mean amount of time in seconds (mean±SE) spent under shelter between the control and CO_2-treated groups of gobies

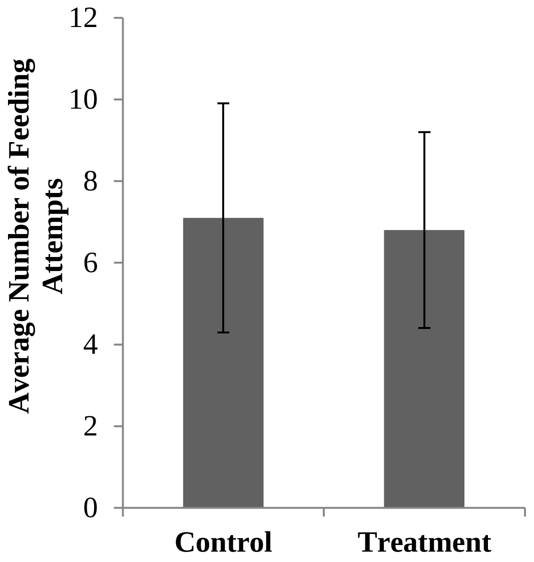

Fig. 2 Comparison of the mean number of feeding attempts (mean±SE) observed between the control and CO_2-treated groups of gobies

A significant difference in mean amount of time spent motionless after adding chemical cue between the control and treatment groups was found ($t_{1,16}$=2.12, p=0.038; Fig. 3). The control group spent a significantly longer mean amount of time motionless after addition of injured conspecific chemical cue than the treated group. Other bold behaviors were observed during testing of the treatment group such as failure to actively avoid the net while being transferred to the test aquarium and relatively increased aggression towards one another once transferred.

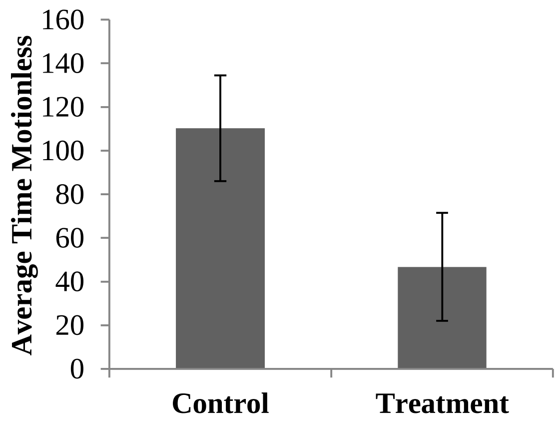

Fig. 3 Comparison of the mean amount of time in seconds (mean±SE) spent motionless after chemical cue between the control and CO_2-treated groups of gobies

Discussion

This study examined the effect of ocean acidification on bridled goby antipredator responses. The results demonstrated that there was a significantly shorter amount of time CO_2-treated gobies remained motionless after exposure to chemical cue as compared to the control group. Overall, the results support the hypothesis that gobies treated in elevated CO_2 levels exhibit less anti-predator behavior than normal and do not effectively avoid the olfactory chemical cues of an injured conspecific.

This result is important in analyzing anti-predator behavior because decreased movement is a common anti-predator response in goby species (Marsh-Hunkin et al. 2013). In this case, the gobies that were treated in control levels sensed the implied predatory threat of the chemical cue, and remained motionless for a longer time. The gobies treated in elevated CO_2 waited a significantly shorter amount of time before beginning to move around the tank and continue feeding. This result raises important questions about the ability of these treated fish to interpret dangerous cues and their willingness to exhibit bold behavior while there is still an implied threat present.

Chemical cues released by an individual's damaged tissue during an attack by a predator have been shown to elicit anti-predator responses in all major groups of aquatic organisms (Wisenden 2003). If the chemical cue of a conspecific had really been a signal of a hungry predator nearby, the treated gobies would not have waited as long for the threat to leave the vicinity. It appears the treated gobies determined it was beneficial to start moving sooner than did the untreated gobies. Chemical cues can be a particularly important signal of predation in a reef environment, since visual cues are often unreliable due to reef structure forming natural barriers (Marsh-Hunkin et al. 2013).

Field experiments with the presence of natural predators and reef structure could shed new light on goby response to antipredator cues. However, the expected changes in ocean pH will not be observable for many decades. Many factors can change in the marine environment over the next century that can affect the survival of these fish. For instance, the rising prevalence of invasive lionfish, *Pterois* spp., may serve as a novel predator that many fish species are not accustomed to avoiding. A study in the Bahamas documented that densities of *C. glaucofraenum* were significantly reduced once lionfish became established predators on the reefs (Albins and Hixon 2008).

This study provides a tangible example of the detrimental effect elevated CO_2 levels can have on the behaviors of certain fish. Conditions of ocean acidification have been observed to affect many different forms of marine life, from corals to multiple different species of fish. The increased acidity of ocean water and changes in carbonate chemistry hinder the ability of organisms to calcify, affecting corals and benthic invertebrates that produce calcareous skeletal structures (Fabry et al. 2008). The entire functionality of reef ecosystems can be disturbed by ocean acidification. One study found that elevated CO_2 levels affected a pair of damselfish species, in that these conditions caused a reversal of competitive dominance for habitat, especially in degraded ecosystems (McCormick et al. 2013). Reviews of scientific literature on ocean acidification have made the grave prediction that the unraveling of complex reef system structure on many different trophic levels is driving them to a tipping point for functional collapse (Hoegh-Guldberg et al. 2007).

Today, approximately 30% of total human emissions of CO_2 to the atmosphere are accumulating in the ocean (Rhein et al. 2013). This study explored the mounting issues of climate change and ocean acidification, with far-reaching implications for the future of the environment. If anthropogenic CO_2 emissions continue at their current rate, ocean CO_2 levels are predicted to reach an extraordinary 850 μatm, or a pH drop of 0.2, by the end of the century (Rhein et al. 2013). The observed phenomenon of increased acidity disrupting fish anti-predator response could result in dramatic reductions in population size and possible extinction of species as a whole, leading to radical changes in ocean ecosystems on a global scale. Changes in antipredator responses can have effects throughout ecosystems, influencing population dynamics, interspecific interactions, and mechanisms of species coexistence (Miner et al. 2005). By examining the effects of ocean acidification on marine organism behavior and survival, substantial conclusions may be reached about the future of the world's oceans, with a harsh new reality about the need for conservation made increasingly evident.

Acknowledgements I would like to thank my advisor, Dr. Patrick Lyons, for his unrivaled expertise in goby behavior and his consistent guidance. I would also like to thank my advisor Lucien Untersteggaber, M. Sc., and my research partner Taylor Robinson. Special thanks to Sean O'Neill for helping me catch most of the test subjects. A big thanks to all the staff and students at CIEE Bonaire for their advice and support!

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Physis (Spring 2014) 15:36-44

REPORT

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Does nutrient pollution affect the prevalence of dark spots disease in corals on Bonaire, Dutch Caribbean?

Abstract Environmental changes and deterioration have increased coral disease outbreaks, creating a Caribbean hot spot of high disease prevalence and virulence. Dark spots disease (DSD) has an unknown causative agent, although it is suspected to be a result of a biotic pathogen. Variation with anthropogenic stressors and DSD has been limited in research; therefore, the purpose of this study was to determine if a correlation existed between DSD prevalence and nutrient enrichment, in the form of nitrogen concentration. It was hypothesized that DSD prevalence and nitrogen concentration would be highest at shallower depths and that there would be a positive correlation between DSD prevalence and nitrogen concentration. In Kralendijk, Bonaire, sites were surveyed for DSD to calculate the prevalence and water samples were collected to determine the concentration of nitrogen. The results indicated no significant effect of depth and site on DSD prevalence as well as no significant effect of depth on nitrogen concentration. There was a significant effect of site on nutrient concentration as indicated by the significantly higher nitrogen concentration. The pooled data illustrated a weak positive relationship and correlation between DSD prevalence and nitrogen concentration with insignificant results, but one site illustrated a moderately strong positive relationship and correlation with statistical significance. The significant results at that site suggest some correlation between DSD prevalence and nitrogen concentration which requires further investigation *ex situ* to establish a stronger correlation or possible causation.

Keywords Dark spots disease • Nutrient Enrichment • Bonaire

Introduction

Coral reefs are in decline due to anthropogenic effects including sedimentation, nutrient loading, overfishing, and climate change (Rogers 1990; Szmant 2002; Hughes et al. 2003). The environmental consequences that result from these human-induced effects have degraded the reef ecosystem, resulting in a 27% loss of reefs with another 16% at risk (Sutherland et al. 2004). Sedimentation, nutrient pollution, overfishing, and climate change cause adverse effects contributing to a decrease in healthy coral individuals. Sedimentation often smothers coral, reducing light and photosynthetic capabilities, resulting in less energy output (Rogers 1990). Nutrient loading and overfishing, especially of herbivorous grazers, increases macroalgae cover, which outcompete coral for space and light, through eutrophication (Szmant 2002). Lastly, climate change has a varying effect on corals. Ocean acidification degrades the calcium carbonate skeleton of corals, temperatures increase to range outside of habitable conditions and the greater frequency of disastrous storms destroy the structural framework of the reef. (Hughes et al. 2003).

In the Caribbean, this deterioration and changing of the environment has caused an increase of disease outbreaks, or epizootic events, resulting in a hot spot with high prevalence and virulence (Weil 2001; Porter 2011). These epizootic events are a cause for concern because they can be a result of a single anthropogenic factor (e.g. nutrient pollution or sedimentation) or a cause of synergistic combination of the aforementioned sources. Diseases are a source of concern because they can be particularly harmful, especially if they are species-specific such as with the *Diadema* die-off in 1983 or if they affect vital species, such as reef-building corals (Hughes 1994).

Epizootic events have been increasing due to environmental influences that either decrease the immunity of the coral, increase the virulence of the disease, or create a synergistic effect. Corals have a simplistic immune response, only relying on mucus production and scattered phagocytic amoebocytes (Sutherland et al. 2004; Mydlarz et al. 2006). The virulence of the disease is affected by enhancement of the biotic pathogen by abiotic conditions, such as temperature, pH, and dissolved oxygen levels (Sutherland et al. 2004). Bruno et al. (2003) studied nutrient enrichment associated with diseases such as aspergillosis and yellow band disease to confirm that the virulence of the pathogen increased in correlation with greater nutrient levels. Due to the biotic nature of aspergillosis and yellow band disease, this study leads to the hypothesis that several biotic pathogens are affected by nutrient enrichment. For DSD, a correlation between prevalence and nutrient enrichment has not been established; however, it has proven to correlate with several other abiotic conditions (Sutherland et al. 2004).

Dark spots disease (DSD), a prevalent coral disease in the Caribbean, has an unknown causative agent, although it is suggested to be biotic in origin. Dark spots disease is characterized by irregular spots of purple to brown coloration and can lead to tissue death and depression of the colony (Sutherland et al. 2004). The disease spreads in a clumped distribution, which may be a result of the spatial distribution of the susceptible corals or due to a pathogen transmission (Gil-Agudelo and Garzon-Ferreira 2001). Important reef-building corals, such as *Siderastrea siderea*, *Orbicella annularis*, *Orbicella faveolata*, and *Stephanocoenia intersepta* are often affected by DSD. A study on Bonaire done by Weil (2001) quantified average disease incidence for major reef-building corals and found that *S. siderea* had 16.5%, *O. annularis* had 12.55%, *O. faveolata* had 9.05%, *S. intersepta* had 5.61%. Dark spots disease prevalence has reached alarming levels with *S. siderea* at 53% as of 1997-1998 in the Caribbean; therefore, the mechanisms behind DSD prevalence need to be further examined (Cervino et al. 2001). It has already been determined that DSD is influenced by high temperatures and shallow depths due to prevalence of infected species in less than six meters of water (Sutherland et al. 2004), however no correlation has been established with anthropogenic influences.

This study investigated the correlation between DSD and nutrient enrichment on *O. annularis*, *O. faveolata*, *S. intersepta* and *S. siderea*. Previous research has indicated a correlation between depth and temperature on the prevalence of DSD, in which higher temperatures and lower depths create favorable conditions for greater occurrences (Sutherland et al. 2004). Dark spots disease is a prevalent coral disease in Bonaire (Cervino et al. 2001) and causes deterioration of the reef ecosystem; therefore protection of the reef is of high importance. The subsequent hypotheses were tested:

H_1: Dark spots disease will have a higher prevalence in shallower depths

H_2: Nitrogen concentrations will be higher in shallower depths

H_3: Positive correlation will exist between DSD prevalence and nitrogen concentration

DSD has shown higher distribution patterns in shallower depths due to the high abundance of susceptible corals (Gil-Agudelo and Garzon-Ferreira 2001) and is suggested to be biotic in origin, therefore it may uptake nitrogen from the environment, increasing its virulence (Bruno et al. 2003). Furthermore, nutrient concentrations are greater along the shore and become increasingly diluted as distance increases (Bolton-Richie 2006).

Materials and methods

Study sites

Three sites along the west coast of Kralendijk, Bonaire were surveyed for DSD prevalence and nitrogen concentrations (Fig. 1). Bonaire is an island in the Southern Caribbean, approximately 235 km northwest of Caracas, Venezuela. The study sites, Something Special (12°09'41.8"N 68°17'00.8"W), Yellow Submarine (12°09'36.5"N 68°16'55.2"W), and Kas di Arte (12°09'21.4"N 68°16'45.3"W), were chosen based on reports of varying nutrient concentrations (Wieggers 2007).

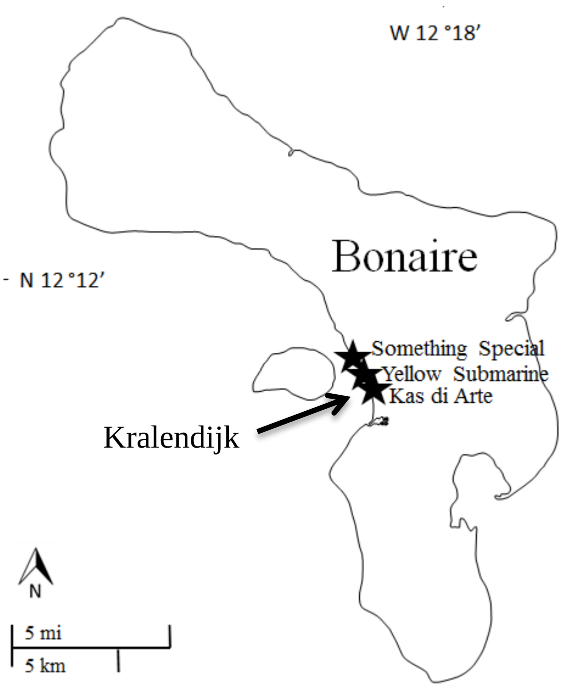

Fig. 1 Map of Study Sites in Kralendijk, Bonaire

The fringing reef has a sand patch from shore to the reef crest that slopes down at approximately six meters. Temperatures and currents were fairly constant from day to day. Something Special is the northernmost study site and lies just south of a marina. Yellow Submarine is just south of Something Special and is often a docking site for fishing and tourist boats. Kas di Arte is the southernmost study site, closest to the main hub of Kralendijk, and lies near a drainage ditch that occasionally empties into the marine system. Based on the varying characteristics (e.g. boat docking, marina traffic, drainage), these sites had the potential to demonstrate varying amounts of nutrient concentrations and disease prevalence.

Coral cover data collection

Data on coral cover was collected using video analysis in order to determine the percentage of live coral at 8 m, 11 m, and 14 m. At each site, three 20-m transects were laid using SCUBA at each of the depths. A Sony Handycam HDR-SR7 video camera with underwater housing was used to record the substrate at 50 cm above the substrate estimated using a metal wand. Videos were assessed for coral cover using Coral Point Count 4.1 (Kohler and Gill 2006). Fifteen randomly selected points were analyzed and sorted into categories of live coral, recently dead coral, old dead coral, sand/rubble, other, or unknown. Data was compared on disease prevalence based on different percentages of live coral at depths and sites.

Dark spots disease data collection

Data on the prevalence and percent cover of DSD was collected using transects at the three sites using SCUBA. At each site, a 15x2 m belt transect was randomly laid at the depths of 8 m, 11 m, and 14 m and headed north parallel to shore. A 1-m T-Bar was used to estimate the two meter belt where the data was collected. *Orbicella annularis, O. faveolata, S. intersepta,* and *S. siderea* colonies that were greater than 5 cm were counted to determine total population of the area. Any colony with DSD was recorded along with dimensions and percent cover. The data collection was repeated a second time at each depth subsequently, but with a southward transect. The methods are based on a study of disease prevalence along the Great Barrier Reef conducted by Lamb and Willis (2010), in which 15x2 m belt transects were laid in order to identify and classify the health of coral colonies. The research methods of this study differed by the limited coral species examined and the recording of only DSD data.

Nutrient data collection

Water samples were taken along the same 15-m transect described above with three water samples per depth at each site. Water samples were taken at the beginning and end of the first transect, and at the end of the second transect during DSD data collection. The samples were collected using an inversion method. Prior to collection, the bottles were rinsed using 10% HCL and filled with deionized water. The nine 100-mL bottles were placed in a mesh bag for easy transportation and were taken to depth. Each bottle was inverted to release water, filled with air from an octopus regulator, and corrected again to fill with seawater. The process was repeated to ensure that the entire sample was seawater. The samples were taken back to the lab and placed in a refrigerator until analyzed for nitrogen (nitrate) concentrations using a Turner Trilogy fluorometer (Strickland and Parsons 1972). Samples were processed using LaMotte Nitrate Test Kit Protocol and absorbance values were taken with the fluorometer. The nitrogen concentration was determined using a standard calibration curve ($y=3.6913x + 0.005$, $R^2=0.9968$) with a minimum nitrogen detection level at 0.03 µM.

Statistical analysis

Video data was analyzed using Coral Point Count to determine relative percentages of each category to compare between sites and depths. Interactions between depth and site on nitrogen concentration and disease prevalence were analyzed using two 2-factor ANOVAs. The ANOVAs compared the effects of site and depth to determine any significant effects on disease prevalence or nitrogen concentrations. A multiple comparisons test was used to determine significance between treatments tested at $\alpha=0.05$. The relationship between DSD prevalence and nitrogen concentration was analyzed using linear regression and correlation to determine the direction and strength of relationship.

Results

Coral coverage

To assess the differences in live coral cover at the three depths, a ratio of percentage of live coral cover was determined. Live coral cover was four times greater at 14m and three and half times greater at 11m than 8m (4.04:3.55:1). Furthermore, Yellow Sub and Something Special had approximately twice as much live coral as compared to Kas di Arte (2.28:2.05:1).

Effects of depth and site on disease prevalence and nitrogen concentration

Trends in average nitrogen concentrations between site and depth varied between trends with lower concentration at 11m or higher concentration at 11 m between sites (Fig. 2). Trends in average dark spots disease prevalence between site and depth varied in a similar pattern to nitrogen concentration for the three sites (Fig. 3).

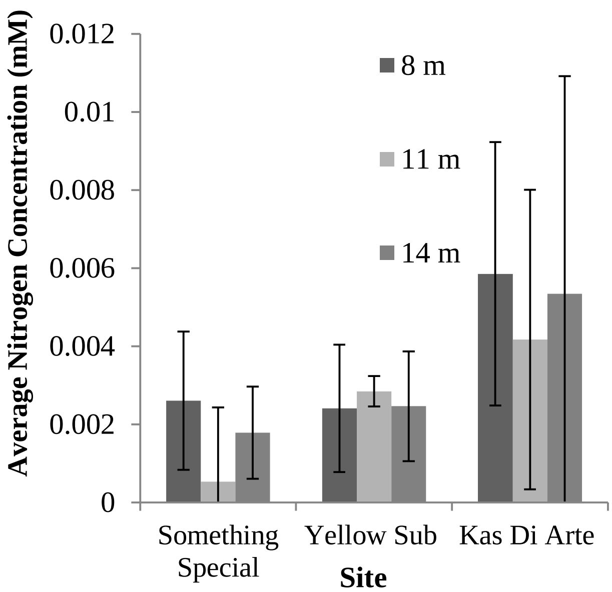

Fig. 2 Average nitrogen concentration in mM (± SD) at each depth interval per site

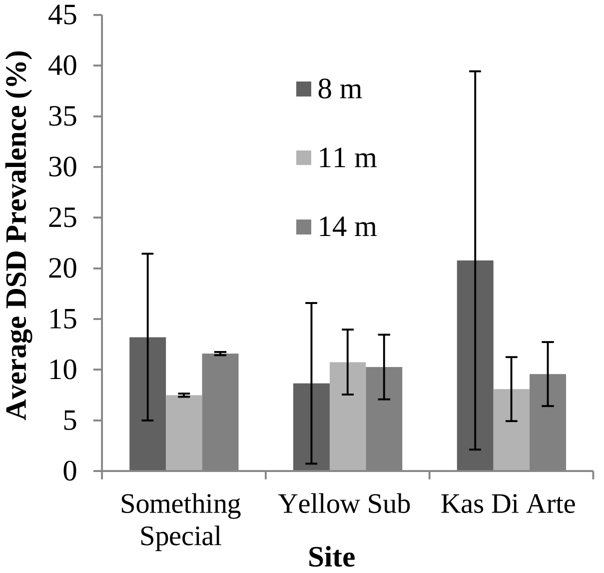

Fig. 3 Average DSD prevalence (± SD) at each depth interval at per site

A significant effect of site on nitrogen concentrations (Table 1a) was determined. Statistically significant variation on nitrogen concentration between Yellow Sub and Kas di Arte and Something Special and Kas di Arte was determined through a multiple comparisons test (Table 1b). No significant effect was found for depth on nitrogen concentration as well for site and depth on average DSD prevalence (Table 2).

Relationship between disease prevalence and nitrogen concentration

Linear regression and correlation determined the strength and direction of the relationship between DSD prevalence and nitrogen concentration. A weak positive relationship and a weak correlation between nutrient concentration and disease prevalence were indicated by the pooled data which were statistically insignificant (Fig. 4a). Yellow Sub data indicated a weak relationship similar to the very weak relationship of Kas di Arte (Fig. 4b & d). The correlations of Yellow Sub and Kas di Arte were also weak, although there is a stronger correlation at Yellow Sub. The results for both of the sites were not statistically significant. The data at Something Special indicated a moderately strong positive relationship with a strong correlation with statistical significance (Fig. 4b)..

Discussion

The purpose of this study was to determine if a relationship existed between nitrogen concentration and DSD prevalence. It was found that a weak insignificant relationship existed for all pooled data, whereas a significant moderately strong relationship existed at Something Special. It was hypothesized that DSD prevalence and nitrogen concentrations would be higher at shallower depths. It was found that both nitrogen concentration and DSD prevalence were not affected by depth.

Table 1 a.Two-way ANOVA testing the effects of depth (14, 11, 8 m) and site (Yellow Submarine(YS), Something Special (SS), Kas di Arte (KDA)) on the nitrogen concentration (mM).b. Multiple Comparisons testing the significance within ANOVA (α=0.05)

a.	Source of Variation	SS	df	MS	F	P-value
	Site	0.0001	2	5E-05	6.744	0.004
	Depth	3.01E-06	2	1.5E-06	0.202	0.817
	Site and Depth	5.02E-06	4	1.26E-06	0.169	0.952
	Within	0.0002	27	7.42E-06		
	Total	0.000308	35			

b.	Site	Mean Diff.	Critical Diff.	P-Value
	YS, SS	-0.001	2.073	0.090
	YS, KDA	0.002	2.073	0.051
	SS, KDA	0.003	2.073	0.007

Table 2 Two-way ANOVA testing the effects of depth (14, 11, 8 m) and site (Yellow Submarine, Something Special, Kas di Arte) on the dark spots disease prevalence

Source of Variation	SS	df	MS	F	P-value
Site	53.936	2	26.968	0.489	0.618
Depth	185.513	2	92.756	1.682	0.204
Site and Depth	278.400	4	69.600	1.262	0.308
Within	1488.541	27	55.131		
Total	2006.393	35			

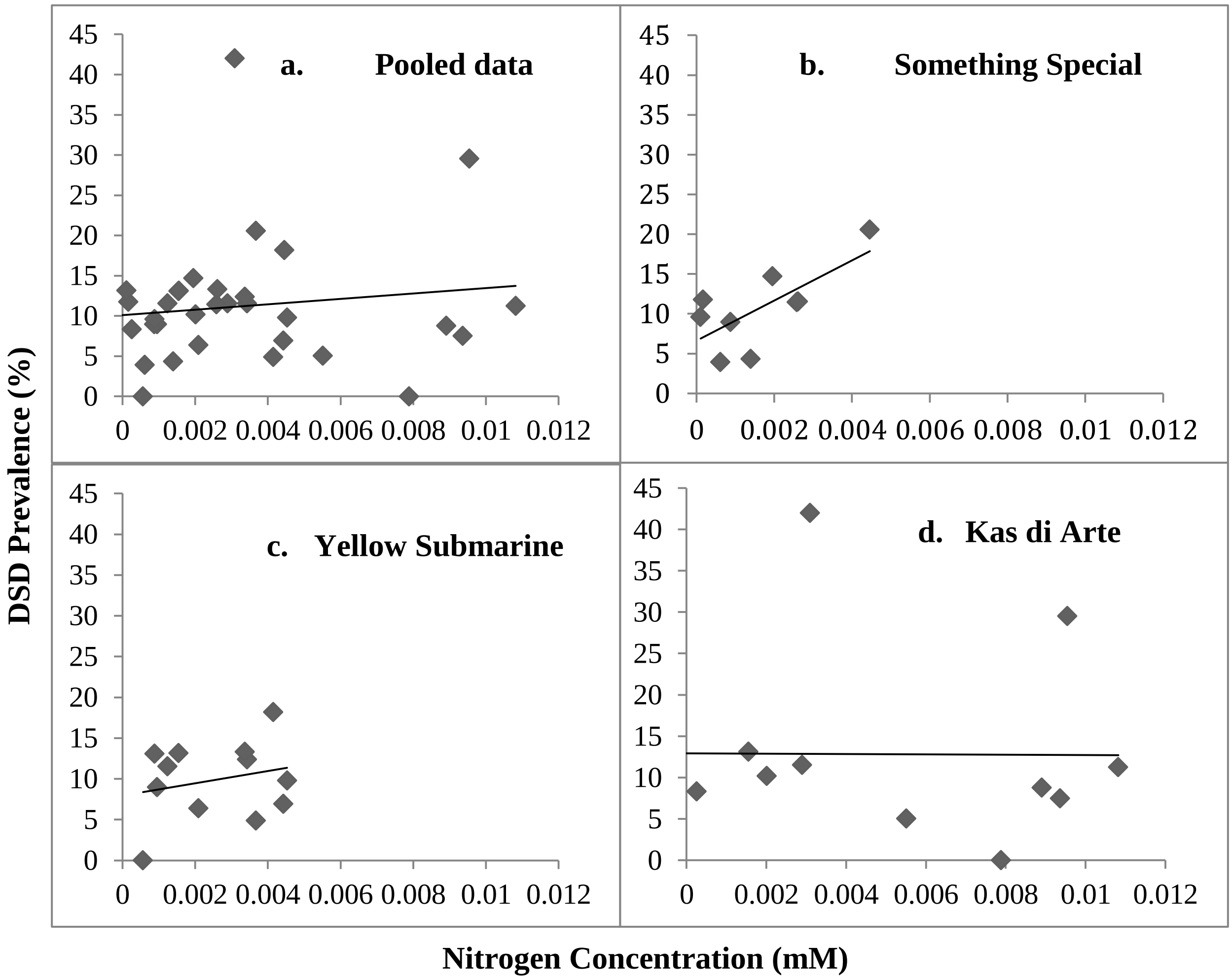

Fig. 5 Linear regression comparing nitrogen concentration (mM) and DSD prevalence (%) for a. Pooled Data of all sites (n=33, p=0.4794,r=0.1275), b. Something Special (n=9, p= 0.0343, r= 0.7039), c. Yellow Submarine (n=12, p=0.4582, r=0.2370), and d. Kas di Arte (n=12, p=0.9835, r=-0.0067)

The results indicate significantly higher nitrogen concentrations at Kas di Arte as compared to Yellow Sub and Something Special. Additionally, there was high variation in nitrogen concentration at Kas di Arte which could be a result of the drainage pit. The pit may have overflowed and leaked into the system during water sampling as inferred by rain patterns. Water collections for nutrient analysis by chance occurred within 72 hrs of the last rain fall which may have increased drainage and resulted in elevated nitrogen concentrations.

There was no significant effect for nitrogen concentration between Yellow Sub and Something Special, most likely a cause of their proximity (0.146 km). These results between Yellow Sub and Something Special are consistent with Wieggers (2007) report on nutrient enrichment in Bonaire, in which he stated that most of the tested sites did not have statistically different results. The results with Kas di Arte are not consistent with Wieggers (2007) results in which Kas di Arte had statistically different nitrogen concentrations.

The interaction between nitrogen concentration and depth resulted in no significant effect. These results are inconsistent with the findings of Bolton-Richie (2006) which found that total nitrogen concentrations were greater at distance of 20 m from shore as compared to distances at 50 m, 100 m, and 500 m. The difference between this study and previous studies results from the closeness of the transects that were separated only by approximately 3 m, potentially disrupting the ability of nitrogen to dissipate.

The results indicate no significant effect of site or depth on average DSD prevalence. The lack of effect of depth on DSD prevalence is due to great variation between depths at Yellow Sub and Kas di Arte, even though Something Special had little variation at 11 m and 14 m. Dark spots disease prevalence differed greatly based on transect location, thereby not illustrating a clear pattern for depth and prevalence. Although there was higher live coral cover at deeper depths, the variation in the prevalence caused no significant effect. Between 11 and 14 m, there were very similar percentages for live coral cover and spatial distribution of susceptible corals, which is a possible driver of the distribution of DSD (Gil-Agudelo and Garzón-Ferreira 2001); therefore, there is little difference between those depths. Eight meters, the transect closest to shore, had the greatest variation in DSD prevalence. This could be a cause of the effects of human impacts (e.g. pollutants, sedimentation) that could reduce the immunity of corals (Rogers 1990, Martin et al. 2010;), thus causing high prevalence while the low percentage of live coral cover could have caused low prevalence. The low live coral cover at 8 m could be a result of the death of susceptible corals by DSD or a result of the environmental conditions that do not favor coral at that depth.

The lack of effect of site on DSD prevalence is a result of the similarity of spatial distribution of corals between sites and the high variation of prevalence. Each of the sites had similar composition of corals at each depth; therefore, there were no apparent differences in the reefs. Variation caused no significant effect between the sites. The greatest variation was seen at Kas di Arte with lesser degrees of variation at Yellow Sub and Something Special. The cause of the variation was the clumped distribution of DSD (Gil-Agudelo and Garzón-Ferreira 2001). Depending on where the transect was laid, there may be high prevalence or low prevalence as a result of the distribution. For instance, the clumped distribution caused one transect at Kas di Arte to have a very high prevalence (42%) whereas another had 0% prevalence.

Linear regression and correlation values indicated an overall weak relationship for the pooled data with the individual sites ranging from very weak to moderately strong relationships. Kas di Arte had a very weak relationship between nitrogen concentration and DSD prevalence, which implies that nitrogen concentration does not affect DSD prevalence. Something Special had a moderately strong relationship and a strong correlation value, implying that nitrogen concentration and DSD prevalence are correlated and nitrogen concentration may affect DSD prevalence. As previously stated, there was large variation in both variables which may have shifted the pooled data linear regression, especially with the very weak relationship at Kas di Arte. Based on the results, the hypothesis that there is a positive correlation between nitrogen concentration and DSD prevalence was not supported because of the lack of a strong relationship in pooled data and statistically significant results. The relationship illustrated by the linear regression at Something Special, though, implies correlation, which could be an indicator of causation, which cannot be proven by this study. Nitrogen enrichment could cause greater prevalence of DSD because, if it is caused by a nitrogen-limited biotic pathogen, the excess nitrogen from the environment could be taken up, thus increasing its virulence and leading to a greater prevalence (Sutherland et al. 2004; Bruno et al 2003). The limitations of the study result from the *in situ* observational design in which a myriad of variables could not be controlled. It is possible that other conditions (e.g. temperature, sedimentation, algal overgrowth) could have decreased coral immunity or increased virulence, but the data still implies some sort of correlation. It is possible that synergistic effects occur between nutrient enrichment and other environmental influences.

The importance of the study lies with conservation of coral reefs on which human impacts are causing considerable damage. Green and Bruckner (2000) illustrated that in the Caribbean, 97% of study sites in which disease was found had medium to high human impacts. In Bonaire, human impacts are expected to rise with increases in both tourist and resident populations and nutrient production (van Kekem et al. 2006). These impacts will ultimately influence the reef and may cause rises in coral disease. Since 1965, total coral disease has been exponentially increasing (Sutherland et al. 2004) and in Bonaire, it has been determined that 53% of *Siderastrea siderea* were infected as of 1997-1998 (Cervino et al. 2001); therefore, the total percentage is now projected to be higher. In order to conserve the reef environment, it is important to understand the effects of anthropogenic stressors. Further studies need to be pursued to gain a better understanding of DSD. To begin, the causative agent needs to be identified to determine if it is a biotic pathogen or the result of abiotic conditions. With the knowledge of what is causing the disease, better management strategies can be taken to limit its prevalence. Furthermore, an *ex situ* nutrient enrichment experiment, similar to Bruno et al.'s (2003) study on aspergillosis and yellow band disease, should be applied to DSD in order to account for all variables and determine causation.

Acknowledgements I would like to thank the following people for their role in the production of this paper: Dr. Patrick Lyons and Lucien Untersteggaber, M.Sc. for invaluable advising and assistance in writing and data collection, Sarah Bruemmer for her commitment to helping collect data on countless dives, Nicole Kleinas and Brooke Davis for their assistance in data collection, and Molly Gleason, M.Sc. for her assistance in the laboratory with all of the water samples. Funding for this project was partially provided by the State University of New York College of Environmental Science and Forestry Honors Program.

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Physis (Spring 2014) 15:45-51

REPORT

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Bioaccumulation of run-off pollutants: An evaluation of marine sponges as bioindicators

Abstract Runoff and sewage discharge present serious consequences if left unchecked in coral reef ecosystems. Eutrophication and the introduction of harmful chemicals to the environment can lead to the destruction of coral reefs. Phosphates and polycyclic aromatic hydrocarbons (PAHs) are well known components of runoff that are detrimental to the reef ecosystem. As such, the ability to monitor the concentration and spatial distribution of these chemicals is of great interest. These pollutants may be detected using bioindicators. Bioindicators are organisms that can be used to monitor the health of an ecosystem. In this study, sponges were assessed as bioindicators for phosphate and PAHs in coral reef environments. *Holopsamma helwigi, Ircinia strobilina*, and *Pseudoceratina crassa* are common Leuconoid sponges that were tested for pollutant contaminations using fluorometric analysis. The sponges were collected along a transect spanning the northern coast of Kralendijk, Bonaire. A known runoff site at 'Kas di Arte' (12° 9' 19.9362" N, 68° 16' 44.5434" W) was selected as the starting point for the transect. The sponges bioaccumulated both phosphates and PAH compounds. Concentrations of the pollutants were not found to decrease as the distance from the runoff site increased suggesting that sponges assessed here are not capable of showing short-term variation in spatial trends of pollutant concentration. In order to better understand how the sponges accumulate pollutants, a thorough exploration of the kinetics of pollution bioaccumulation should be pursued in future studies.

Keywords Bioaccumulation • Marine sponges • Pollution

Introduction

Runoff and sewage discharge give rise to serious consequences if left unchecked in coral reef ecosystems. It has long been known that corals are extremely susceptible to minor changes in chemical concentrations in the water column (Bell et al. 1989). Pollutants can lead to a decrease in reef biodiversity and changes in species composition (Pastorok and Bilyard 1985; McKenna et al. 2001). Rather than attempting to monitor every component of runoff pollutants, time and money are better-spent examining key chemical constituents. Variations in phosphate on the micromolar level can create devastating eutrophication effects in coral reef ecosystems (Bell et al. 1989). While some studies have found that increased phosphate levels promote coral growth, an influx of phosphate may allow macroalgae to outcompete corals and dominate the reef ecosystem (Wheeler and Bjornsater 1992; McClanahan et al. 2007; Dunn et al. 2012). Phosphate pollution can also stimulate the growth of phytoplankton, reducing the availability of light to corals (Walker and Ormond 1982). Polycyclic aromatic hydrocarbons (PAHs) are another component of runoff pollutants that can wreak havoc when introduced to marine ecosystems. PAHs are typically produced through the burning of fossil fuels or by direct introduction of crude or refined oil into the environment. PAHs are known to cause certain cancers and birth defects in marine fauna (Kelly et al. 2009). The deleterious effects of PAH compounds and harmful effects caused by runoff stress the need for a way to monitor concentrations in the ecosystem.

Bioindicators are an extremely useful tool for monitoring the presence and spread of runoff pollutants. Bivalves, mainly due to the bioaccumulation of pollutants in their tissue as a result of filter feeding, have been extensively studied as successful bioindicators in marine and aquatic ecosystems (Park and Polprasert 2008; Manganaro et al. 2009). Moreover, the filtration pressure of bivalves has been found to greatly mediate eutrophication (Manganaro et al. 2009). In Bonaire, Dutch Caribbean, sponges could be used as bioindicators since bivalves are not as prevalent. Sponges meet many of the ideal criteria for a potential bioindicator of runoff pollutants: they are highly diverse, abundant, and have the capability of altering the water column by filtering particles from dissolved to particulate sizes (Reiswig 1971; Diaz and Rützler 2001).

Sponges are already known to act as bioindicators for metal pollution (Rao et al. 2008; Genta-Jouve 2012). Metal contaminant concentration in sponges has a clear decreasing gradient as distance from the pollution source increases (Perez et al. 2005). The ability to detect a spatial gradient makes for a very strong bioindicator. This study focused on phosphates and PAHs, as they are well-known components of anthropogenic pollutants. Some sponges have been found to bioaccumulate PAH compounds at levels similar to those found in their immediate environment (Batista et al. 2013). Studies have also found that sponges tend to bioaccumulate PAH compounds of a higher molecular weight than the compounds accumulated by bivalves (Baussant et al. 2001; Batista et al. 2013).

This study aimed to determine if selected marine sponges in Bonaire have the capability of bioaccumulating phosphate and PAH compounds. Sponges have not been studied as bioindicators in the Dutch Caribbean, though there is a great need for monitoring the runoff and sewage discharge present in the area. The current literature contains sparse information concerning the bioaccumulation of phosphate in marine sponges and the spatial gradient of non-metal bioaccumulated contaminants in relationship to a runoff site. It was hypothesized that sponges around Bonaire are capable of bioaccumulating both phosphates and PAH compounds. Furthermore, it was anticipated that the concentration of the bioaccumulated compounds would decrease as the distance from the runoff site increases.

H_1: Sponges around Bonaire are capable of bioaccumulating both phosphates and PAHs

H_2: The concentration of the bioaccumulated compounds will decrease as the distance from the runoff site increases

Materials and methods

Study sites

The northern coast of Kralendijk (the capital of Bonaire on its west coast) was examined for this study. The site used ran from the small drainage ditch adjacent to ‘Kas di Arte’ (12° 09' 21.4" N 68° 16' 45.3" W) to the Yellow Sub dive site (12° 09' 36.5" N 68° 16' 55.2" W; Fig. 1). A transect was set up between these two sites between 10 m and 15 m depth. The ‘Kas di Arte site is known to have elevated runoff levels (Kekem et al. 2006). A study conducted in the fall of 2013 confirmed the presence of PAHs at the ‘Kas di Arte’ runoff site (Mason 2013).

Sponges for analysis

Leuconoid sponges (Class Demospongiae) have a complex internal structure and the highest tissue density of all sponges, making them ideal candidates for bioaccumulation of pollutants. Three species of Leuconoid sponges were chosen for this study based on their prevalence at the run off sites: *Holopsamma helwigi* (Lumpy Overgrowing sponge), *Ircinia strobilina* (Bumpy Ball sponge), and

Pseudoceratina crassa (Branching Tube sponge).

Sample collection

Sponge samples were collected every 15 m along the ‘Yellow Sub’ – ‘Kas di Arte’ transect between 10 m and 15 m depth. There was a sampling gap between the 225 and 520-meter marks due to time limitations (Fig. 1). A 1-cm^3 piece was collected from each sponge sampled and stored in HCl-washed 100 mL plastic containers. The containers were filled with water from the collection site when the sponge was taken. All sponges under examination in this study were collected within a one-meter radius of the collection point. Samples were labeled based on distance from the runoff site. Immediately after being transferred to the lab, samples were placed in a -20ºC freezer until further analyses were performed.

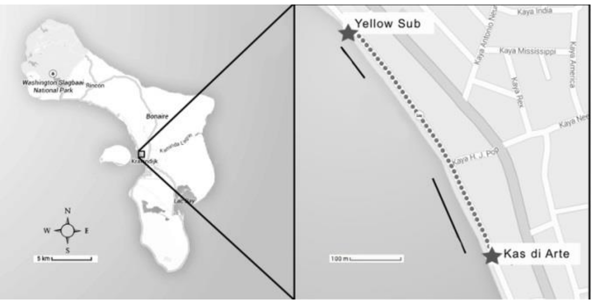

Fig. 1 Site map of Bonaire and the coast of Kralendijk. The coastal transect ran between Yellow Submarine (12°09'36.5"N 68°16'55.2"W) and the runoff site at Kas di Arte (12°09'21.4"N 68°16'45.3"W). The gap between 225 m and 520 m in the coastal transect is shown

Bioaccumulation analysis

Samples were analyzed for inorganic and organic phosphate and PAH compounds. Sponges were ground with a mortar and pestle and analyzed using a Turner Designs Trilogy Fluorometer. The procedure for phosphate analysis followed the protocol of Strickland & Parsons (1972) using the Turner Designs phosphate module. The procedure was modified by filtering the assay through a Whatman GF/F filter before the reagents were added to remove particulate matter that might interfere with fluorometric analyses. PAH compounds often fluoresce at the same wavelengths as crude oils, meaning the crude oil module from Turner Designs could be used to analyze samples. Because PAHs fluoresce naturally, no reagents were added for analysis.

Water quality analysis

To determine if the concentration of compounds in the sponges accurately reflected the concentrations in the water column, water samples were assessed with the same procedures used for bioaccumulation analysis. Ambient water samples were taken every 30 m along the entire coastal transect between 10 m and 15 m depth. All water samples were taken within one day to avoid being skewed by temporal variables. These samples aid in the establishment of a possible spatial gradient for the concentrations of runoff pollutants. The baseline concentration of runoff pollutants in the water was compared to the concentrations in the sponge samples to establish if the sponges were bioaccumulating pollutants.

Data analysis

Bioaccumulation data were analyzed using linear regressions relating distance from runoff site and concentration of compound to determine if the correlation was statistically significant. Concentration of pollutants in sponge samples were compared with the concentration of pollutants in the water samples to establish that bioaccumulation was occurring, rather than simply a measurement of the pollutants within water contained in the sponge.

Results

This study used three species of sponges, *Holopsamma helwigi, Ircinia strobilina,* and *Pseudoceratina crassa*, to explore the possibility of sponges bioaccumulating runoff pollutants in a coastal coral reef environment. The correlation between distance from runoff

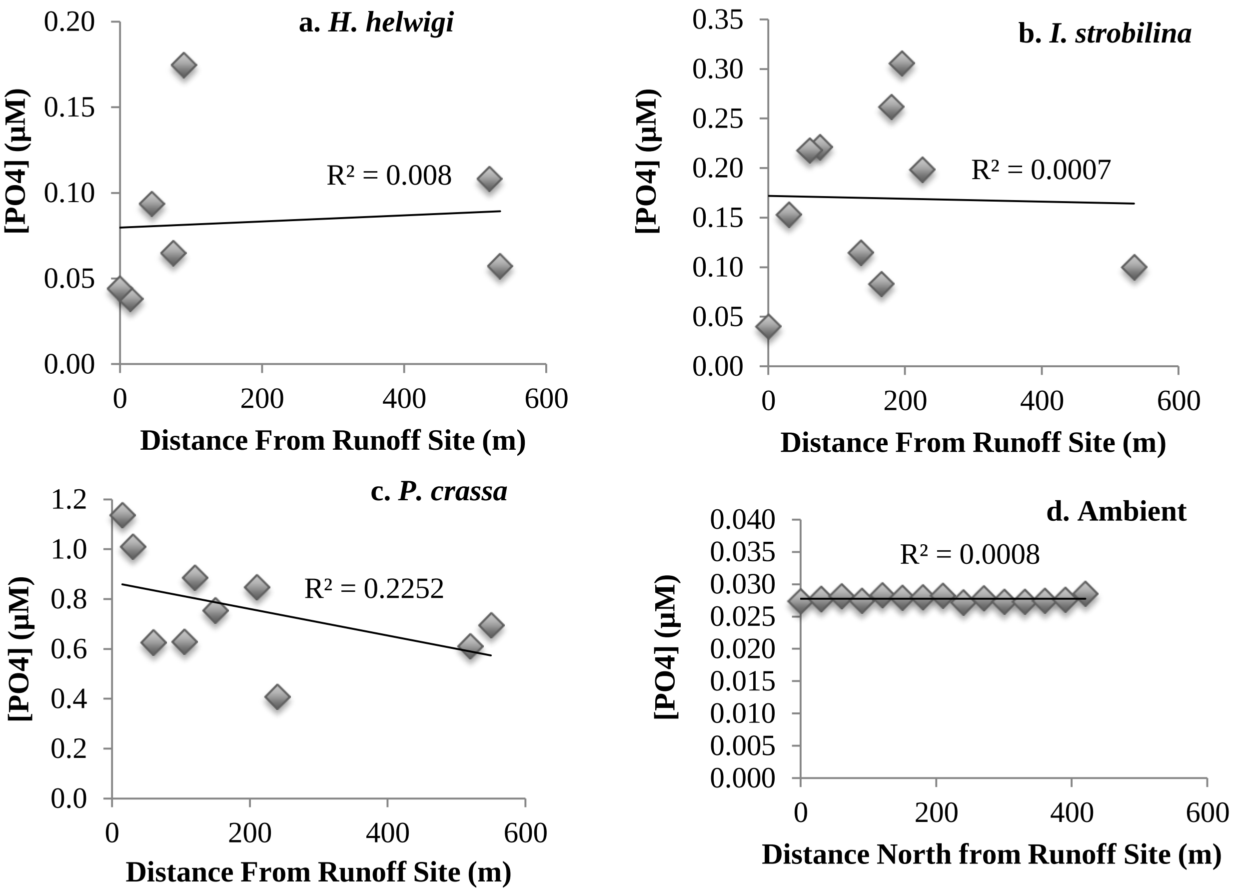

Fig. 2 Correlation plots between the distance from the runoff site at Kas di Arte (12°09'21.4"N 68°16'45.3"W) and $[PO_4]$ (µM) for a. *H. helwigi*, b. *I. strobilina*, c. *P. crassa*, and d. the ambient levels in water samples

site and $[PO_4]$ was found to be very weak for *H. helwigi* and *I. strobilina* (R^2=0.009 and R^2=0.001, respectively) and only slightly stronger for *P. crassa* (R^2=0.225; Fig. 2a-c). Data for the points south of the runoff site were not plotted in order to maintain a linear relationship (as concentration should have theoretically decreased as distance from the runoff site increased in either direction). The correlation between $[PO_4]$ and distance from the runoff site was not found to be statistically significant for *H. helwigi* (p=0.849), *I. strobilina* (p=0.943), or *P. crassa* (p=0.166). Water samples were found to have the same $[PO_4]$ throughout the transect (Fig. 2d). *Holopsamma helwigi* and *I. strobilina* had $[PO_4]$ on the same order of magnitude as the concentration in the water samples (2.6 times that of ambient levels and 6.4 times more, respectively), while *P. crassa* had $[PO_4]$ an order of magnitude greater than ambient levels (28.6 times more; Fig. 4a).

The concentration of crude oil was also examined. Crude oil contains many polycyclic aromatic hydrocarbons (PAHs) and was used as a proxy for direct PAH measurement. *Holopsamma helwigi*, *I. strobili,* and *P. crassa* all had weak correlations between crude oil and distance from runoff site (R^2=0.024, R^2=0.038, R^2=0.047, respectively) that were not found to be statistically significant (p=0.714, p=0.943, p=0.550, respectively; Fig. 3a-c). Water samples were found to have the same concentration throughout the transect (Fig. 3d). Sponge samples had crude oil levels consistently higher than the ambient water levels, with both *H. helwigi* and *I. strobilina* having concentrations of crude oil an order of magnitude greater than the ambient levels (32.3 and 61.6 times more, respectively). *Pseudoceratina crassa* had especially high

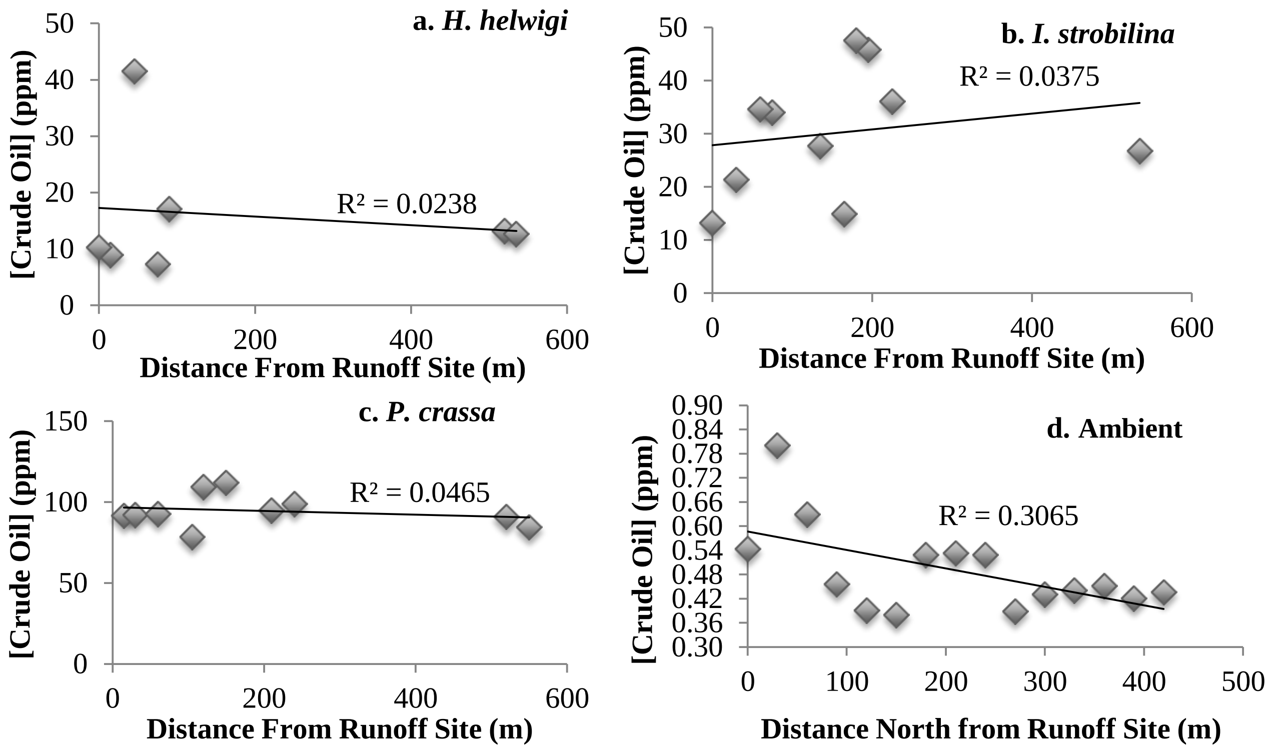

Fig. 3 Correlation plots between the distance from the runoff site at Kas di Arte (12°09'21.4"N 68°16'45.3"W) and [Crude oil] (ppm) for a. *H. helwigi*, b. *I. strobilina*, c. *P. crassa*, and d. the ambient levels in water samples

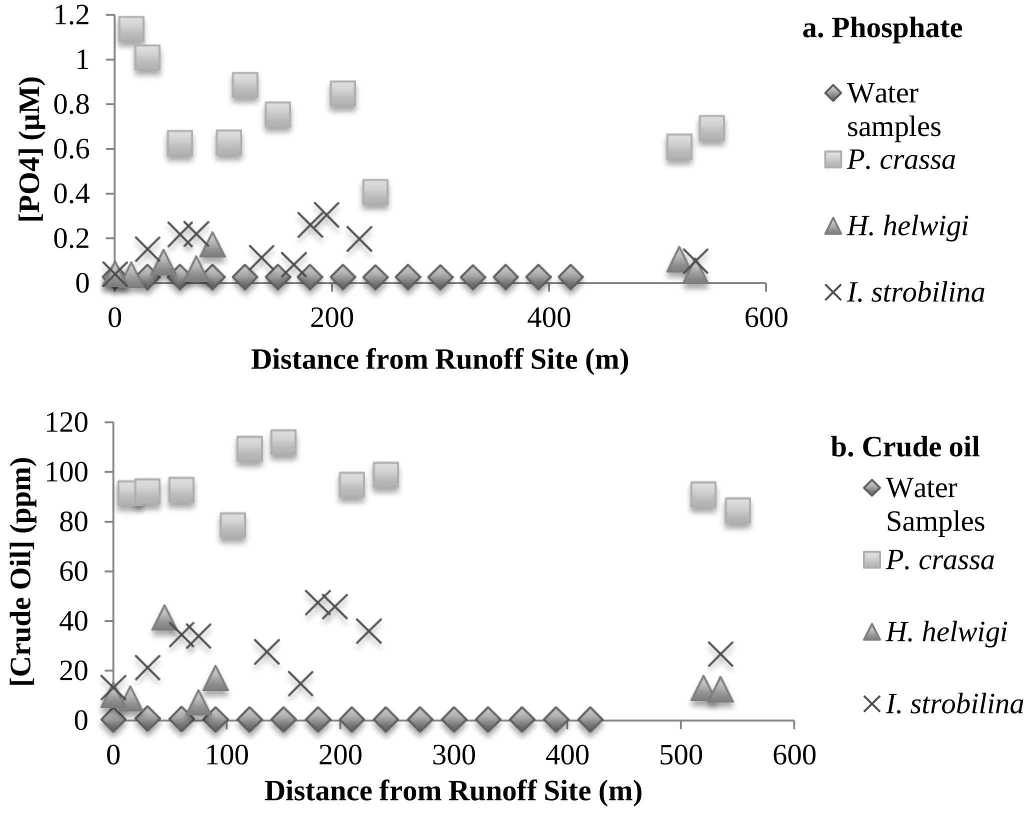

Fig. 4 Comparison of concentrations of runoff pollutants in sponge tissue and ambient levels in environment for a. phosphate andb. crude oil

accumulation, reaching concentrations 192.8 times greater than ambient levels (Fig. 4b). Although there was no spatial variability in crude oil concentrations in sponge samples, there was a significant negative correlation between ambient crude oil concentrations and distance from the runoff site ($n=15$, $r=-0.055$, $F=5.745$, $p<0.05$; Fig. 3d).

Discussion

Holopsamma helwigi, *Ircinia strobilina*, and *Pseudoceratina crassa* are all capable of bioaccumulating both phosphate and crude oil. While *H. helwigi* and *I. strobilina* accumulated both pollutants at levels slightly higher than ambient concentrations, *P. crassa* had concentrations an order of magnitude larger for both pollutants. This difference in bioaccumulation between *P. crassa* and the other two sponges indicates that there may be mechanistic difference in the amount of water each species is able to filter. If *P. crassa* has a higher water intake capability it might allow the sponge to bioaccumulate more pollutants. Furthermore, although all three sponges are Leuconoid sponges with similar internal structure, *P. crassa* is the only sponge that grows vertically, while the other two grow near to the bottom. The difference in morphology may allow *P. crassa* to be more influenced by the efflux of pollutants from the runoff site that may not settle to the bottom. In addition to possible differences in the amount of water filtered and the amount of pollutants that actually reach each type of sponge, there may also be differences in the retention times of the chemicals. Perhaps all three sponges experience the same nutrient load but have different turnover rates of the pollutants. If *P. crassa* retains pollutants for a longer period of time than *H. helwigi* or *I. strobilina,* it would lead to higher concentrations of the pollutants in the sponge. A thorough exploration of the kinetics of pollutant bioaccumulation should be undertaken in future studies to determine the specific manner in which each species handles pollutants.

Although all three sponges were capable of bioaccumulating phosphate and crude oil, it appears that they do not accurately portray the spatial distribution of these pollutants. The concentration of phosphate showed some spatial variation in all three sponge samples, but not in the ambient water (Fig. 2a-d). This is likely because of the possible differences in bioaccumulation kinetics discussed above. The ambient water samples were collected over the duration of three hours on a day with strong current to the north. This snapshot in time was valuable for establishing ambient concentrations of pollutants, but not for examining the actual average spatial gradient for pollutant concentrations. Despite this, the correlations between the sponges and distance can still be considered as indicators of the long-term spatial trends in concentrations. *Holopsamma helwigi*, *I. strobilina,* and *P. crassa* show concentrations of phosphates and crude oil that do not vary significantly over distance. However, in the case of *P. crassa*, if more samples had been collected the correlation may have shown a significant relationship between concentration of phosphate in *P. crassa* tissue and distance from the runoff site.

To further determine if the sponges are bioaccumulating phosphate and crude oils, future research should be conducted using chemical analysis to positively identify the compounds within the sponge tissue. This type of analysis could also be used to identify specific PAHs, rather than the broader range of crude oil compounds that were considered in this study. Furthermore, future studies should look into the possibility of filtering the samples through filters below the 0.7µM nominal pore size. Ideally, nutrient analysis uses filters with a 0.2µM. Additionally, *ex situ* experiments could be run using known inputs of nutrients and analysis over a time period in order to determine the turnover rates of each sponge species. This was not possible within the scope of this study due to time constraints. Because of their bioaccumulation ability, all three sponges should be considered for future monitoring of runoff pollutants.

Acknowledgements I would like to thank Dr. Enrique Arboleda and Colin Howe for advising me throughout my research, CIEE for the opportunity to do research on Bonaire, and everyone at the research station for their support and help. Special thanks to Molly Gleason for endless lab talks, Belle Perez for being a replacement dive buddy and Alice Vejins, dive buddy extraordinaire.

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Physis (Spring 2014) 15:52-57

REPORT

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Can the lionfish, *Pterois* spp., differentiate between colors?

Abstract Since the invasion of red lionfish, *Pterois* spp., in the Western Atlantic and Caribbean, many studies have evaluated the predatory behavior of the species both in the field and in the laboratory. There is still relatively little knowledge regarding the intraspecific behaviors of the species, which could be successfully studied with the use of visual models. However, information is lacking regarding visual ability of the species. One study has confirmed that the lens of the eye of the lionfish eye is adapted to transmit visible light, but further anatomical evidence is required to confirm the use of color vision in the species. Color vision has recently been assessed in reef fish using stimulus-reward methods. Similar methods were repeated with lionfish in this study. Preference tests were performed following a week-long reward-stimulus training regime. The difference between time spent with the "training color" and the "distraction color" was not significant, but the group as a whole spent more time on the correct side than the incorrect side of the aquarium. Although there was no significant data to support color vision in the species, the behaviors of each individual are discussed in detail. The study, however, does not rule out nor confirm color vision in lionfish. It can be concluded that lionfish do not learn to associate colors with food rewards as readily as other reef fish species.

Keywords Lionfish • Color vision • Training

Introduction

Lionfish as an invasive species

Pterois volitans and *Pterois miles* are invasive in the Caribbean and Western Atlantic. The current hypothesis is that they were introduced from the aquarium pet trade (Morris et al. 2008). They are a hardy species, which is exemplified by their invasion success. Lionfish are easily acquired for research in their invasive range, which makes them a practical study species.

Since their invasion, studies have emerged discussing lionfish predatory behavior, both in the field and in the lab (Green et al. 2011; Albins and Lyons 2012). However, studies regarding intraspecific interactions are lacking. Successful control of an invasive species requires a multi-level understanding of the organism's behavior (Holway and Suarez 1999), including intraspecific social behaviors. A thorough understanding of these interactions may be helpful in assessing patterns of spread based on mating behaviors and territoriality (Holway et al. 1998).

Implications of color vision for the use of models

Intraspecific behaviors are readily studied in laboratory conditions with live individuals and the use of visual models (Rowland 1997). Visual models control for the behavior of a conspecific and are therefore helpful in evaluating the behavior of an individual. Methods involving models would be useful in evaluating intraspecific behaviors in lionfish, such as mate preference and aggression. Before behavior can be evaluated with the use of models, it must first be established whether the species in question will respond to models appropriately. Information regarding visual abilities of lionfish is lacking, making it difficult to design a plausible visual model system for the species. The first step to understanding the visual abilities of a species is to determine whether they are able to differentiate colors.

Lionfish hunt during low-light hours (Green et al. 2011), so it is possible that they do not require color vision. The anatomy of the lionfish eye was evaluated by Karpestam et al. (2007), which determined that the species has a multifocal lens that could be representative of color vision, but they did not determine the photoreceptive abilities of the genus. They found that the anatomy of the eye confirms their hunting patterns; their lenses have high light-gathering ability and lower magnification abilities.

Behavioral testing of color vision

Due to the conspicuous coloration of coral reef fish, the visual abilities of many species have been studied. Marshall (2000) used color measurements from the field and mathematical modeling based on depth and distance to determine that the bright colors of many coral reef fish are highly visible to nearby conspecifics, but can also serve as camouflage from predators from afar. Behavioral assessments can also be designed to determine visual abilities, as demonstrated by Sieback et al. (2008). This study used behavioral testing to demonstrate that the damselfish *Pomacentrus amboinensis* can differentiate between blue and yellow stimuli. Many of the damselfish were able to complete reward-based tests by the second trial. However, damselfish are relatively active compared to lionfish, and therefore the training period of this study was extended due to the reluctance of the lionfish to earn the reward.

Demonstrating that lionfish acquire a preference for the color to which they are trained to associate with food would confirm both color vision and associative learning in the species. Confirming color vision in lionfish will open the doors for using models as a method for behavioral testing.

H_1: Lionfish will spend significantly more time on the side of the aquarium with the “training color” than with the “distraction color.”

Materials and methods

Training period

Individuals were obtained via SCUBA and snorkeling in shallow waters surrounding Kralendijk, Bonaire, Dutch Caribbean. Lionfish were housed in individual aquaria. Four lionfish were collected and trained, the smallest of which was 5.90 cm and the largest of which was 8.00 cm. The average size was 6.75 cm. Each day at 17:30 hrs a feeding apparatus was placed in the aquaria. The apparatus consisted of a PVC pipe attached to either an open or close-bottomed clear chamber. Local juvenile fish were placed in the chambers as prey items. The apparatus had a 2 cm band of color above the clear chamber. Each individual was trained to associate one color (“training color”) with the food reward, while a different color (“distraction color”) served as a distraction.

Tubes of both colors (“training color” and “distraction color”) were placed in the tank for each feeding interval. The pray item was released only when the individual began displaying predatory behavior toward the prey in the “training color” tube. Because lionfish blow water at their prey prior to striking (Albins and Lyons 2012), this behavior was used as the predation indicator.

Preference tests

After one week of training, preference tests were performed. The test aquaria were divided length-wise into three sections of 15 cm. Prior to the test, the lionfish were contained in the center section for an acclimation period of 5 min while the feeding apparatuses were placed on opposite sides of the tank. The observer used JWatcher (Version 1.0) to record the time spent on each side of the aquarium over a 10 min testing period. The tests were performed each day at 17:30 hrs, which was consistent with the feeding time during the training interval. The procedure was repeated the following two days, while the alternating which side on which the respective colors were placed. This was done to control for any association between the reward and the physical side of the aquarium.

Statistical analysis

A paired t-test was performed with the individuals to evaluate whether they approached the side of their "training color" first. A paired t-test was performed with the time spent on each side of the test aquarium.

Results

There was not a significant difference between the average time spent on the correct and incorrect side of the aquarium ($t=1.0482$, $df=11$, $p=0.3171$). The group as a whole spent the most time in the center of the aquarium during the 10 min testing interval (Fig. 1). The average time in the center of the aquarium was 367.5 ± 225.8 s, while the time on the correct and incorrect side of the aquarium was 163 ± 224.7 s and 69.5 ± 137.1 s, respectively. However, when divided by individual subject there were three out of four individuals that, on average, spent more time on the correct side of the aquarium than the incorrect side (Fig. 2). The first individual spent the most time in the center of the aquarium (418.3 s), and the least amount of time on the incorrect side (0 s). The second individual spent approximately equal time in each section of the aquarium: correct (198 s), center (208.3 s) and incorrect (194 s). The third individual spent the entire test period in the center of the aquarium (600 s). The fourth individual spent the most time on the correct side of the aquarium (272.7 s), and the least amount of time on the incorrect side (84 s).

The approximate routes of each individual were also analyzed (Fig. 3, Fig. 4, Fig. 5, Fig. 6). Individual 1 spent the majority of the test

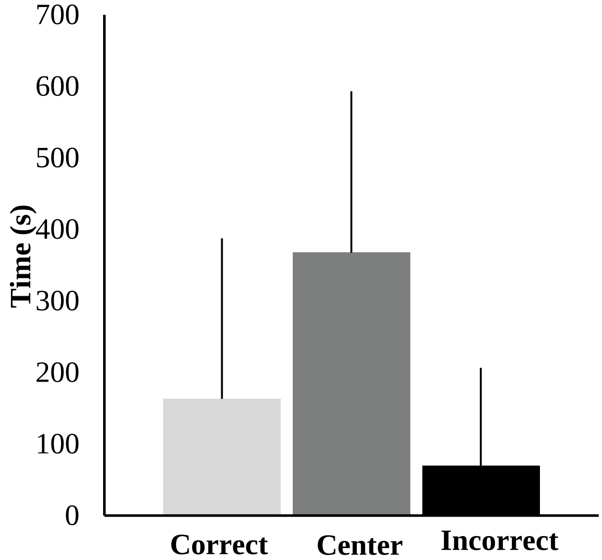


Fig. 1 Average time spent on the correct side, the center and the incorrect side of the aquarium for all individuals. Lionfish spent the most time in the center of the aquarium, and the least time on the incorrectside of the aquarium. Error bars indicate standard deviation

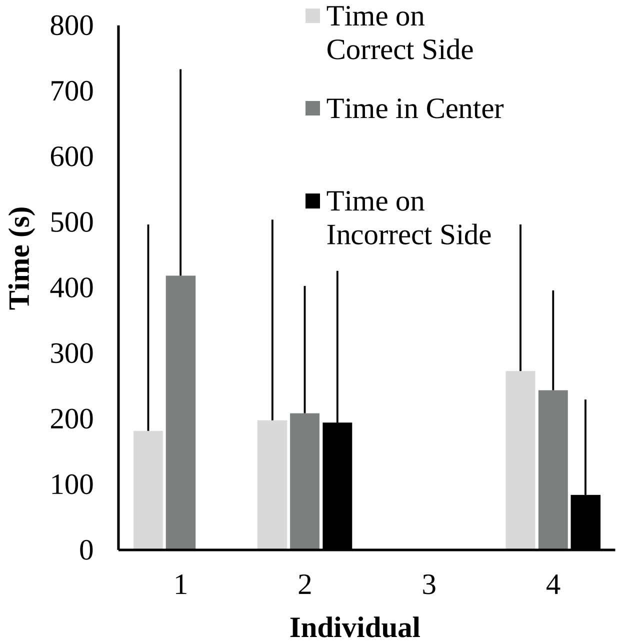
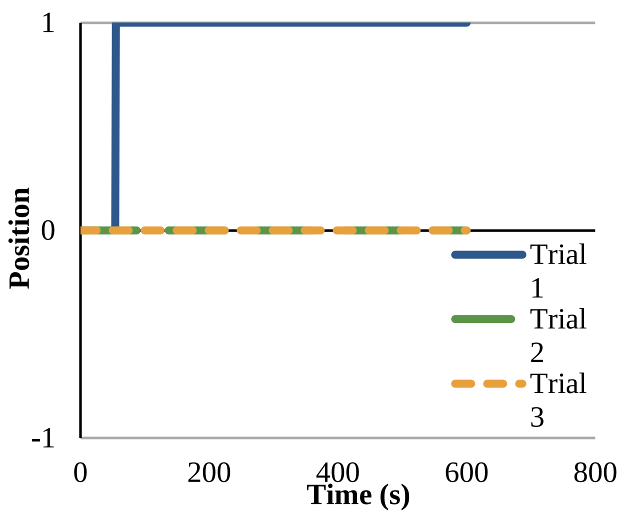


Fig 2. The average time from three trials that each individual spent on the correct, the center and the incorrect side of the aquarium. Error bars represent standard deviation



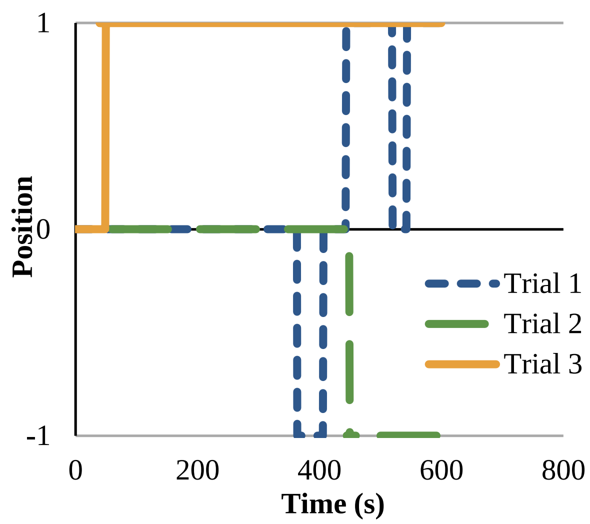
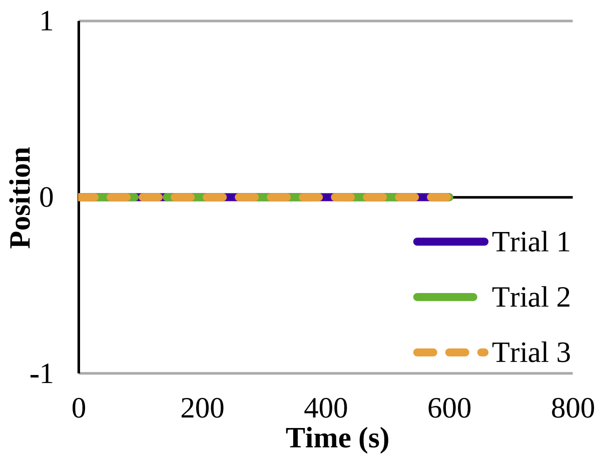


Fig. 3 Approximated route travelled by Individual 1. Position value of "1" indicates the correct side of the aquarium, "0" corresponds to the center, and "-1" represents the incorrect side of the aquarium. All three trials are depicted in different colors

Fig. 4 Approximated route travelled by Individual 2. Position value of "1" indicates the correct side of the aquarium, "0" corresponds to the center, and "-1" represents the incorrect side of the aquarium. All three trials are depicted in different colors



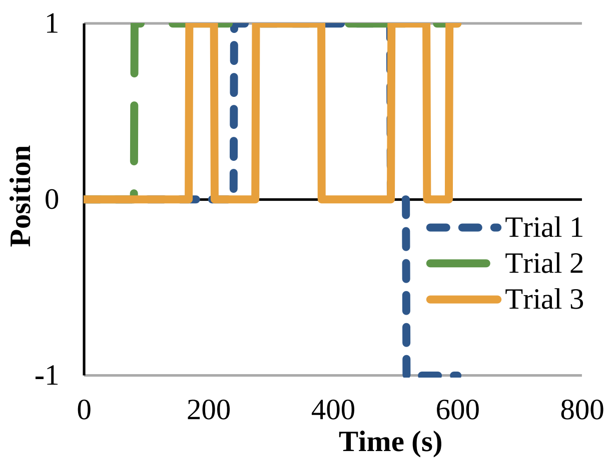


Fig. 5 Approximated route travelled by Individual 3. Position value of "1" indicates the correct side of the aquarium, "0" corresponds to the center, and "-1" represents the incorrect side of the aquarium. All three trials are depicted in different colors

Fig. 6 Approximated route travelled by Individual 4. Position value of "1" indicates the correct side of the aquarium, "0" corresponds to the center, and "-1" represents the incorrect side of the aquarium. All three trials are depicted in different colors

period during the first trial on the correct side of the aquarium after the initial movement at 55 s (Fig. 3). However, during the second and third trial, the individual spent the entire test period in the center of the aquarium. Individual 2 travelled sporadically across trials (Fig. 4). During the first trial, Individual 2 travelled initially to the incorrect side of the aquarium, then travelled to the correct side. Individual 3 remained in the center section of the aquarium during all three trials (Fig. 5). Individual 4 spent the most time on the correct side across trials, but ended the first trial on the incorrect side (Fig. 6). Individual 4 travelled to and remained on the correct side for the duration of the test only during trial 2.

Discussion

Although there was not a significant preference for the trained color within the group of subjects, there was a visible trend among individuals to spend more time on the correct side of the aquarium than the incorrect side. The lack of significance may have been due to a limited sample size, wherein one individual did not respond to the stimuli. It is more advantageous to draw conclusions from the qualitative observations from individual trials rather than the group as a whole.

Individual 1

In the first trial, Individual 1 moved to the correct side of the aquarium and remained on that side (Fig. 3). However, in the following trials, it did not move from the center region. During the preference tests, the prey items were not released when the subjects displayed predatory behavior, as they were during the training period. It is possible that the individual learned that they would not be rewarded for the same behavior and therefore did not act accordingly. This seems unlikely, considering the time period required for the subjects to learn the rewarded behavior initially. It is also possible that the individual's motivation for food decreased because they were fed daily.

Individual 2

The second individual's movement patterns demonstrate a continued learning curve. During the first trial, Individual 2 initially moved to the incorrect side, then switched to the correct side (Fig. 4). This is likely due to the realization that the lionfish was not rewarded upon approaching the incorrect side. During the second trial, the fish only moved to the incorrect side, but did so after remaining in the center region for 450 s. As mentioned previously, this may indicate a lack of motivation due to regular feeding. In the third trial, Individual 2 moved to the correct side and remained there for the duration of the trial. This trial alone demonstrates a preference for the correct stimulus, which may have been consistent had there been continued trials.

Individual 3

The behavior of Individual 3 did not support the hypothesis, as this individual either did not learn the reward-stimulus or was not motivated by the prey. The three trials spent entirely in the center (Fig. 5) did not affect the results of the paired t-test, because the time spent in the center was not included. However, the behavior of the individual did increase the average time spent in the center for the entire group.

Individual 4

The behavior of Individual 4 exemplified continued learning. During the first trial, the individual moved to the correct side but ended the trial on the incorrect side, possibly due to not receiving the reward after more than 200 s (Fig. 6). In the second trial, the individual moved to the correct side and remained there for the duration of the test period. During the third trial, the lionfish moved repeatedly between the center and the correct side, but did not spend any time on the incorrect side. Since this individual only spent time on the incorrect side of the aquarium during the first trial, the trend indicates that they did learn to associate the "training color" with the prey reward.

Conclusions

The results of this study can neither confirm nor negate color vision in lionfish, but some conclusions can be drawn from the response of the lionfish to reward-based training. The data were unable to support the hypothesis. The lionfish were less motivated by prey than the damselfish studied by Sieback et al. (2008). It is possible that a week of training is inadequate for a predator species, or that the lionfish did not adjust to captivity as readily. It is also possible that the lionfish were able to associate the color-specific behavior with receiving the reward during the training period, but not during the preference tests because of the delayed release of the prey item.

Color vision may be lacking in lionfish for a number of reasons. As mentioned, they hunt almost exclusively during crepuscular periods where color vision would be unnecessary due to inadequate light for color reflection (Green et al. 2011). Green et al. (2011) also demonstrated that invasive lionfish do not have a clear preference for one species or family of prey. Morris and Akins (2009) determined that lionfish consume prey from a diverse array of families and morphologies, indicating that color vision may not be required for prey identification. If color vision is not present in lionfish, they may use other cues for conspecific recognition and intraspecific behaviors, such as patterns or chemical cues.

Future studies should continue to investigate the visual abilities of lionfish to develop methods for studying intraspecific behaviors. Behavioral assessments should be more specifically adapted to the natural behaviors of the lionfish to ensure representative testing of color vision.

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Physis (Spring 2014) 15: 58-65

REPORT

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Cascading effects of nutrients on macroalgae and herbivorous fish on coral reefs in Bonaire, Dutch Caribbean

Abstract The island of Bonaire has significant contamination from anthropogenic sources such as sewage and landfills, which can cause excess nutrients in groundwater that will eventually enter the ocean. Nutrients have been suggested to increase macroalgal growth. The amount of nutrients and abundance of herbivores play a key role in maintaining a healthy coral dominated reef system. The major objective of this study was to determine the health of Bonaire's reefs by assessing various bioindicators, evaluating bioacummulation of macroalgae, assessing the biocontrol mechanisms, and determining the presence of phase shifts. This study looked at the relationship of herbivorous fish, nitrogen content and abundance of macroalgae to make inferences regarding the overall health of the reef. Two study sites, Kas di Arte and Something Special, were chosen for research over the course of four weeks. Data collection included abundance of herbivorous fish, substrate composition and nutrient level in water and algae samples. No inferences could be determined from the nutrient tests due to the varying concentrations found in both water and macroalgae. The herbivorous fish abundance and macroalgae were found to be inversely proportional. This study is important to determine whether herbivorous fish or nutrient input control phase shifts on Bonaire's reefs and can aid in identifying similar issues in reefs all over the Caribbean.

Keywords Herbivorous fish • Macroalagae • Nutrients

Introduction

The island of Bonaire suffers from many sources of anthropogenic pollution. This type of pollution can cause excess nutrients, oils, sediments and other discharges that can disturb reef ecosystems (Pearson and Rosenberg 1975). For example, Bonaire lacks a permanent wastewater treatment plant and a lined landfill. The untreated sewage water currently sits in unlined ditches. The unlined landfills and sewage ditches contaminate the groundwater through runoff and percolation which may bring nutrinets to coral reefs (Wieggers 2007, Peachy 2009). Due to these factors and other anthropogenic sources the ocean water in Bonaire may have excess nutrients.

Nutrient rich waters are a threat to tropical marine ecosystems. A nutrient threshold above ~1μM dissolved inorganic nitrogen and ~0.2μM dissolved phosphorus are considered eutrophic conditions on coral reef ecosystems (McCook 1999). Research suggests that an increase in the ratio of nitrogen and phosphorus leads to an increase in macroalgae growth, which is an indication of an unhealthy reef (Valiela et al. 1990; Weigger 2007). Increased algal cover can be so dramatic that the algae may cause phase shifts in coral reefs. A phase shift is when a dominate substrate in a reef ecosystem changes form a major group of establised speices to another. In this case, from a coral dominated to an algal dominated system (Hughes 1994). The first documented phase shift from a coral to an algal dominated reef occurred in Waikiki, Hawaii (Littler 1973) where eutrophication was suggested to cause the change from a copious coral cover to a frondose algae (Littler et al 2006). Excessive nutrient enrichment may also increase macroalgae cover and productivity via bottom-up control, creating an environment controlled by nutrient concentration (Lapointe et al.1997).

Grazers are essential to the coral reef ecosystem because they are in charge of keeping the algae population at a healthy level for coral growth (Litter 1973). The level of herbivory affects what type of algae can be present in a given area. Large standing crops of macroalgae only occur in areas of low herbivory (McCook 1999; Littler and Litter 2007). Herbivory rates can also indirectly affect many other parts of the reef. For example, high herbivory acts indirectly on fleshy algae through reduced competitive abilities, whereas lowered herbivory and elevated nutrients also indirectly inhibit corals and coralline algae by fleshy algal competition (Littler and Littler 2007). It is also suggested that a decline in keystone herbivorous fish may cause a macroalgal bloom, leading to a phase shift (Hughes et al. 1999; McCook 1999). Parrotfish (Scaridae) and surgeonfish (Acanthuridae) are very important to the coral reef ecosystem because they are grazers that control the amount of algae on the reef (Littler and Litter 2007). This study aimed to determine if the abundance of these species indicates whether the reef is algal dominated or coral dominated. The following hypotheses were tested:

H1: Nutrient composition in the water is directly proportional to macroalgae growth

H2: Macroalgae can bioaccumulate

H3: The abundance of macroalgae and herbivorous fish are inversely proportional

Materials and methods

Study site

Research was conducted on Bonaire, a small island located in the Dutch Caribbean. The fringing reef in Bonaire starts 50 m off the coast, at 8 m deep after a sandy back reef area. The shorelines of Bonaire consist mostly of coral rubble. The study was conducted at two different sites (Fig. 1). The first site, Something Special, (12° 09' 21.4" N 68° 16' 45.3" W), served as the control site and ranged from the entry point to 120 m south and 200 m north just before a large boat harbor. This site has a small coral rubble beach and a few moored boats within the sandy back reef area. The second site, Kas di Arte (12° 09' 41.8" N 68° 17' 00.8" W), ranged from the entry point to 120 m north. At this site, there is a sewage pit that may cause increased nutrient levels in the surrounding waters. Additionally, there is a cruise ship dock 400 m to the south of this site. This site has an entrance that is next to a sea wall with a greater number of moored boats, which also may increase the amount of nutrients in the area.

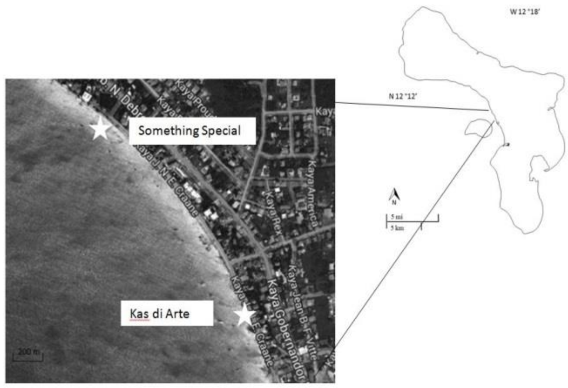

Fig. 1 Map of Bonaire. The stars represent each site. Site 1: Something Special is located (12°09'41.8"N 68°17'00.8"W). Site 2: Kas di Arte is located at (12°09'21.4"N 68°16'45.3"W). Image taken from GoogleMaps

Data collection

Macroalgae cover

A baseline percentage of macroalgae cover was determined for each site. This was completed by taking a video transect of the coral and macroalgae cover for 45 min at a depth of 13.7 m with a Sony Handycam video camera in an Ocean Images housing held 50 cm above the substrate.

Herbivorous fish abundance

Two divers conducted fish surveys once a week for 3 weeks. Dives lasted 45 min along each reef site, at a depth of 13.7 m. The number of parrotfish and surgeonfish observed were recorded. Diver A only recorded fish observed at depths greater than 13.7 m and diver B recorded fish only observed at depths 13.1 m or less.

Nutrients in macroalgae and water

Water samples were collected in bottles at the start, middle, and end of each herbivorous fish abundance dive. Each bottle was prepped with HCl and distilled water before going out in the field. The bottles full of distilled water were taken to each site. The distilled water was let out of the bottle, at depth, at each location. The bottle was then inverted and cleared out with air from the researcher's octopus. The bottle was once again inverted right side up to collect the seawater.

Three algae samples were collected from each site. The macroalgae samples were taken at the start, middle, and end of each dive. The macroalgae samples consisted of *Dictyota* spp. because of its abundance on the reef. Both the seawater samples and the macroalgae samples were tested for nitrate concentrations, using a Trilogy Laboratory Fluorometer-Version 2.1. The concentrations were determined using the methodology proposed by Stickland and Parsons (1968). The algae samples were ground down using a mortar and pestle, diluted (20 mL with distilled water), filtered using a vacuum filter (0.2 µm), and then tested for nitrogen using a fluorometer.

Reassessment of macroalgae cover

A reassessment of macroalgae cover was also taken into consideration. This was completed by conducting a second video transect at each site after a four-week long period, at the same depth of 13.7 m. This determined the change in the coral and macroalgae cover over the course of the research. The video provided evidence to see if the herbivorous fish or nutrients have had any recent effect on the reef.

Data analysis

The herbivorous fish data consisted of the parrotfish and surgeonfish counts from all depths separated by site. A descriptive analysis was completed for this data, to calculate average herbivorous fish abundance and standard deviation.

The initial and final substrate cover after four weeks between the sites were compared. The videos were analyzed using Coral Point Count (CPCe) (Kohler and Gill 2006). Using CPCe analysis, 100 randomized frames with 15 randomized points were sorted into the various categories; coral, other, sand, unknown, *Cladophora* spp., *Dictyota* spp., cyanobacteria, and turf algae. All videos were 35 min long and spanned 120 m in length, however one video was only 25 min long due to technical difficulties. Therefore, 100 frames were taken from a shorter amount of time in this one video, causing unequal sampling.

Results

To compare the health of each reef, the average number of herbivorous fish at each site was compared (n=3; Fig. 2). The values at Kas di

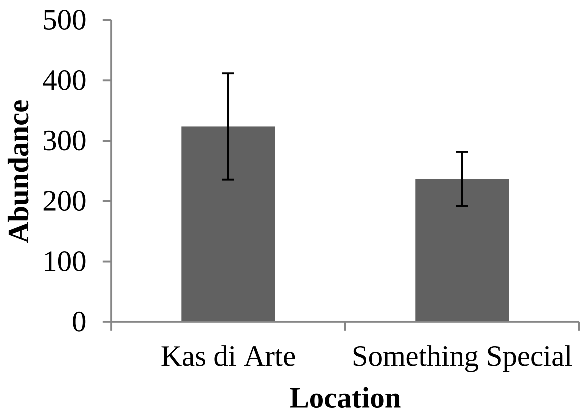

Fig. 2 Average number of herbivorous fish found at each site (n=3). Herbivorous fish consisted of parrotfish and surgeonfish observed from both above and below 13.7 m. Error bars represent standard deviation

Arte portrayed a greater abundance of herbivorous fish (162 individuals, ± 87.8), than Something Special (118 individuals, ± 45.1).

The nutrient concentrations in macroalgae and water samples at the same site were compared to determine if macroalgae contained more nutrients than water (Fig. 3). On average, the macroalgae had a slightly higher nitrogen concentration (1.9E-4 mM L-1) than the surrounding water. The samples did not show a clear pattern at Something Special, which had the most variance (ranging from 1.0E-3 to 1.0E-2 mM L-1). During the first collection, all algal samples collected at Something Special had nitrogen concentrations below the minimum detection limit of 3.0E-5 mM L-1. However during the last collection, the concentrations in the algae superseded the nitrogen concentration in the water (1.0E-2, 6.0E-3, 3.0E-3 mM L-1).

To compare the amount of macroalgae at each site, the substrate cover was analyzed by performing video transects at the start of data collection (time zero) and after four weeks. The macroalgae cover at Kas di Arte decreased over time (Fig. 4a). This was indicative in both *Dictyota* spp. and *Cladophora* spp. (2.7% and 2%). At Something Special, the same trend was observed (Fig. 4b). *Dictyota* spp. decreased by 2.0% while *Cladophora* spp. decreased by 0.4%.

a.

b.

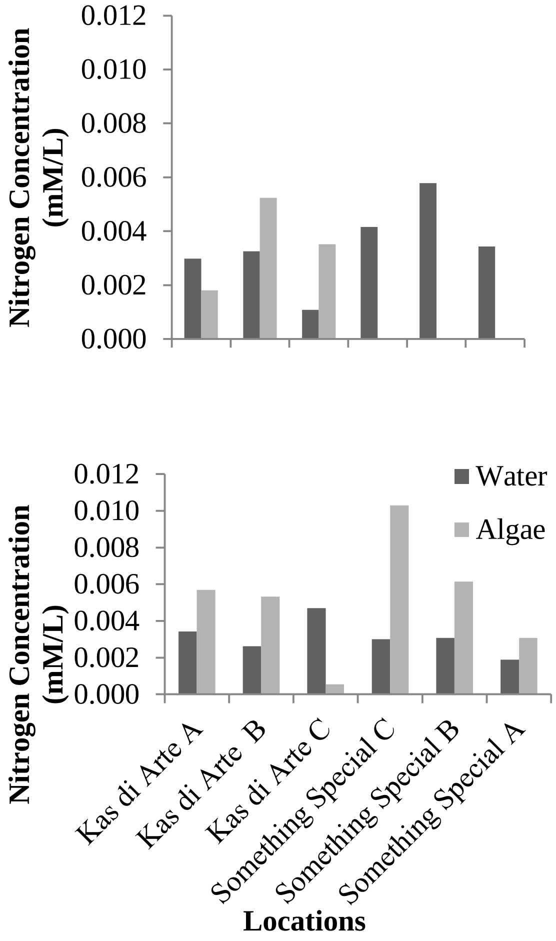

Fig. 3 Nitrogen concentration (mM/L) in water and macroalgae samples. Locations are organized from north to south for each site. A. Concentration of nitrogen (mM/L) during the first collection on 9th March 2014. b. Concentration of nitrogen (mM/L) during the last collection on 23rd March 2014

a.

b.

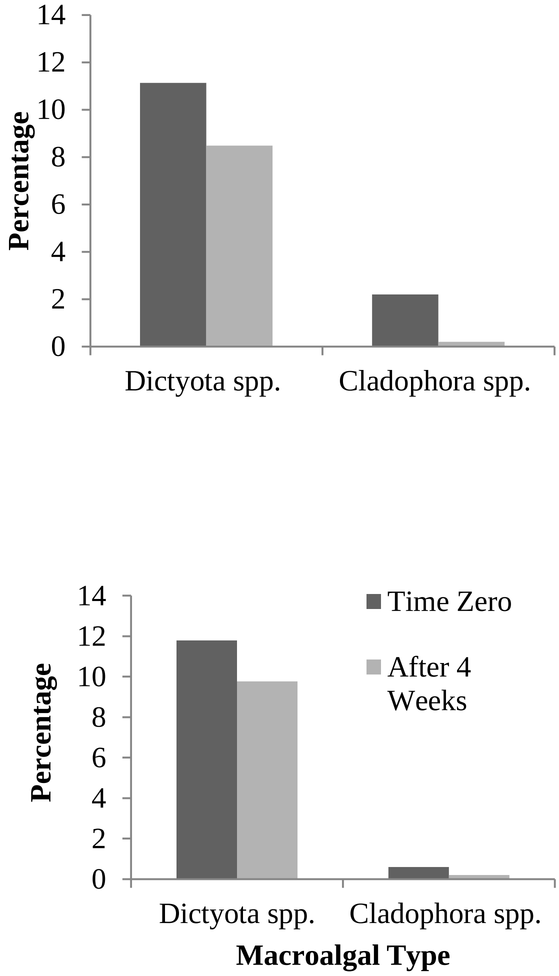

Fig. 4 Percentage of marcoalgal type, at time zero and four weeks later, from video transects at a. Kas di Arte and b. Something Special

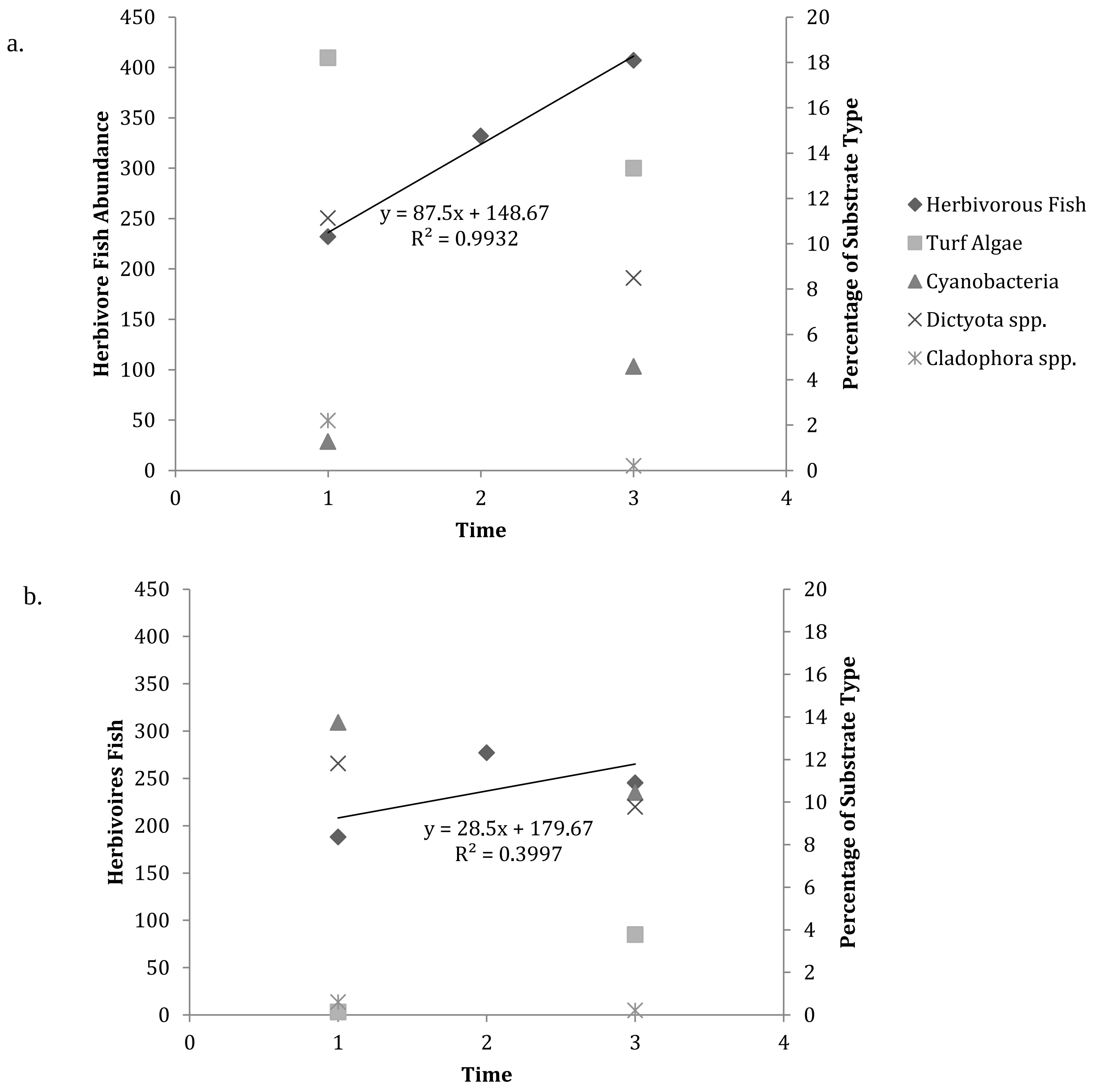

Fig 5: Herbivorous fish abundance compared to percentage of substrate type (cyanobacteria, turf algae, *Dictyota* spp. and *Cladophora* spp.) at a) Kas di Arte and b) Something Special

Fish abundance and percentage of substrate type were compared to determine how one affects the other at each site over time. At Kas di Arte, values showed a clear increase (by 175 individuals) while the amount of macroalgae decreased (Fig. 5a). The abundance of *Dictyota* spp. decreased by 2.7% and *Cladophora* spp. by 2.0%. Throughout the course of the study, turf algae declined by 3.0%, while cyanobacteria increased by 3.3%.

A similar trend was evident from the data collected at Something Special. The relative abundance of herbivorous fish increased less dramatically (by 57 individuals) than the previous site (Fig. 5b). *Dictyota* spp. had a greater decrease in abundance than *Cladophora* spp. (2.0% and 0.4%). Over the course of the study, turf algae declined by 3.3% while cyanobacteria increased by 3.6%.

Discussion

The purpose of this research was to examine the cascading effects of nutrients on the macroalgae and herbivorous fish in Bonaire. The results showed that nutrient composition in both the water and in the algae varied, allowing no pattern to be drawn. The hypothesis stating the inverse relationship between herbivorous fish and macroalgae supported that Bonaire's reefs are top-down controlled; that is, an environment controlled by the predation of grazers (Lapointe 1997) where herbivorous fish are a key indicator of macroalgal growth. As explained before, by exerting a top-down control, herbivorous fish play an important role in preventing a phase shift to an algal dominated reef.

The first hypothesis was that nutrient composition in the water was directly proportional to macroalgal growth. There was insufficient data to support this hypothesis due to the fact that the nitrogen levels in the water varied over time and could not be used as a control to evaluate macroalgal growth (Fig. 3). Nutrient concentration variance could be due to a variety of factors including anthropogenic input, which may include sewage (840 kg N yr-1), animal feed import (21600 kg N yr-1), and municipal solid waste (43000 kg N yr-1);(Gijzen 2004). Distribution of nutrients could have been affected by variation in ocean currents as well as other life forms, such as turf algae, that may be utilizing these nitrites for their own survival (Littler et al. 2006).

There was insufficient data to support the second hypothesis that macroalgae can bioaccumulate nitrogen. The nitrogen concentrations in the macroalgae were compared to the concentrations found in the surrounding water and no clear pattern was observed (Fig. 3). When an average of the concentrations was taken, the macroalgae showed slightly higher nitrogen concentrations than the water, however this difference was too minuscule to be considered significant. Although *Dictyota* spp. was not shown to bioaccumulate, there is still a chance that other species of macroalgae can. For instance, other studies have suggested that algal tissues, such as *Codium isthmocladum*, may gain nutrients over time (five months; Grice et al. 1996; Laponte 1997; McCook 1999). However *Dictyota* spp. may not bioaccumulate due to morphological or genetic differences compared to other macroalgal species.

The herbivorous fish and macroalgae abundance were inversely proportional at both sites, thus supporting the third hypothesis (Fig. 5). Other studies have also shown that large standing crops of macroalgae can only occur in areas of low herbivory (McCook 1999; Littler and Litter 2007). It is important to note that *Cladophora* spp. had the most significant decrease when there was a large number of herbivorous fish present (Fig. 5). This may suggest that *Cladophora* spp. is a food preference for many of Bonaire's herbivorous fish. The supported hypothesis suggests that the herbivorous fish are a key indicator of macroalgal growth, especially *Cladophora* spp.

The decrease of turf algae showed a similar trend as the decrease of the percent of macroalgae on the reefs, suggesting that herbivory may also control the amount of turf algae in Bonaire. Another observation noted was the increase in the abundance in cyanobacteria when the macroalgae decreased. This may be due to interspecific competition; with a decrease in macroalgae, the cyanobacteria may have room to grow over the substrate (Sammacro 1973; Thacker et al. 2001).

This study was important because it helped to answer the question of whether Bonaire's reefs are a top-down controlled environment. The varying amounts of nitrogen in the water suggest that Bonaire's reefs are not bottom-up controlled. A bottom-up controlled environment usually has a steady stream of nutrients coming in, such as eutrophication from sewage and agriculture (Littler et al. 2006). Bonaire's reefs are instead top-down controlled, as evidenced by greater amounts of herbivores associated with lower amounts of macroalgae. Litter et al. (2006), suggested that oligotrophic coral systems will have the effects of top-down inhibitory controls due to intense herbivory. As explained by top-down control, the herbivorous fish had a major role in preventing a phase shift from a coral dominated to an algal dominated reef. The abundance of these fish is an important bioindicator of the reef's overall health. It is important to note that herbivorous fish were the only grazers examined during the study, since other grazer populations (*Diadema antillarum* and other echinoids) are too small to make any significant difference on the percentage of macroalgae reef (Alves, 2012).

This study also stresses the importance of the herbivorous fish to the reefs of Bonaire. The abundance of these fish is crucial in preventing a phase shift. Fortunately, Bonaire has implemented laws to protect their reefs. It is now unlawful to fish for herbivores, such as parrotfish (Steneck et al. 2013). It is important that these rules are strictly enforced to preserve the marine environment. Other top-down ecosystems should have similar laws put into place, which will help keep these systems from entering a phase shift and ultimately destroying the reef.

To address issues with nutrient variability, suggestions for future research include testing sites further apart with larger constant nutrient influxes over a longer time frame. Another suggestion is to test for nutrient composition by using a different method or using a more sensitive fluorometer. The minimum detection limits on the fluorometer used in this experiment limited the collection of nitrogen data and prevented testing for phosphorus. Further studies should also evaluate a wider range of macroalgal species (especially green algae) to determine if any species of macroalgae can bioaccumulate, since previous studies have suggested that green algae can bioaccumulate nitrogen. Lastly, it is suggested that future studies look at food preference of herbivorous fish, because the fish seemed to have a preference for *Cladophora* spp. rather than *Dictyota* spp., which may have affected the results.

Acknowledgements I thank Ben Gulmon for helping collect data. Thank you to Molly Gleason from CIEE for assisting in the lab work. Thank you to my intern advisor, Colin Howe from CIEE, for reading and editing my paper. A special thank you to Dr. Arboleda from CIEE who without his guidance this project would not have been possible. A final thank you to the rest of the staff at CIEE and my home university of Roger Williams.

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Physis (Spring 2014) 15:66-78

REPORT

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Cultural perceptions of environmental degradation, management, and accountability in conservation in Bonaire, Dutch Caribbean

Abstract Conservation is predicated on local support, and if scientists and resource managers wish to develop effective programs the cultural values and perceptions of surrounding communities need to be considered. As a result, researchers have shifted their attention to ethnography as a means to improve human-environment interactions and garner support for conservation. Bonaire serves as an ideal study site to explore the intricate relationship between cultural perception and environmental programs. Despite being a leader in conservation, current waste management programs greatly undermine island-wide environmental efforts and few successful solutions to address this discrepancy have been suggested. This study explored the cultural perceptions held by different subgroups on Bonaire (i.e. NGOs, divers, and conventional households) and provides valuable insight into how the community views environmental health and current management practices. Ethnographic methods were used to examine how three subgroups perceive environmental degradation, major factors contributing to ecological degradation, current management, and possible solutions, both individual and collective. Overall, 85% of all respondents identified environmental degradation as a problem on Bonaire. Salience values demonstrated that, overall, subgroups considered 'humans', 'sewage' and 'garbage' as the top environmental threats. Additionally, nearly all participants expressed a negative perception of management. Not only does this research highlight a general awareness of environmental issues by Bonaireans, but it also reveals that there exists a widespread feeling of support for conservation. It is imperative that the ideological and cultural differences identified through this research are noted and incorporated into future management plans.

Keywords Cultural perception • Ethnography • Conservation management

Introduction

An often overlooked and highly important phenomenon is that the fates of our world's biological and cultural diversity are intrinsically intertwined. It is estimated that indigenous territories cover nearly 24% of the Earth's land surface, and within this land exists almost 80% of the world's remaining global biodiversity priority areas and healthy ecosystems (Watanabe 2008). The data provide evidence that a significant spatial convergence exists between cultural and biological diversity, and this remarkable overlap presents great opportunities and poses tremendous challenges for scientists. In order to implement conservation management strategies that will exhibit not only short-term but also long-term success, scientists must take a multidisciplinary approach. It is no longer practical to ignore the cultural values and practices of local people. Conservation is predicated on local support and if scientists and resource managers wish to develop effective programs the cultural values and perceptions of surrounding communities need to be incorporated (Debrot and Nagelkerken 2000; Pomeroy 2004; Lockwood 2010; Bennett and Dearden 2014).

In response to the recognition that local support and involvement are major determinants of conservation success, attention has shifted to incorporate ethnography of to improve human-environment interactions and garner support for conservation (Debrot and Nagelkerken 2000; Kuriyan 2002). Studies conducted worldwide have demonstrated the importance of cultural perception of resource state and management for long-term conservation success (Debrot and Nagelkerken 2000; Kuriyan 2002: Sekhar 2003; Bennett and Dearden 2014). By using methods often exclusive to the life sciences (i.e., semi-directive interviews, participant observation, questionnaires), these case studies have shown that conservation program design and implementation can be greatly improved by increasing local involvement and generating cultural benefits that no longer limit participation in conservation by monetary compensation or economic benefits (Kuriyan 2002). Additionally, there exist intricate links between public perception, conservation, and management in maintaining environmental health and promoting healthy community-government relationships (Bennett and Dearden 2014). By documenting local knowledge, scientists, government institutions, and resource managers can gain valuable insight into how surrounding communities perceive conservation programs. Consequently, this awareness of cultural perceptions can highlight local environmental priorities and facilitate governing bodies in addressing problems that are most likely to be supported by the community.

Using ethnographic studies for conservation has shown to be most effective in communities with positive perceptions of wildlife and the environment (Kuriyan 2002). A leader in conservation, Bonaire (Dutch Caribbean) fosters a population invested in the environment, particularly in marine health (Lacle et al. 2012; Roth 2013). It has been demonstrated that Bonaireans view the environment as intrinsically valuable and that coral reefs are not only an important asset to the island's tourism industry, but local perceptions of them have changed such that coral reef health is an important component in the Bonairean cultural system (Lacle et al. 2012; Roth 2013). Thus, due to the generally positive perception of conservation and environmental health held by Bonaireans, the island serves as an ideal study site for ethnographic research intended to improve conservation practices. Additionally, Bonaire provides an interesting setting for study because despite the tremendous strides the island has made to protect its environment (e.g. the establishment of two MPAs and designation of the entire coastline as a marine sanctuary) there exist certain practices and series of threats that greatly undermine the success of current conservation management efforts. The island currently lacks an adequate waste management system and, consequently, untreated sewage and waste run freely into the groundwater and onto coral reefs (Lacle et al. 2012). Recommendations have been made to address the disconnect between environmentally detrimental waste treatment practices and the largely successful conservation strategies throughout the island. However, these recommendations seem to have failed to produce substantial or tangible results.

This study aimed to explore the cultural perceptions of Bonaireans concerning environmental degradation, governance and management, and responsibility in finding solutions to conservation problems. By examining how Bonaireans of different subgroups (NGO members and representatives, divers and conventional households) perceive current environmental threats and management processes, this study can provide valuable insight into how Bonaireans believe the current waste management system may be improved.

H_1: Different subgroups possess different perceptions pertaining to environmental degradation, management, and accountability.

H_2: Ideological and cultural differences will allow for the identification of common ground and prompt collective conservation action from the different subcultures.

Materials and methods

Study site

Bonaire is an island in the Dutch Caribbean located approximately 50 km from the northern coast of Venezuela. Covering an estimated area of 288 km^2, Bonaire is home to a permanent population of roughly 16,000 individuals (Lacle et al. 2012). Bonaire's economy is primarily based on tourism, mainly from diving and cruise ships, and the island attracts a staggering 80,000 visitors each year (Lacle et al. 2012). As a result, the island's marine environment plays an essential role in the local economy. Additionally, the marine environment and the resources that it contains are integral to the cultural system of Bonaireans; a large portion of permanent residents relies on Bonaire's marine environment for fish consumption and recreational activities (Lacle et al. 2012). Research efforts were concentrated within the capital of Bonaire, Kralendijk, due to convenience and strong likelihood of gathering a sufficient data set.

Data collection

Ethnographic data was collected using a mixed-methods approach in which semi-directive oral interviews and questionnaires examined local perceptions of environmental degradation, management, and accountability (Huntington 2000; Bennett and Dearden 2014). Participants were grouped into three subgroups: 1) NGO members and representatives, 2) divers, and 3) conventional households. Subgroup 1 (NGOs) was comprised of individuals involved with local nongovernmental organizations. Subgroup 2 (divers) included instructors, dive masters, and interns employed at local dive shops throughout the island; tourists and visiting recreational divers were not taken into account. Subgroup 3 included individuals, both short- and long-term residents, located within the major Kralendijk area.

Questionnaires

Self-administered questionnaires were constructed to examine local perceptions of four main topics: (1) environmental degradation, (2) major sources of and factors contributing to ecological degradation, (3) current governance and management, and (4) possible solutions, both individual and collective (See Appendix I). Questionnaires were modeled after those used by Lacle et al. (2012), which contained similarly structured questions. The use of questionnaires helped to quantify responses and simplify data analysis of the differences and similarities in target subgroups' perceptions. Additionally, questionnaires provided participants with the opportunity to respond anonymously, a factor that may make respondents more comfortable and, as a result, produce more accurate results compared to in-person interviews (Huntington 2000). Questionnaires included a combination of closed and free response questions. The former included multiple-choice and rating scale questions; rating scales asked respondents to assign a score from 1 to 5 on a given topic with 1 and 5 corresponding to a low and high score, respectively. Free response questions were included to account for unanticipated insights that may have been prompted by the questionnaire (Huntington 2000). Questionnaires were distributed to divers and conventional households, exclusively; NGO members and representatives were not given questionnaires due to time constraints and scheduling difficulties.

Semi-directive interviews

Key informants from all three groups were identified through recommendations made by local community members and individuals; after which, semi-directive interviews were conducted. Interviews were recorded using QuickVoice® Recorder (nFinity Inc. 2008).

Questions for the in-person interview were extracted from the self-administered questionnaire. In addition, free list prompts were issued to ascertain respondent comprehension and ensure all themes of the interview were addressed (i.e. environmental degradation, sources and factors contributing to ecological degradation, current governance and management, and possible solutions). Interviews were structured to provide participants the opportunity to present unforeseen insight into the study topic and serve as a strong, detailed baseline from which ethnographic data were built upon.

Data analysis

Questionnaire responses were compiled according to subgroup as well as into a single data set to represent the sample population as a whole. Multiple-choice answers were summed for each question and percentages of total responses were calculated. Rating scale answers were also summed and percentages were calculated based on the total score respondents gave for a single question. With respect to free response questions, responses were ranked according to the protocol provided by Quinlan (2005). These rankings were used to produce salience values. Furthermore, reviewed recordings of oral interviews and free response answers were visually displayed and quantified using the free Internet program Wordle to generate word clouds based on the frequency of questionnaire responses (http://www.wordle.net/).

Results

Questionnaires

Environmental degradation

Eighty-five percent of all respondents (n=41) identified environmental degradation as a problem on Bonaire. Responses between subgroups did not differ dramatically; however, divers (n=17) always perceived environmental degradation as a problem whereas conventional households (n=24) did not. Within the conventional households subgroup, 75% felt it is a problem while 25% did not know. The subgroups did differ, however, in how important they considered environmental degradation to the health of the marine and land environment in Bonaire. In general, divers rated environmental degradation as very important (scores 4-5) while conventional households assigned more varied scores (scores 1-5). Although over half of conventional households (54%) rated environmental degradation as very important, roughly one-fifth (21%) rated it as neutral to not important at all (scores 1-3; Fig. 1a).

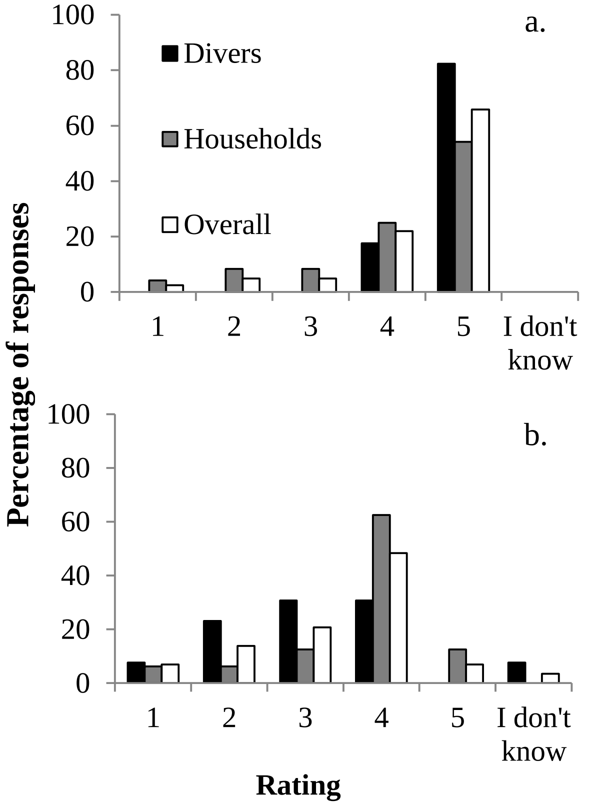

Fig. 1 Rating scores of respondents to questions on environmental degradation on a scale of 1 to 5 (n=41). a. Respondents were asked to score the importance of environmental degradation to Bonaire's environment. b. Respondents were asked to rate the decline in environmental health. A score of 1 means the decline is very low and 5 very high

Subgroups also differed in their ratings of the "marked decline in the health of the marine and/or land environment." Conventional households rated the decline much higher than divers; 75% of conventional households rated it as high to very high (scores 4-5) whereas only approximately 31% of divers scored it similarly (Fig. 1b). The majority of divers (~61%) rated the decline as very low to moderate (scores 1-3).

Factors of degradation

With respect to potential threats facing Bonaire's marine and land environments, sewage and solid waste, such as plastic and glass bottles, were consistently identified as the top two most important impacts (n=40). Additionally, these threats were recognized as having most noticeably changed the environment (n=35). Percentages were calculated out of total possible score values to determine the threats enacting the most change. Higher percentages denote more change while lower percentages imply little to no change. Thirty percent of total scores corresponded to sewage (Fig. 2). Similarly, 29% corresponded to solid waste (Fig. 2). Subgroups differed in their ratings of coastal

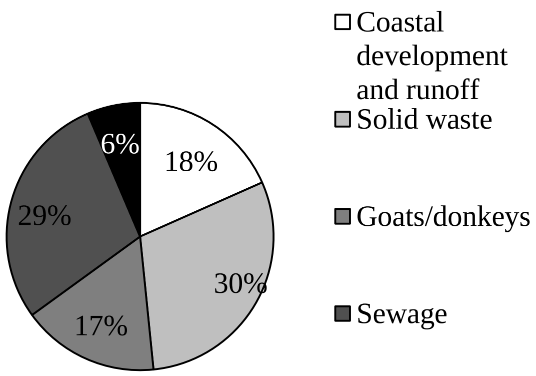

Fig. 2 Percentages out of total score values assigned by respondents (n=35) for threats most noticeably having changed the environment. Higher percentages indicate more change and lower percentages indicate less change

development and runoff; conventional households and divers assigned lower and higher scores, respectively.

Nearly all respondents (n=40) were able to identify at least one threat facing the marine and land environment in a free list response question. From these free listed threats, a word cloud was generated to visually represent the most frequently identified threats. The size of the words in the word cloud corresponds to the frequency in which they appeared in responses. The threats most often identified by respondents were 'humans', 'sewage', 'garbage', and 'cruise ships' (Fig. 3). In instances where respondents listed more than one threat, responses were ranked according to the protocol provided by Quinlan (2005).

Fig. 3 Word cloud created from free list responses of the top threats facing the marine and land environment (n=40). The size of words corresponds to the frequency in which they appeared in responses

Salience values closer to 1 imply threats that were listed more frequently and ranked higher by respondents. The top five most frequently provided and highly ranked threats were: 'landfill/garbage', 'humans', 'sewage', 'cruise ships' and 'climate change' (Table 1). Based on salience values, subgroups differed in their rankings of the top three threats. For divers the most frequently listed and highest ranked threats were 'humans', 'cruise ships', and 'landfill/garbage'. The top three threats as identified by conventional households are identical to the overall (divers and conventional households combined) top threats: 'landfill/garbage', 'humans', and 'sewage' (Table 2).

Management

Perceptions of management differed between divers and conventional households. When asked who they thought was in charge of current sewage treatment practices, the

Table 1 Salience values for top threats as they appeared in a free list response question for all respondents

Threats	Sum of ranked values	Salience values
Landfill/garbage	11.3	0.28
Humans	11.0	0.28
Sewage	6.5	0.16
Cruise ships	6.3	0.16
Climate change	4.3	0.11
Industry and development	4.3	0.11
Fishing	4.0	0.10
Pollution	3.5	0.09
Plastic and solid waste	3.0	0.08
Tourism	3.0	0.08
Other	2.8	0.07
Oil companies	2.5	0.06
Human waste	1.7	0.04
Habitat destruction	2.3	0.06
Divers	1.0	0.03

Table 2 The top three threats facing the marine and land environment as identified by different subgroups in a free list response question

Threats	Overall (n=40)	Diver (n=17)	Conventional households (n=23)
1	Landfill/ garbage	Humans	Landfill/ garbage
2	Humans	Cruise ships	Humans
3	Sewage	Landfill/ garbage	Sewage

subgroups did not identify the same authoritative body. Approximately half of all divers (47%; n=17) could not identify an individual management body and, in those instances where divers did recognize one, 26% identified the federal and 26% the local government. In contrast, more than half of all conventional households (54%) specified the federal government. Additionally, subgroups differed in who they preferred to be in charge of sewage practices. Divers preferred to have the federal (33%) or local (27%) government in charge. The majority of conventional households (42%) did not know their preference, and those that did chose government (federal and local) only after community leaders (~17%) and nongovernmental organizations (~13%). Moreover, after identifying the perceived responsible management party in charge of sewage treatment practices, respondents were asked to score management's performance. Conventional households typically assigned lower scores than divers. A higher percentage of conventional households perceived the responsible management party as doing a poor job (30%; score of 1) compared to divers (6%). Overall, roughly 64% of all respondents assigned ratings of poor to fair (scores 1-2). With respect to improving management, respondents provided suggestions in a free list response question. In general, the most common suggestions listed by respondents (n=29) related to transparency, time management, organization, trustworthiness, and a sense of self-motivation to care for the environment. Moreover, respondents from conventional households suggested privatizing sewage treatment facilities as a means to improve management.

Solutions

Support for several possible environmental management activities varied between conventional households and divers. Fifty percent of conventional households (n=22) stated they were not in favor of placing restrictions on coastal and inland development (Fig. 4a). On the other hand, approximately 86% of divers (n=14) were in favor of development restrictions and none of the respondents in this subgroup stated they were not in favor (14% replied 'I don't know'; Fig. 4b). Although conventional households were not willing to restrict development, they did indicate their support for limiting areas of free-grazing goats (82%) and sterilizing free-roaming donkeys (73%; Fig. 4a). Divers did not share the same opinion and only 32% and 36% of respondents within this subgroup were in favor of limiting goat grazing and sterilizing donkeys, respectively (Fig. 4b). Despite these subgroup differences, 100% of conventional

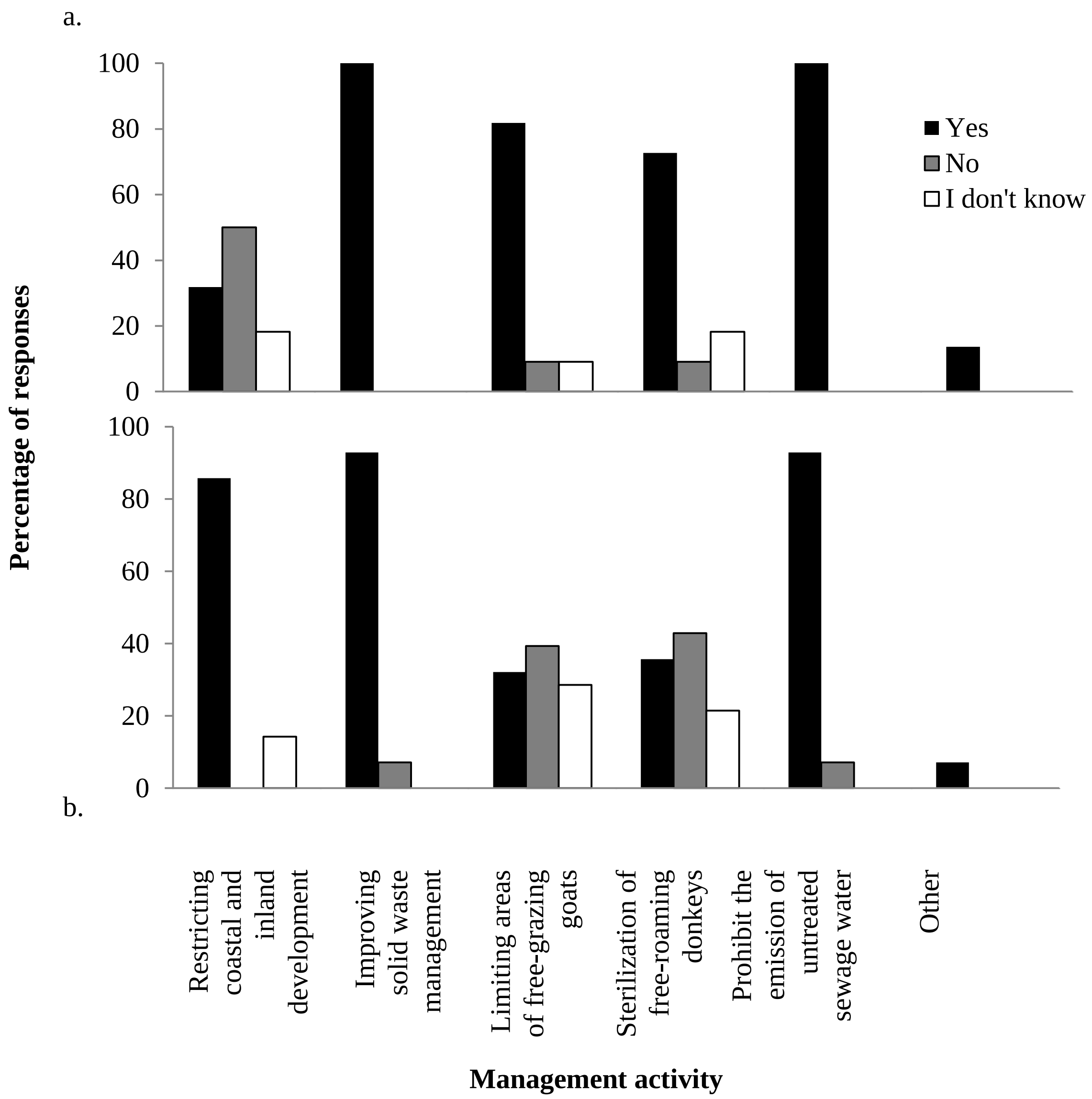

Fig. 4 Respondents in favor of, not in favor of, and unsure of using various management activities to improve environmental health. a. Responses from conventional households (n=22). b. Responses from divers (n=14)

households and 93% of divers were in favor of improving solid waste management and prohibiting untreated sewage emissions (Fig. 4a-b).

Questionnaire respondents also listed the top three most important things they believe can be done to protect the environment (n=30). For both subgroups, the most common words as they appeared in free list responses were ‘education’, ‘recycling’, and ‘raise awareness’. Other frequent responses included ‘better waste system’, more control’, and ‘restrict boats’ (Fig. 5).

Semi-directive interviews

Environmental degradation

Overall, interviewees from all three subgroups stated there has been an evident decline in the health of the environment, both marine and terrestrial. Similar to questionnaire respondents, all individuals (n=7) who participated in semi-directive interviews recognized environmental degradation as a problem on Bonaire. However, in some cases interviewees from different subgroups differed with respect to how much the environment has declined as a result of degradation over time.

Fig. 5 Word cloud created from free list responses of the most important things that can be done to protect the environment (n=30). The size of words corresponds to the frequency in which they appeared in responses

For example, a representative of a local NGO mentioned that although Bonaire has one of the best coral reefs in the Caribbean, the marine environment has experienced noticeable degradation (Oral Interview). Additionally, the representative indicated there has been a decrease in native tree species and an increase in invasive plant species and run off. The same NGO participant emphasized that degradation on the island is not only noticeable, but also exacerbated by Bonaire's small size and limited resource pool. On the other hand, an interviewee from the dive subgroup stated that although it is well known that corals reefs have changed over the decades, the changes occur so gradually that they are not noticeable. Despite agreeing that the marine and land environments on Bonaire have declined, the subgroups disagreed with respect to the degree of the declines (i.e. whether the decline is or is not noticeable).

Factors of degradation

When asked to identify major threats to Bonaire's environment, interviewees mentioned similar responses to those listed by questionnaire respondents in free response questions. Among the interviewees from the dive subgroup (n=2), 'sewage', 'humans', and 'traffic' (i.e. cars) were the threats discussed most at length. The divers stressed that the lack of an adequate sewage system capable of treating waste from throughout the island significantly threatens the environment. Participants from the NGO subgroup (n=4) also identified 'sewage' as a top threat. In addition, NGO representatives and members also listed 'oil companies', 'landfill', 'and 'political will' as major environment threats. Similarly, the interviewee from the conventional household subgroup (n=1) identified 'sewage' and 'humans' as some of the most important threats. Regardless of subgroup, the threats identified by interview participants greatly overlapped indicating that they are considered important to nearly all individuals included in this portion of the study.

Management

Interviewees within the NGO subgroup (n=4) all identified the local government as the authoritative body in charge of current sewage treatment practices. They demonstrated a deep understanding of the political and social systems by explaining that the Dutch federal government, in the end, governs the local government due to Bonaire's status as a special municipality of the Netherlands. As a result, NGO participants stated that both forms of government ultimately exert control at some level. The conventional household participant also recognized the local government as being in charge of waste treatment, with no mention of the federal government. Interviewees within the dive subgroup (n=2) held mixed opinions. One participant, similar to the other subgroups, identified the local government as the main management body while the second participant stated they did not know. However, despite mixed responses to who they thought was in charge, all interviewees expressed a negative perception of current management. When asked to rate management's performance, interviewees assigned low scores (scores 1-2) indicating they perceived the responsible management party as doing a poor to fair job. All participants expressed the need to improve current management by increasing transparency, focusing on both short- and long-term goals, acting with urgency, and recruiting knowledgeable personnel who are familiar with waste treatment processes. Furthermore, interviewees from the dive (n=2) and NGO subgroups (n=4) stated that good management requires effective communication to the public and consistent monitoring of duties and projects to ensure real progress is being made in a timely manner.

Solutions

Interview participants stated that it is the 'small things' that are most important in protecting the environment. Several interviewees covering all three subgroups stated that 'recycling', 'taking individual responsibility', and encouraging 'good attitudes' are key in maintaining environmental health. Interviewees also mentioned subgroup-specific measures that can be taken to protect the environment. For example, one NGO participant expressed the need to subsidize after school educational programs (lead by their organization) to motivate children about conservation issues and encourage environmental stewards. The interviewee also stated that research, environmental monitoring, and law enforcement are important assets to environmental protection. The majority of respondents, however, identified 'education' as the single most important factor influencing the protection and preservation of the environment.

Discussion

This study identified important differences in the subgroups' perceptions of environmental degradation, major threats contributing to ecological degradation, governance and management, and potential solutions. thus, the findings of this research project support H1. Although nearly all study participants recognized environmental degradation as a problem on Bonaire, the subgroups differed with respect to how noticeable they perceived the degradation to be. For example, divers perceived the decline as less noticeable compared to conventional households and NGO members and representatives. Despite a significant percentage of conventional households scoring the decline of the marine and land environments as high to very high, this subgroup placed less importance on environmental degradation to overall environmental health than did divers. It is possible that although conventional households have noticed a more marked decline, they do not feel it takes precedence over other important issues such as politics, economics, and tourism. Or perhaps there exist other unapparent conservation issues that conventional households perceive as more important to overall environmental health than degradation.

Subgroups also differed with respect to their perceptions of major threats and attitudes toward potential management activities. Conventional households did not perceive coastal development to be as important to the health of the marine and land environments as divers did and, as a result, the majority of respondents within this subgroup were not willing to limit coastal and inland development. In contrast, respondents from the dive subgroup perceived costal development as having noticeably degraded the environment and were largely in favor of limiting development (85%). Moreover, the subgroups identified different sets of top environmental threats, which further highlight differences in conservation priorities. Conventional households placed more significance on 'landfill/garbage' and 'sewage' while divers listed 'cruise ships' near the top of their list. These perceived top threats are likely products of the environment with which each subgroup is most familiar. For instance, conventional households may spend more time in the terrestrial environment and, consequently, overlook cruise ships as potential ecological threats. Similarly, divers may be more familiar with the marine degradation caused by cruise ships and perceive 'landfill/garbage' and 'sewage' as less environmentally threatening because of their relatively oceanic exclusivity. Regardless from where these perceptions stem, the differences between the two subgroups highlight potential areas of conflict and serve as an important reference tool in developing effective conservation management strategies that address a diverse and appropriate set of perceived environmental issues (Debrot & Nagelkerken 2000; Stump & Kriwoken 2006; Broad & Sanchirico 2008).

From the answers provided in both questionnaires and oral interviews, it is apparent that regardless of the subgroup, study participants possess a negative perception of current management and waste treatment practices. Attitudes toward government ranged from apathetic to considerably disapproving. Additionally, participants provided a plethora of suggestions they believe would improve management; suggestions ranged from better public communication and transparency to completely privatizing sewage treatment facilities. It is evident from the low performance scores and long list of necessary improvements that perception of management is primarily negative and may greatly hinder local support of conservation initiatives. It has been widely established that conservation success is predicated on local support and positive perception of management and governance (Bennett & Dearden 2014). Despite demonstrating other favorable conditions such as widespread awareness of environmental issues and broad support for conservation action, subgroups on Bonaire do not approve of current management and thus, are less likely to comply with management-proposed strategies (Debrot and Nagelkerken 2000; Kuriyan 2002: Sekhar 2003; Bennett and Dearden 2014). Similar to the neighboring island of Curaçao (Dutch Caribbean), poor governance may prove to be "the single greatest threat to modern resource stewardship" (Debrot and Nagelkerken 2000).

It is critical to mention that the top threats identified by participants in this study greatly differed from those identified in a very recent ethnographic study conducted on Bonaire (Roth 2013). According to Roth (2013), 'pollution', 'divers', and 'invasive species' were the top ranked and most frequently mentioned threats to the island's coral reefs by Bonaireans. In contrast, participants in this study placed more importance on 'humans', 'sewage', 'garbage', and 'cruise ships' as potential threats to Bonaire's environment (Fig. 3). The differences in ranked threats emphasize the transient nature of environmental perceptions and the importance of consistently measuring and assessing current attitudes to determine the best courses of conservation action. It is imperative that scientists, policy makers, and other major stakeholders continuously evaluate local perceptions if conservation strategies are to efficiently and accurately address the most up-to-date environmental priorities. By targeting current perceived threats, management can develop programs that will garner the most public support and ultimately exhibit not only short-term, but long-term success. For instance, it is evident from the increase in salience value of 'sewage' that waste treatment is now a top priority for Bonaireans, regardless of occupation or subgroup category (Roth 2013).

As a result, Bonaire should place emphasis on this perceived threat. However, it is important that other real environmental threats are not ignored. Management should develop programs that prioritize efforts in such a way that perceived threats are emphasized, but less apparent or recognized ones are still tackled. Organizing conservation objectives in this manner may allow for a strategy that not only addresses updated perceptions, but updated threats as well, while at the same time acquiring substantial local support.

This study establishes a valuable and informed foundation upon which new directions for management and conservation strategies may be based. Moreover, these findings provide insight into the perceptions of three important Bonairean subgroups with respect to conservation priorities and possible solutions to environmental problems. The information provided by this research offers stakeholders, community members (including subgroup members), and decision makers a useful tool when developing conservation management strategies, especially sewage treatment practices. Such information enables these various groups to predict which measures will garner the most support and how different subgroups, and the general population as a whole, will perceive certain actions. This knowledge allows for the identification of potential conflicts as well as favorable opportunities, and management strategies can be designed accordingly "in terms of legislation, general information and educational programs" (Debrot and Nagelkerken 2000).

The results of this study illustrate a broad feeling of approval from principle subgroups (divers, conventional households, and NGOs) for improved conservation, particularly in regards to waste treatment management. As a result, conditions are favorable to develop conservation strategies that are well received by a large subset of Bonaire's population; however, Bonaire's potential conservation success lies heavily with management perception. Bonaire is fortunate to have a population that is aware of recent environmental declines and willing to support various management strategies, but despite these ideal circumstances, the public's negative public perception of management greatly hinders implementation of new strategies. It is imperative that the ideological and cultural differences identified through this research are noted, and that these differences are incorporated into future management plans. Furthermore, it is crucial that the different subgroups actively initiate collective conservation action based on the common grounds identified by this study if conservation priorities are to be addressed in a timely manner. Given the significant and ever increasing influence humans have on the environment, it is essential that individuals understand that accountability does not lie exclusively with certain subgroups; rather, we are all responsible for the influence we exert and, as a result, we must prompt individual and collective conservation action before it is too late.

Acknowledgements I thank Dr. E. Arboleda and Colin Howe for their support and help with the project. I also thank Shannon Wood for her support in the field. A special thanks to my parents who made my time in Bonaire possible. Finally, I thank the entire staff at CIEE Research Station in Kralendijk for guiding me through the scientific process.

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Appendix I

Questionnaires distributed to survey participants

Section I: Environmental degradation
1. Is environmental degradation a problem on Bonaire?
2. How important do you consider environmental degradation to the health of the marine and land environment on Bonaire? (1 being not important at all and 5 being very important)
3. Have you noticed a marked decline in the health of the marine and/or land environment?
4. How would you rate the marked decline in the health of the marine and/or land environment? (1 being very low and 5 being very high)
5. In your opinion, what is the definition of environmental degradation?

Section II: Potential threats and their sources
6. What do you think are the three biggest terrestrial/anthropogenic threats to the marine and land environment?
7. In your opinion, what are the main sources of the three biggest threats listed above?
8. How important do you consider the following potential threats facing the marine and land environment on Bonaire? (1 being not important at all and 5 being very important)

	Not important at all ←→ Very important					I don't know
Coastal development and runoff	1	2	3	4	5	0
Solid waste (plastics, glass bottles, etc.)	1	2	3	4	5	0
Wild roaming goats and donkeys	1	2	3	4	5	0
Sewage pollution and runoff	1	2	3	4	5	0
Other, specify:	1	2	3	4	5	0

9. How much have the following potential threats <u>noticeably</u> changed the environment? (1 being not at all and 5 being significantly)

	Not at all ←→ Significantly					I don't know
Coastal development and runoff	1	2	3	4	5	0
Solid waste (plastics, glass bottles, etc.)	1	2	3	4	5	0
Wild roaming goats and donkeys	1	2	3	4	5	0
Sewage pollution and runoff	1	2	3	4	5	0
Other, specify:	1	2	3	4	5	0

Section III: Perceptions of management

10. Who is in charge of current sewage treatment practices?
11. Who would you prefer to be in charge of waste treatment practices? Why?
12. In your opinion, how well is/are the responsible management parties doing to manage sewage? (1 being poor and 5 being excellent)
13. How do you think management can be improved?

Section IV: Accountability and possible solutions

14. Who do you think is responsible for protecting the environment?
15. What do you think are the three most important things that can be done to protect the environment?
16. Are you in favor of using the following management activities to improve environmental health?

	Yes	No	I don't know
Restricting coastal and inland development	☐	☐	☐
Improving solid waste management	☐	☐	☐
Limiting areas of free-grazing of goats	☐	☐	☐
Sterilization of free-roaming donkeys	☐	☐	☐
Prohibit the emission of untreated sewage water	☐	☐	☐
Other, specify:	☐	☐	☐

17. Do you believe individual action can make a difference in maintaining the health of the marine and land health?
18. In your opinion, are sewage treatment practices linked to conservation and environmental health?

Section V: Demographics

19. Are you originally from Bonaire?
20. How long have you been living in Bonaire?
21. If not, where are you from?

Physis (Spring 2014) 15: 79-85

REPORT

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Spatial distribution and severity of dark spots disease in Bonaire, Dutch Caribbean

Abstract Corals are the building blocks of coral reefs as they provide countless marine organisms with protection and habitat. However, coral diseases are currently threatening coastal environments by causing tissue loss and, in some cases, death of corals. This destroys the habitats utilized by marine organisms and the biodiversity of given areas. Many factors contribute to the prevalence of coral diseases, but very little is known about the overall impact of anthropogenic stressors on diseases. Dark spots disease (DSD) is a common coral disease found in the Caribbean and was the subject of this study. Dark spots disease prevalence and severity was quantified utilizing video transects and a severity index approximately one kilometer north of downtown Kralendijk on the west coast of Bonaire, Dutch Caribbean. This data was then analyzed for any trends with regards to spatial location and depth. It was observed that DSD is typically more common and severe at deeper depths of 15 m than at shallower depths of 8 m, although no trends were observed in regards to spatial location and DSD distribution. Gaining a better understanding of DSD distribution paves the way for future studies to potentially understand causative agents of DSD; therefore, allowing for more preventative measures and mitigation processes to conserve the health of coral reefs.

Keywords Dark spots disease • Spatial distribution • Severity

Introduction

Coral diseases pose a great threat to all coral reefs around the world by threatening the reefs with complete coral loss. Despite high coral disease prevalence and range, very little is known in regards to specific vectors or pathogens causing certain lethal diseases (Weil et al. 2006). Diseases cause damage to the coral tissue and can eventually lead to complete tissue loss (Richardson 1998). Tissue loss or damage can become lethal for corals. Coral loss leads to habitat destruction and a potential loss of marine organisms that seek shelter among the corals (Weil & Cróquer 2009).

It is know that climate change has played a large role in the increased frequency of coral diseases and the impacts of local stressors on these diseases and corals are beginning to be understood (Ateweberhan et al. 2013). Both microbes and external factors cause coral diseases. Microbes include bacteria, viruses, protozoa, and fungi; external factors encompass temperature changes, toxins, and nutrients (Harvell et al. 2007). Some microbial and external factors can be traced back to human actions, which provide further explanations for the current influx of disease.

The coral disease examined in this study is Dark spots disease (DSD). Dark spots disease is identified by dark purple to gray lesions on the coral tissue. The disease does not always result in complete tissue loss, but it can still be harmful to the coral structure. Currently, there are no known pathogenic causes of DSD (Muller and Woesik 2012). While it is expected that coral disease severity has increased as a result of climate change, there is insufficient information regarding the impact of anthropogenic stressors on local ecosystems. Therefore, this project aimed to analyze the spatial distribution and severity of DSD and to potentially better understand if there is a pattern or correlation in DSD within the Kralendijk area.

H_1: It is hypothesized that the prevalence and severity of Dark spots disease will exhibit a pattern along the west coast of Bonaire.

This study aimed to observe correlations between DSD abundance and severity, allowing for future research to provide more information regarding any potential effects of onshore anthropogenic stressors. It was anticipated that DSD would be more prevalent and severe closer to central Kralendijk due to the increased nutrient input of the city. As there is no known cause for DSD, this study and others could lead to greater awareness in the determination of a causative agent. A better awareness could aid in prevention and mitigation for overall coral reef health, which in turn could benefit surrounding marine habitats.

Materials and methods

Study site

The study sites selected are on Bonaire's fringing reef, which has a sandy sea floor extending from the coast out to the reef at a depth of 7 m, where the reef reaches the crest. The reef then gradually slopes down to 30 m and is primarily composed of massive and plate corals with a wide range of diversity. Bruckner (2011) found that coral cover accounts for 40-60% of the Bonaire's benthic community from 5-15 m.

DSD was selected as the disease of study due to its abundance along the coast of Bonaire. This study took place across three continuous sites (Fig. 1) on the west coast of Bonaire, north of the downtown Kralendijk area. Data collection began at the dive site Something Special (12°09'41.8"N 68°17'00.8"W) moving south to Yellow Submarine (12°09'36.5"N 68°16'55.2"W) to the Fisherman's Dock (12°09'29.6"N 68°16'49.3"W), and ended in front of the Venezuelan Consulate (Kaya Grandi No. 52) (12°09'22.2"N 68°16'45.6"W). Data was collected at each location at two depths: 10 m and 15 m.

Fig. 1 Study sites on the western coast of Bonaire. Markers from North to South correspond to Something Special, Yellow Submarine, Fisherman's Dock, and the Venezuelan Consulate. (Google Maps)

Methods

Both distribution and severity were assessed for this study. Distribution of DSD was evaluated utilizing video transects at each given dive site and depth. These transects ran for a length of 30 m and were approximately 1 m wide. Two video transects were conducted at each study site at 10 m and 15 m. Coral size was also quantified from the video transects by measuring the size of the diseased coral. Disease distribution was assessed based on the number of disease encounters at each specified dive site.

Dark spots disease severity was collected utilizing the rover diver technique at each specified dive site. The rover diver technique consisted of diving at each site for a total of 30 minutes. The first 15 minutes were spent at 15 m and the second 15 minutes at 10 m. During this time, corals with DSD were surveyed and quantified as a percent cover of disease using a 25 cm x 25 cm quadrat. Severity was measured and converted into an index according to the percent of coral tissue affected by DSD. This followed a scale of one through six, where, 1 = <10% of coral affected, 2 = 10-25% affected, 3 = 26-50% affected, 4 = 51-75% affected, 5 = 76-90% affected, and 6 = >90% of the coral was affected by DSD (Vega et al. 2014).

Data Analysis

Video transects were analyzed using Picture Motion Browser (PMB) and Coral Point Counter (CPCe) with 20 randomly scored points per image. Videos were trimmed into a series of individual photos on PMB and then imported to CPCe for analysis. CPCe analysis consisted of identifying all corals by species and marking all additional scores as 'other,' generating a list of all corals present and their relative diversity. The rover diver technique data was analyzed to observe any patterns between depth, location, or severity of DSD. This analysis consisted of comparing the number of instances and severity of DSD at 10 m and 15 m. These comparisons lead to observed trends with regards to DSD and its occurring depths.

Results

This study displayed the impact that depth has on the prevalence and severity of DSD. The compiled video transects data consistently showed that at 15 m DSD was more prevalent than at 10 m (Table 1). The data also yielded overall coral diversity for each dive site (Appendix 1). Coral diversity indicated that *Orbicella faveolata* (OFAV), a massive coral commonly affected by DSD, is typically one of the most abundant coral species and *Siderastrea siderea* (SSID), also a massive coral affected by DSD, is much less abundant than other corals. Data also showed that DSD prevalence was relatively consistent throughout the sites. At Something Special there were 30 reported coral colonies impacted by DSD, followed by 35 at Yellow Submarine, and 34 at both the Fisherman's Dock and at the Venezuelan Consulate.

Rover Diver data also showed that DSD is more prevalent at 15 m compared to 10 m (Fig. 2, Table 1). This data also showed that DSD severity is relatively consistent throughout the area with no apparent trends. Also, DSD is mildly severe as most of the data falls within a severity of one to three (Fig. 2).

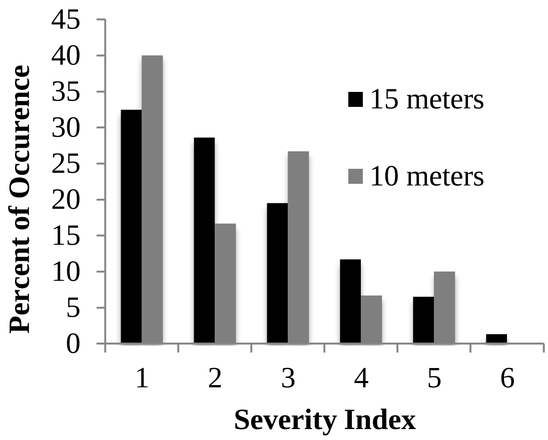

Fig. 2 Overall percent of each severity observed at 15 m (n=77) and 10 m (n=30) across three consecutive locations. Index operates on a scale of 1 being the least severe and 6 as the most severe. 1 (n=37), 2 (n=27), 3 (n=23), 4 (n=7), 5 (n=12), 6 (n=1)

Discussion

Results showed that DSD is typically more common at 15 m than at 10 m, which supports the hypothesis that there would be a pattern in DSD prevalence. There are three possible explanations for this phenomenon. One explanation is likely due to the overall distribution of corals. Average coral cover at 10 m was 13.5% compared to that of 15 m with an average coral cover of 25.5%. Subsequently, it is more likely that DSD will be found at 15 m because there are more corals for the disease to impact. Another explanation for DSD distribution could be attributed to the vector. Although there is currently no known vector for DSD, it is possible that the causative agent is found in higher abundance at deeper depths. Lastly, many diseases are known to fluctuate in regards to temperature (Gil-Agudelo & Garzón-Ferreira 2001). It has been observed that DSD incidences increase during months with warmer temperatures (July-October) (Gil-Agudelo & Garzón-Ferreira 2001). Therefore, it is possible that DSD is temperature specific inhibiting it from consistently growing at certain depths. Awareness of disease distribution offers itself to exploring more options for the causative agent of DSD.

Table 1 Data gathered from the video transects. The total coral cover (%) is the percent of coral over the 30 m transect, OFAV (%) and SSID (%) is the percent of each respective coral of the total coral cover (%), and the number of coral colonies with DSD is then dissected to represent which coral was diseased

Location	Depth (m)	Coral Cover (%)	OFAV (%)	SSID (%)	Number of Colonies with DSD	
					OFAV	SSID
Something Special	15	36.3	20.7	0.2	9	0
Yellow Submarine	15	23.9	35.9	2.6	1	2
Fisherman's Pier	15	26.3	9.1	1.8	6	1
Venezuelan Consulate	15	15.3	16.4	1.4	3	1
Something Special	10	25.6	9.5	1.4	0	1
Yellow Submarine	10	17.0	28.2	0	2	0
Fisherman's Pier	10	9.4	16.4	1.4	1	1
Venezuelan Consulate	10	2.1	16.7	0	0	0

Results also showed that DSD was consistently found on OFAV and SSID. While OFAV is typically one of the most abundant corals, SSID is typically one of the least common corals (Appendix 1). Therefore, it can be concluded that DSD abundance is not just a result of coral prevalence. It may be relevant that both OFAV and SSID are massive, or bouldering, corals. Future research relating to coral diseases and coral morphologies could potentially yield more results in regards to coral disease and host preferences.

Although there were no observed trends in regards to DSD and location along the coast, which does not support the hypothesis, it is possible that this is due to the locations' proximity to Kralendijk, because Kralendijk is an area with a wide gradient of human impacts. The city boasts a varied range of tourist activities from scuba diving to cruise ships. There is also a harbor (Harbour Village) in close proximity to the study sites as well as areas of high wastewater runoff. All of these factors have the potential to influence the distribution and severity of DSD as nutrient loading has been suggested to increase the severity of coral diseases (Bruno et al. 2003).

Overall, coral diseases pose a large threat to coral reef habitats and marine environments. Becoming more aware of causative agents and trends of coral diseases will allow for future work to be done regarding preventative measures. By preventing the spread of coral diseases, marine environments and coral reef habitats will be better maintained and preserved, saving overall biodiversity for future generations.

Acknowledgements I would like to thank Dr. Enrique Arboleda and Colin Howe for all of their support and guidance throughout this process. I would also like to thank B. Davis and N. Kleinas for their research assistance.

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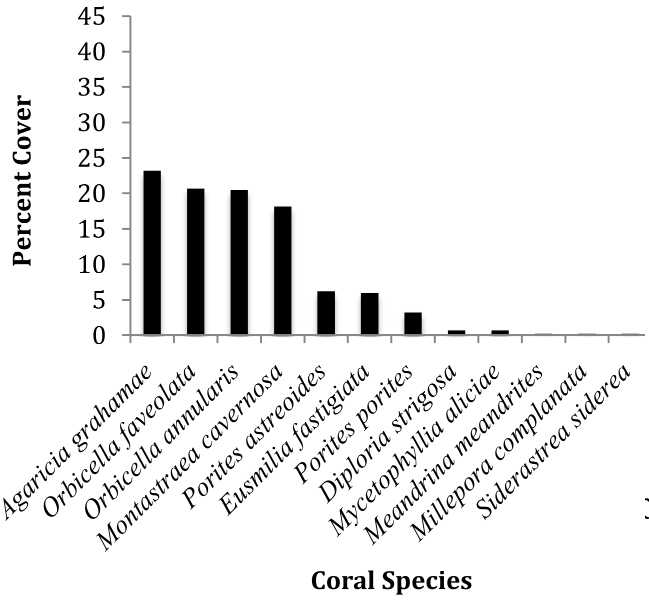
Appendix

a.



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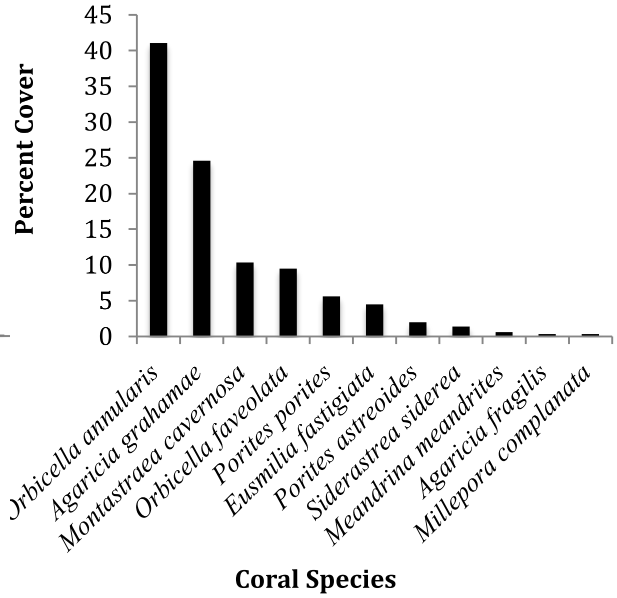

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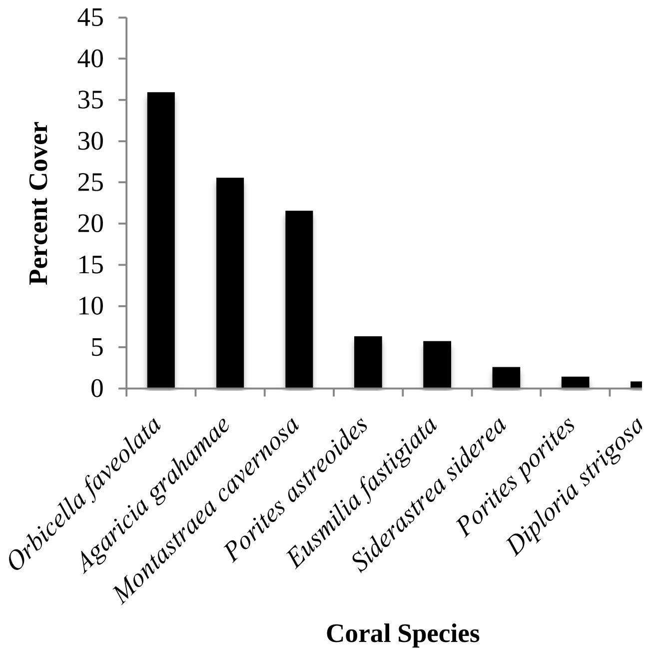

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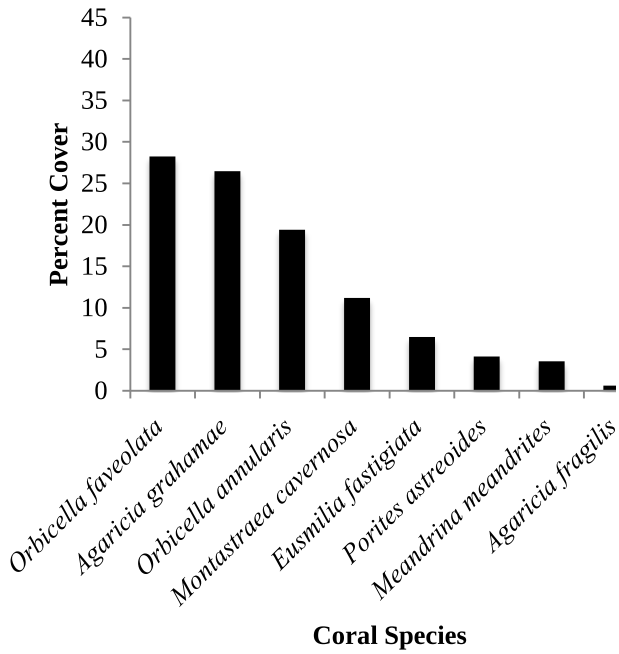

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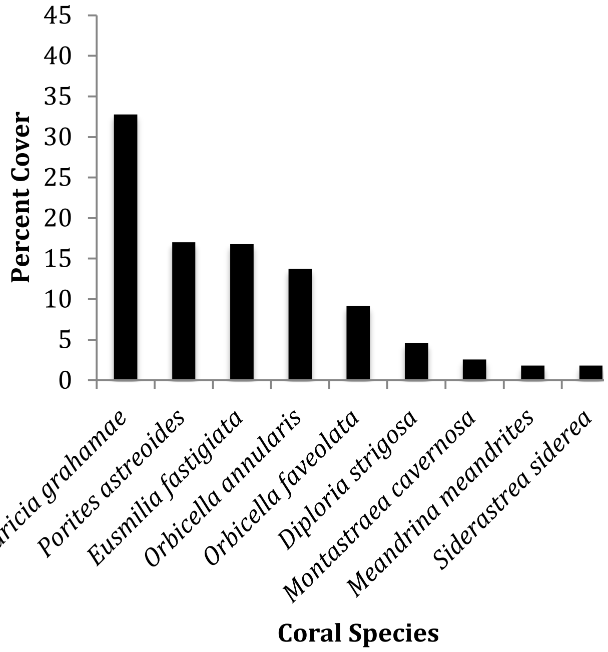

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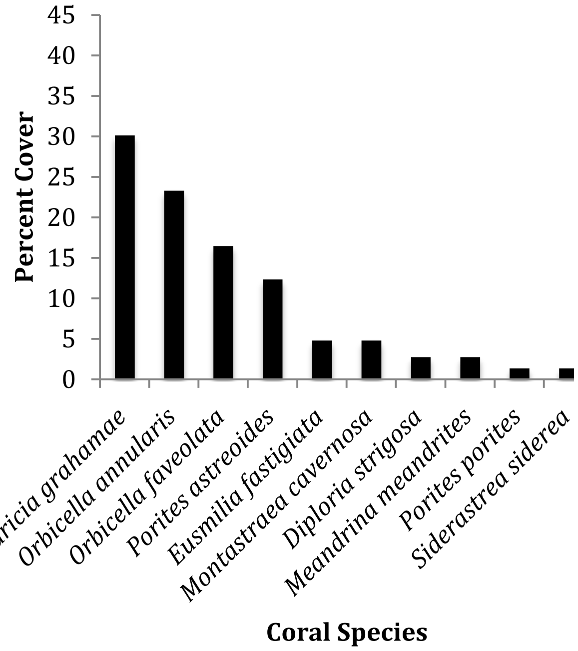

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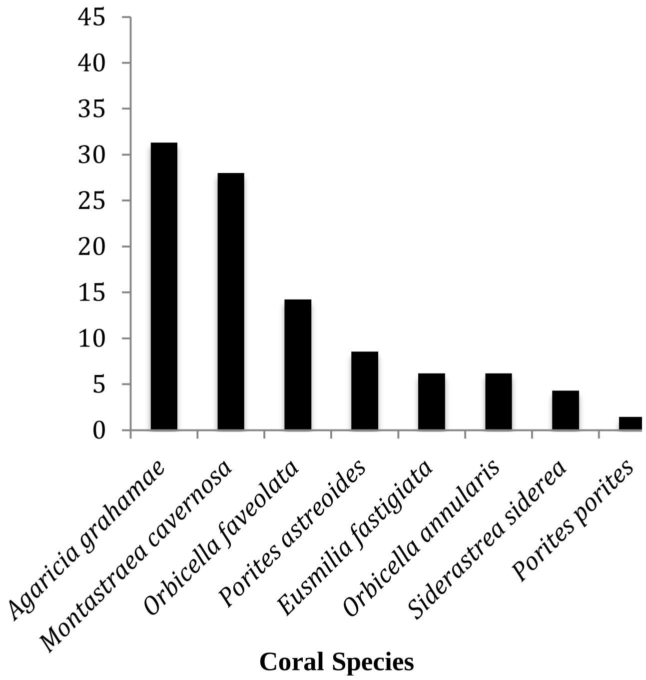

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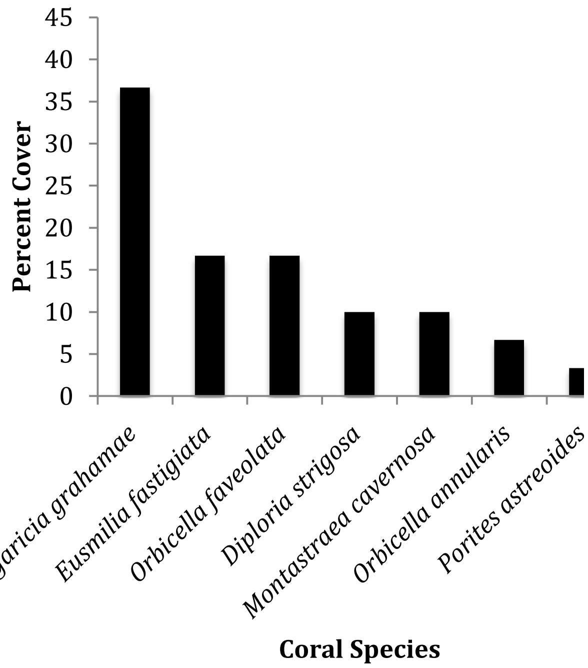

Fig. 1 Coral diversity at each dive site, as calculated with video analysis. a. Something Special at 15 m (n=435). b. Something Special at 10 m (n=358). c. Yellow Submarine at 15 m (n=348). d. Yellow Submarine at 10 m (n=170). e. Fisherman's Pier at 15 m (n=394). f. Fisherman's Pier at 10 m (n=146). g. Venezuelan Consulate at 15 m (n=211). h. Venezuelan Consulate at 10 m (n=30)