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“Good communication is essential for science. By translating complex science into understandable information and providing meaningful context behind the day’s headlines, we can equip decision-makers with the knowledge they need to take action on issues such as climate change, rebuilding our fisheries and protecting our oceans, coasts and Great Lakes. Our responsibility is to communicate clearly and accurately.” –Jane Lubchenco, former Under Secretary of Commerce for Oceans and Atmosphere and NOAA Administrator

Physis is an ancient Greek term for nature and the physical world. As scientists, we are constantly asking questions and seeking answers about the world in which we interact. We attempt to break down a complex system of interactions into smaller, more discernable branches of the whole. We go through years of schooling and research to understand these different branches of science. Each scientific field has developed it’s own dialect of specialized terms that articulate the overarching concepts of their research.

This knowledge, in turn, must be communicated to the political, economic, social, religious, and artistic aspects of society; yet, the terms used by each field are generally not understood by the layman. Too often scientists feel that their advice has fallen on deaf ears. We see this when political bodies set fishing limits that far surpass the levels advised by fisheries scientists. Likewise, only 53% of Americans believe that climate change is partially caused by humans, despite the 2013 IPCC report stating: “It is extremely likely that human influence has been the dominant cause of the observed warming in the mid-20th century.” There is a clear lack of effective communication between the scientific community and the public. As scientists, it is our prerogative not only to study physis, but also to translate that understanding of nature and the physical world to others. If we cannot communicate these findings, we are not succeeding as scientists.

At CIEE, we strive not only to study various aspects of marine ecology, but to communicate our findings with the broader community. Human beings rely on the marine environment for fishing, trade, tourism, and recreation, as well as religious and aesthetic value. Yet, each of us has our own unique interests and understanding of that system. As we begin to consider the various ways in which we all rely this environment, it becomes increasingly apparent how important it is to understand how we interact with our oceans. Through this publication, we attempt to disseminate our findings about specific aspects of the marine environment. Each of us has a duty not only to communicate this knowledge, but also to take the interests and perspectives of others into consideration. Only through effective communication can we support the broader community, as well as the marine ecosystem of which we are all members. We present to you Physis: Journal of Marine Science.

Hannah Rempel
CIEE Research Station Bonaire, Fall 2014
Foreword

The Council on International Educational Exchange (CIEE) is an American non-profit organization with over 150 study abroad programs in over 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 offers a one-of-a-kind opportunity designed for upper-level undergraduates majoring in Biology and other related fields. This program aims to provide an integrated and superlative experience in Tropical Marine Ecology and Conservation. The emphasis on field-based science 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 heavy and comprehensive course load, this program provides dive training that culminates 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 island’s population is concentrated. Students presented their findings in a public forum on 26 November, 2014 at CIEE Research Station Bonaire.

The proceedings of this journal are the result of each student’s research project, which are the focus of the course co-taught by Rita B.J. Peachey, PhD; Patrick Lyons, PhD; and Enrique Arboleda, PhD. In addition to faculty advisors, each student had an intern who was directly involved in logistics, weekly meetings, and editing student papers. The interns this semester were Jack Adams, Noah DesRosiers, Sasha Giametti, and Martin Romain. Astrid de Jager was the Dive Safety Officer and helped oversee the research diving program.

Thank you to the students and staff who participated in the program this semester! My hope is that we succeeded in our program goals and CIEE’s mission, and that the students succeeded in their individual goals as well.

Dr. Rita Peachey
Instructors

Dr. Rita Peachey is the instructor for Cultural & Environmental History of Bonaire, co-instructor of Independent Research, and the CIEE 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. Dr. Peachey is president of the Association of Marine Laboratories of the Caribbean.

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

Dr. Patrick Lyons is the instructor for Tropical Marine Conservation Biology, and co-instructor for Marine Ecology Field Research Methods and Independent Research. He also coordinates outreach activities for CIEE Bonaire including lessons with the Bonaire National Marine Park Junior Rangers and Jong Bonaire children, public talks, and community events. Dr. Lyons’ research interests are broad in scope and include ecology and evolution in marine mutualisms, predator-prey interactions between invasive species and native prey, and diver impacts on coral reefs.
**Staff**

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

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

Molly Gleason is the laboratory technician at the CIEE Research Station. She graduated with a M.S. in Biology from the 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 effects 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 of Bonaire.

Mary DiSanza was born and raised in Colorado, a state with a long-term commitment to protecting the environment. Computers, banking, and law gave way to scuba diving and travel, while skis were traded in for dive gear. Mary worked as a Dive Instructor and Retail Manager for a dive shop on Bonaire for several years before branching out to the resort management side of the business. She is now part of the administrative staff at CIEE Research Station.
Interns

**Jack Adams** is the Marine Ecology Field Research Methods and Cultural & Environmental History of Bonaire co-intern. He studied Environmental Science at the University of Leeds in the UK. Jack has travelled to Indonesia and studied habitat complexity of coral reefs and its effects on fish communities for his final project at the university. After graduating, he completed his Divemaster in Honduras.

**Noah DesRosiers** is the Tropical Marine Conservation Biology and Dive Safety intern. He has his Bachelors in Marine Sciences from the University of Miami / RSMAS. Noah holds a Masters in Fisheries Ecology from the King Abdullah University of Science & Technology in Saudi Arabia where he studied the demographics of grouper populations in the Red Sea. He studied reefs at James Cook University in Australia and has worked as a freelance scuba instructor and zoological collector in the Philippines.

**Sasha Giametti** is the Coral Reef Ecology Intern. She recently graduated from Eckerd College with a B.S. in Marine Science. Her previous endeavors in marine environments include surveying the reefs of Tobago, sailing through the Sargasso Sea, and sampling invertebrates in Hawaiii. She plans to continue her education in biological oceanography through a Masters program in the future.

**Martin Romain** is the Marine Ecology Field Research Methods and Cultural & Environmental History of Bonaire co-intern. Originally from Belgium, he graduated with the Erasmus Mundus Master of Marine Biodiversity and Conservation (EMBC) in 2012. His thesis focused on the juvenile blacktip reef sharks (*Carcharhinus melanopterus*) of French Polynesia. He then joined the team of the Marine Megafauna Foundation where he studied the whale shark (*Rhincodon typus*) population of Mozambique (Tofo).
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Sydney Bickerstaff • University of South Carolina • sydneybickerstaff94@gmail.com

Comparison of foraging strategies and feeding rates of the Atlantic Trumpetfish, *Aulostomus maculatus*

**Abstract** Coral reef fish exhibit remarkably diverse hunting techniques such as solitary hunting, shadow stalking, nuclear hunting, and hunting in schools of fish. This study examines the differences in feeding rates of the Atlantic Trumpetfish, *Aulostomus maculatus*, while it utilizes four dissimilar foraging strategies. Observations were completed in Bonaire, Dutch Caribbean while SCUBA diving to record *A. maculatus* striking at its prey. Feeding rates were calculated from the number of bites at prey during an observation period, in order to rank the strategies. Although consumption of prey was not determined, it is expected that feeding rate will track the number of bites at prey items and is used as a proxy for feeding rate in this study. Solitary foraging was hypothesized to exhibit the highest feeding rate due to its high prevalence on the reef, followed by shadow stalking, nuclear hunting, and hunting in schools. Competition for prey during associations with other fish and rarity of dense aggregations of schooling fish was thought to support the hypothesis. In this study, the feeding rate during solitary foraging was found to be significantly lower than shadow stalking, nuclear hunting, and hunting in schools, which were not significantly different from each other. The results indicate that *A. maculatus* forage more successfully in groups and exhibit multiple foraging strategies to exploit prey most efficiently. The hunting behavior of *A. maculatus* affects prey and other associated species, thus understanding this behavior may lead to further knowledge of other predatory fish and interspecific interactions.

**Keywords** Group hunting • Shadow stalking • Solitary hunting

**Introduction**

Fish exhibit a wide variety of hunting behaviors to capture prey as efficiently as possible. Some predatory fish have evolved to utilize physical camouflage to avoid recognition by prey (Darimont and Child 2014). Bar jacks, *Caranx ruber* (Carangidae), swim among prey for several hours until the prey no longer recognize the bar jack as a threat (Hobson 1975). Lizardfishes (Synodontidae) avoid early detection by camouflaging with the substrate, remaining immobile, and striking when prey come within range (Hobson 1975). In addition, moray eels (Muraenidae) remain in crevices on coral reefs to take advantage of prey fish that also use crevices for shelter from other predators (Hobson 1975).

*Aulostomus maculatus*, the Atlantic Trumpetfish, is a piscivore that uses a wide range of foraging strategies including solitary hunting, shadow stalking, nuclear hunting, and hunting in schools of other fish (Aronson 1983; Helfman 1989; Kaufman 1976). Solitary *A. maculatus* hover among gorgonians, sponges, and mooring lines to stalk and ambush prey at short distances (Aronson 1983; Auster 2008). To remain undetected by prey, *A. maculatus* may shadow stalk parrotfish (Scaridae) or Spanish hogfish (Labridae) by following along the dorsal fins of other similarly sized fish species on the reef (Kaufman 1976; Lukoschek and McCormick 2000). *A. maculatus* may also forage with bar jacks (Carangidae), sharptail...
eels (Ophichthidae), and goatfish (Mullidae) in a strategy known as nuclear hunting (Deloach and Humann 1999; Lukoschek and McCormick 2000). In addition, *A. maculatus* hunt for prey among schools of chromis (Pomacentridae) and surgeonfishes (Acanthuridae), which allows *A. maculatus* to remain concealed from prey (Auster 2008). Each strategy, with the exception of solitary foraging, involves multiple species, often between members of different trophic levels (Lukoschek and McCormick 2000).

Previous studies have demonstrated that *A. maculatus* hunt primarily in solitude (Aronson 1983). Shadow stalked fish, such as parrotfish; often display aggression towards *A. maculatus* possibly causing shadow stalking to be a less effective strategy than foraging solitarily (Aronson 1983). Nuclear hunting engages other fish that consume the same prey as *A. maculatus*, resulting in competition for food in this foraging strategy (Lukoschek and McCormick 2000). Thus, *A. maculatus* may not have as many opportunities to bite in comparison to foraging solitarily or shadow stalking. The lowest feeding rate could occur when *A. maculatus* hunt in schools because this behavior is only effective when schools are extremely dense. Chromis and surgeonfishes, primarily schooling reef fish, are rarely present in large enough aggregations for *A. maculatus* to successfully feed (Auster 2008). Previous observations and current knowledge have led to the hypothesis that compares the feeding of *A. maculatus* utilizing different foraging strategies.

**H1:** It was hypothesized that *A. maculatus* will exhibit the highest feeding rate while foraging solitarily, followed by shadow stalking, nuclear hunting, and hunting in schools.

Prior studies have focused on individual techniques and other fish with which *A. maculatus* associate. This study provided a new perspective on the feeding rates of *A. maculatus* utilizing the four foraging strategies. A comprehensive view of each foraging strategy and its corresponding overall feeding rate were compared to assess the most efficient strategy by analyzing the number of bites at prey. This comparison is novel and will lead to more knowledge on why *A. maculatus* exhibit this combination of foraging strategies. Understanding the variation in predatory behavior of *A. maculatus* contributes to overall knowledge of unique strategies adopted by predators to exploit prey (Auster 2008).

**Materials and methods**

**Study site**

This experiment was completed at three sites on the island of Bonaire, Dutch Caribbean. Bonaire is located in the southeastern Caribbean and is known for the high biodiversity found in the fringing coral reefs surrounding the island (Sommer et al. 2011). The study locations included dive sites Yellow Submarine (12°9’33”N 68°16’55”W), Something Special (12°09’40.1”N 68°17’00.0”W), and Margate Bay (12°3’3”N 68°16’20”W). Yellow Submarine is roughly 200 m south of Something Special, and both sites are off the coast of the populous city Kralendijk on the western side of the island. At both sites the coral reefs begin 20 m away from shore at a depth of 6 m, extending to a depth of 30 m. Margate Bay is located on the southwestern point of the island, away from human populated areas. The coral reef begins 25 m away from shore at a depth of 6 m, extending to a depth of 30 m. The primary substrate at all three sites between the shore and reef crest is sand, where *A. maculatus* are commonly observed.

**Study organism**

*A. maculatus* inhabit coral reefs in the West Atlantic ranging from a depth of 5 to 25 m. *A. maculatus* have long, thin bodies that vary in size from 15 to 70 cm and exhibit colorations including brown, yellow, and pale purple and can darken or lighten the initial coloration. *A.*
A. maculatus often display vertical and horizontal stripes, dark spots, and blue snouts. A. maculatus primarily consume small fish such as juvenile grunts (Haemulidae) and chromis (Pomacentridae) while solitary hunting, shadow stalking, nuclear hunting, and hunting in schools of other fish (Deloach and Humann 1999).

Data collection and analysis

Observations of foraging A. maculatus were recorded at Yellow Submarine, Something Special, and Margate Bay while SCUBA diving. Randomly selected individuals were observed for a maximum of 5 minutes and number of bites, foraging strategy, depth, coloration, and size of the fish were recorded. Foraging strategies were recorded as solitary, shadow stalking, nuclear hunting, or hunting in schools of other fish. Bites min\(^{-1}\) were calculated and compared using a one-way analysis of variance (ANOVA; \(\alpha = 0.05\)) and a Tukey pairwise comparisons post-hoc test (Tukey 95% CI).

**Results**

A total of 12 h was spent observing 60 A. maculatus foraging behaviors including; solitary foraging (n=21), shadow stalking (n=20), nuclear hunting (n=11), and hunting in schools of other fish (n=8). A. maculatus exhibited the highest feeding rates (mean ± SD bites min\(^{-1}\)) during nuclear hunting (1.27 ± 0.85 bites min\(^{-1}\)), hunting in schools (1.25 ± 1.00 bites min\(^{-1}\)) and shadow stalking (1.06 ± 0.85 bites min\(^{-1}\)). Solitary foraging exhibited the lowest feeding rate of the four strategies observed (0.34 ± 0.37 bites min\(^{-1}\)) (Fig. 1).

Feeding rates were compared among the foraging strategies with a 1-way ANOVA and a Tukey pairwise comparisons test. There was a significant difference among feeding rates while utilizing the different foraging strategies: solitary foraging, shadow stalking, nuclear hunting, and hunting in schools (ANOVA; \(df = 3, p = 0.002\)). The results of the post-hoc test indicated that solitary hunting feeding rate was significantly lower than shadow stalking, nuclear hunting, and hunting in schools (Tukey 95% CI).

![Mean feeding rates of A. maculatus](image)

**Discussion**

A. maculatus exhibited the lowest feeding rate while foraging solitarily. Shadow stalking, nuclear hunting, and hunting in schools of fish had higher feeding rates. These results refute the initial hypothesis that A. maculatus would have the highest feeding rate while foraging solitarily, followed by shadow stalking, nuclear hunting, and hunting in schools. The contrast between the initial hypothesis and results may be caused by including solitary A. maculatus that were not actively foraging. Solitary hunters often displayed zero bites min\(^{-1}\) and it is possible that A. maculatus was not actively hunting at all times when solitary. Using field observations, it may be impossible to distinguish between solitary hunting and resting behavior of A. maculatus.

One potential benefit of shadow stalking over solitary hunting is that it provides cover to gain access to prey that is difficult to locate and cautious of predation (Lukoschek and McCormick 2000). However, costs or benefits of this interaction have yet to be determined.
hunting, shadow stalking, nuclear hunting, and efficiency of forage more successfully. It appears that different strategies of foraging are being adopted by various species. The present study suggests that multiple foraging strategies may be beneficial, as they can help in locating and catching otherwise unattainable prey. For instance, Acanthurus coeruleus forages more efficiently in damselfish territory than Sparisoma aurofrenatum because A. coeruleus has a preference for group hunting.

Individuals benefit by foraging in groups to avoid aggression from other organisms and increase consumption of prey. A study conducted on wolves, Canis lupus, demonstrated a preference for group hunting over solitary foraging. Although wolves are forced to share food in large packs, the benefit of avoiding losses of prey to other aggressive scavengers and increased prey capture outweigh the cost of sharing food (Vucetich 2004). Similar to the observed behavior of wolves, a review was conducted on multi-species foraging in fishes that concluded locating and catching otherwise unattainable prey was more successful during group feeding than solitary feeding (Lukoschek and McCormick 2000). The advantages of social foraging were most beneficial when multiple species combined searching skills (Lukoschek and McCormick 2000). According to the results of the present study, A. maculatus appear to be adopting similar strategies while nuclear hunting and hunting in schools to forage more successfully.

Future studies could assess the capture efficiency of A. maculatus during solitary hunting, shadow stalking, nuclear hunting, and hunting in schools using high-speed videography. Feeding rates are only an estimation of foraging strategy success, whereas capture efficiency denotes exactly which strategy allows A. maculatus to consume the most prey. Another future analysis could compare capture efficiency while A. maculatus forages with fish from different guilds, such as Spanish hogfish or parrotfish, to provide more insight into advantages and disadvantages of different hunting strategies.

In the present study, four different foraging strategies that A. maculatus utilizes were compared by assessing mean feeding rates. Shadow stalking, nuclear hunting, and hunting in schools exhibited higher feeding rates than solitary foraging. This comparison had not been completed before and leads to a new perspective on the advantages of group hunting in predatory fish when compared to solitary hunting.

**Acknowledgements** I would like to thank my advisor Dr. Peachey for her guidance and advice on my project. Intern and research buddy Sasha Giampietri deserves a big thank you for her assistance in finding trumpetfish in the field and for her overall helpfulness. Thanks to CIEE Research Station for making this study possible. Further thanks to Katie Engel in assisting me in a research dive. Final thanks is extended to Allison Frey and Elizabeth Gugliotti for their moral support that kept me sane throughout this process.

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Investigation of depth-dependence of pumping rates and filtration efficiencies in two Caribbean reef sponges

Abstract Suspension feeders perform a crucial role in uniting the benthic and pelagic environments in coral reef ecosystems. Of suspension feeders, sponges are one of the most highly abundant, widespread, and efficient filter-feeding organisms. However, suspension feeding in sponges is not completely understood. Previous studies have looked at the effect of temperature on pumping rate as well as the effect of particle size on retention rate. The purpose of this study was to investigate the depth-dependence of pumping rates and filtration efficiencies in two Caribbean reef sponges, *Aplysina archeri* and *Aplysina lacunosa*, at two depth profiles. Videos were taken of sponges pumping fluorescein dye to obtain pumping rates, and turbidity measurements were taken of both inhalant and exhalant water samples that were collected in situ via syringes in order to estimate filtration efficiency. The results revealed a species-specific interaction with depth for both pumping rate and filtration efficiency. *Aplysina lacunosa* was found to have both a faster pumping rate and increased filtration efficiency at the shallower depth, while no differences were observed across depths for *A. archeri*. Additionally, correlations were found between pumping rate and filtration efficiency for both species, suggesting the development of distinct filter-feeding strategies. *Aplysina lacunosa* had a positive correlation between pumping rate and filtration efficiency, while the correlation in *A. archeri* was found to be negative. Understanding the effect of depth on the filter-feeding mechanism of sponges is important to understanding the greater implications of the benthic-pelagic coupling process of sponges and suspension feeders in general.

Keywords *Aplysina archeri* • *Aplysina lacunosa* • Suspension feeding

Introduction Suspension feeders are a dominant presence in the benthic marine environment and are responsible for a majority of the energy flow from the pelagic zone to the benthic zone. They have evolved a unique trophic strategy that allows them to filter out and capture living organisms and particulate matter in the water column (Gili and Coma 1998). However, in order to fulfill their nutrient requirements, filter feeders must filter large quantities of water because of the dilute nature of the food particles in suspension (Riisgard and Larsen 1995). Despite this, suspension feeding is an extremely efficient process with no energetic input required for passive suspension feeding and only a 4% energy demand for pumping in active suspension feeders (Gili and Coma 1998). Additionally, the ability of both active and passive suspension feeders to share the same habitat makes this community of benthic suspension feeders one of the most efficient communities in the marine environment in terms of obtaining and processing energy, in what is known as benthic-pelagic coupling (Gili and Coma 1998).

The process of and mechanisms behind suspension feeding have been heavily studied, specifically in bivalves (Jorgensen 1975). Riisgard and Larsen (1995) have described in
detail the energetics and engineering principles behind the pump and filter system of various filter-feeding organisms. They have shown that pumping rates and temperature are positively correlated in bivalves (Riisgard and Larsen 1995). Pumping rates have also been shown to vary across habitats in a number of epifaunal and infaunal bivalves (Jorgensen 1975). Additionally, Gili and Coma (1998) have pointed out various other factors that influence capture rates of bivalves, including food concentration, energy availability, and organism size, while also suggesting that depth may have an impact on suspension feeding.

Of the suspension feeders, sponges are one of the most abundant and widespread organisms in marine ecosystems (Diaz and Rutzler 2001; Gili and Coma 1998). They provide a crucial role by filtering large volumes of water and extracting nutrients, suspended particles, and other food items such as free-living bacteria and phytoplankton from the water column (Bell 2008; Lesser 2005). The combination of their high abundance in many benthic habitats and their contribution to benthic-pelagic coupling make them an extremely important link between the benthic and pelagic environments. The importance of this complex interaction is evident as abrupt declines in sponge population have the potential to cause cascading disturbances in an ecosystem, including changes in water chemistry and loss of commensal species (Bell 2008; Butler et al. 1995).

Despite their critical role of coupling the benthic and pelagic environments, sponges and their suspension feeding systems are not completely understood (Bell 2008). Like other suspension feeding organisms, sponges have been shown to have elevated pumping rates at higher temperatures (Riisgard et al. 1993). However, compared to other filter feeders, sponges have lower pumping rates. Thomassen and Riisgard (1995) suggested that this is accounted for through increased retention of small particles as compared to other filter-feeding invertebrates. A study by Duckworth et al. (2006) supports this, showing that sponges display an increased percent retention for smaller particles (Duckworth et al. 2006). Other studies have found that sponges acquire more energy, use less of it, and grow significantly more at depth than at shallow sites because of greater food availability (Lesser 2006; Trussell et al. 2006).

This study aims to further these investigations by analyzing the impact of depth on pumping rate and filtration efficiency in two Caribbean reef sponges, Aplysina archeri and Aplysina lacunosa, in order to expand the realm of knowledge surrounding suspension feeding in sponges. Based on previous research, the following hypotheses have been developed:

H<sub>1</sub>: Sponges at depth will exhibit greater pumping rates and higher filtration efficiencies
H<sub>A</sub>: Sponges will exhibit greater pumping rates and higher filtration efficiencies at a shallower depth
H<sub>0</sub>: Sponges will exhibit no difference in pumping rates or filtration efficiencies across depths

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**Materials and methods**

**Study site and study organisms**

All data were collected at the Yellow Submarine dive site (12°09’36.3”N, 68°16’54.9”W) in northern Kralendijk, located on the western coast of Bonaire, Dutch Caribbean. The experimental work was conducted on the reef slope at two different depth ranges: 6-12 m (shallow) and 20-26 m (deep). Due to their high abundance at the study site and an ability to grow at both depth categories, two species of demosponges were selected for this study: A. archeri (stove-pipe sponge) and A. lacunosa (convoluted barrel sponge).

Both A. archeri and A. lacunosa are large, upright tube sponges. Oftentimes, multiple tubes extend from a single base. Aplysina archeri generally reach heights of 0.5-1.5 m with fewer tubes, whereas A. lacunosa are
generally shorter at 0.3-1.0 m with a greater number of tubes. At Yellow Submarine, both species can be found across both depth categories; however, they are both seemingly more abundant in the shallow depth range, where they appear to be equally established. Conversely, at depth, *A. archeri* appears to be more prevalent than *A. lacunosa*. Additionally, it is key to note that *A. lacunosa* is frequently covered in filamentous macroalgae, (with this occurring more often in shallower water) while *A. archeri* is not.

Measurement of sponge pumping rate

Sponges were filmed *in situ* using a GoPro Black Hero 3+ video recording device. A SCUBA diver ejected fluorescein dye from a syringe near the base of the sponge. The pumping action of the dye out of the osculum was recorded, with a ruler placed in the frame as a scale. The movement of the dye-front was measured using frame-by-frame analysis in QuickTime X. Pumping rate was calculated using the known values of distance and time and reported in m s\(^{-1}\). Three dye-fronts were tracked for each individual and the calculated pumping rates were averaged.

Inhalant-Exhalant (InEx) water sampling

A modified InEx method, as described by Yahel et al. (2005), was used for the collection of inhalant and exhalant water samples. Two SCUBA divers simultaneously drew water samples using 50ml syringes from next to the ostial surface (inhalant) and from within the osculum (exhalant), without physically contacting the sponge. These samples were then brought to lab for turbidity analysis and stored at 4°C.

Turbidity analysis

For turbidity analysis, a Turner Designs Trilogy Laboratory Fluorometer was used. Turbidity readings were given in nephelometric turbidity units (NTU). The fluorometer was calibrated using 0.1, 1, 10, and 100 NTU solutions, which were prepared from a 1000 NTU stock solution via serial dilutions. Water samples from each individual were then directly transferred to plastic cuvettes and the turbidity was measured using the fluorometer. Percent reduction in turbidity between the inhalant and exhalant samples was calculated for each individual. This was used as an estimate for filtration efficiency of suspended particles.

Data analysis

Two separate two-way analyses of variance (ANOVA) were performed to analyze the effect of sponge species and depth on both pumping rate and percent reduction in turbidity. Subsequent post-hoc unpaired t-tests were performed to investigate the significance of the effect of depth on both response variables. Additionally, a linear regression was run to examine the relationship between pumping rate and percent reduction in turbidity.

**Results**

**Pumping rate**

Sponges were injected with fluorescein dye and the pumping process was filmed. Three dye-fronts were traced for each individual and the calculated pumping rates were averaged for both species at both depths. A two-way ANOVA found that pumping rate was not affected by species (F = 0.523, p = 0.474), but rather by depth (F = 9.75, p < 0.01). Additionally, there was a significant interaction between species and depth (F = 4.44, p < 0.05). *Aplysina archeri* was found to have no significant change in pumping rate across depth categories (deep: 0.162 ± 0.015 m s\(^{-1}\); shallow: 0.180 ± 0.012 m s\(^{-1}\); t = -0.91, p = 0.713; Fig. 1). Conversely, *A. lacunosa* exhibited a significantly lower pumping rate at depth (deep: 0.130 ± 0.021 m s\(^{-1}\); shallow: 0.218 ± 0.016 m s\(^{-1}\); t = -3.33, p < 0.05; Fig. 1).
Turbidity

InEx water samples were measured for turbidity using a fluorometer. A decrease was observed between the average turbidity of inhalant and exhalant water samples for A. archeri at depth \((t = 2.57, p < 0.05)\) and A. lacunosa at the shallow site \((t = 4.93, p < 0.01; \text{Fig. 2})\). However, the InEx turbidity values were not significantly different for A. lacunosa at depth \((t = 1.84, p = 0.116)\) and were only marginally significant for A. archeri at the shallow site \((t = 2.19, p = 0.065; \text{Fig. 2})\). Further, the inhalant turbidity values were significantly different for A. lacunosa across depth categories \((t = -3.38, p < 0.01; \text{Fig. 2})\). This was not observed in A. archeri or in the exhalant turbidity values for A. lacunosa (Fig. 2).

Percent reduction in turbidity was calculated for each sample and averaged for both species at both depths. A two-way ANOVA found that percent reduction in turbidity was not affected by depth \((F = 1.71, p = 0.202)\) or species \((F = 0.03, p = 0.871)\), but was found to have a significant interaction between the two \((F = 4.39, p < 0.05)\). Aplysina archeri demonstrated a higher percent reduction in turbidity at depth \((53.52 \pm 10.30\%; t = 0.38, p = 0.712)\), while the opposite was observed in A. lacunosa \((27.97 \pm 13.79\%; t = -2.73, p < 0.05; \text{Fig. 3})\).

Relationship between pumping rate and percent reduction in turbidity

Pumping rates were plotted against percent reductions in turbidity for both A. archeri and A. lacunosa. Linear regression analysis was performed for each species. A moderate positive correlation was observed between pumping rate and percent reduction in turbidity for A. lacunosa \((R^2 = 0.355; \text{Fig. 4})\). On the other hand, pumping rate and percent reduction in turbidity were found to have a negative correlation for A. archeri \((R^2 = 0.539; \text{Fig. 4})\). Both regressions were found to be statistically significant \((A. archeri: F = 6.06, p < 0.05; A. lacunosa: F = 14.04, p < 0.01; \text{Fig. 4})\).
Discussion

This study sought to determine the impact of depth on pumping rate and filtration efficiency in two sponge species. The results reveal a species-specific interaction with depth for both pumping rate and filtration efficiency. Previous research has shown that sponges acquire more energy, use less of it, and grow significantly more at depth (Lesser 2006; Trussell et al. 2006). This could suggest increased filtration efficiency at greater depths. Yet, other studies have found that temperature and pumping rate are positively correlated in sponges (Riisgard et al. 1993) and bivalves (Riisgard and Larsen 1995). This would suggest increased pumping rate in warmer, shallower water. However, these studies did not directly examine the effect of depth and observing such a temperature change at the current study site would require going to depths beyond the scope of this study.

Contrary to expectation, A. lacunosa was found to pump faster and filter more efficiently at a shallower depth, while A. archeri was found to have no difference in either pumping rate or filtration efficiency across depths. These results disagree with the primary hypothesis posed in this study, which postulated that an increase in pumping rate and filtration efficiency at depth would be observed. Instead, the results show that the interaction with depth is species-specific and offer support for both the alternate and the null hypothesis for A. lacunosa and A. archeri, respectively.

Additionally, the results highlight a distinct difference in filter-feeding mechanisms between the two species. Aplysina lacunosa demonstrated a positive correlation between pumping rate and filtration efficiency, while A. archeri exhibited a negative correlation. This indicates that A. archeri is more efficient at filtering at a slower pumping rate, while A. lacunosa filters more efficiently when pumping faster. Thus, the lower pumping rate of A. lacunosa that was observed at depth corresponds with inefficient filtration. On the other hand, A. archeri does not experience a depth-dependent difference in pumping rate or filtration efficiency. Combined, this suggests that A. archeri is more of a generalist, while A. lacunosa is more specialized to the shallower habitat. Previous studies have indicated that

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**Fig. 4** Relationship between pumping rate and percent reduction in turbidity for two sponge species, *A. archeri* (shaded) and *A. lacunosa* (unshaded), at two different depth categories, deep (circles) and shallow (squares). Linear regression lines are plotted for both species, *A. archeri* (solid) and *A. lacunosa* (dashed). R-squared statistics are displayed for each.
certain sponges are capable of specializing to different habitats, while others (generalists) are more widely distributed (Diaz et al. 2004). This apparent specialization of *A. lacunosa* for the shallow depth might account for the perceived drop in its relative abundance at depth compared to *A. archeri*. The inefficient filtration of *A. lacunosa* found at greater depths might prevent it from extracting the nutrients it needs from the water column. Thus, the consistency of efficient filtration in *A. archeri* might allow for it to be better suited for deeper environments than *A. lacunosa*. Gili and Coma (1998) put forward that such an observed change in strategy of suspension feeding along a depth gradient is commonplace. However, a possible alternative explanation for the observed shifts in abundance is provided by Wulff (2000), who has shown that sponge predators can induce compositional changes in sponge communities. While more information must be collected to make such a definitive claim for the reasoning behind the observed shifts in abundance of *A. archeri* and *A. lacunosa*, it is evident that the two species of sponges have developed different filter-feeding strategies. However, more studies are required to investigate the differences between and implications of these two different strategies in these sponge species.

Other avenues for future research could include expanding the repertoire of species studied. This study shows that the effect of depth on pumping rate and filtration efficiency is species-specific. Understanding how depth affects the suspension feeding system in a wide range of sponge species could provide useful insight into the structure of sponge communities within the greater picture of coral reef communities. Additionally, future studies should consider diversifying the methods used to estimate filtration efficiency. Analyzing the particle size content of both inhalant and exhalant water samples across depth categories could offer supplementary information regarding the distribution of the items being filtered out, in addition to providing another estimate of filtration efficiency. An investigation into the compositional change in the bacterial community that passes through different sponges at various depths could also contribute to the understanding of the microbial communities that are harbored within marine sponges, while serving as another measure for filtration efficiency.

Despite their high abundance on many coral reef communities around the world and the many functional roles they play for the marine ecosystem, sponges are underrepresented in the research world (Bell 2008). Specifically, their suspension feeding system and the factors that influence the normal pumping process are understudied and much is still unknown. This study provides evidence for the species-specific effect of depth on both pumping rate and filtration efficiency in two Caribbean reef sponges. From this study, it can be deduced that *A. archeri* and *A. lacunosa* have developed different filter-feeding strategies, which may explain their differing depth profiles. Understanding the effect of depth on the filter-feeding mechanism of sponges is important to understanding the greater implications of the benthic-pelagic coupling process of suspension feeders, which is responsible for a majority of the energy flow from the pelagic to the benthic community on a coral reef ecosystem.

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The effects of density and size on the hiding response of Christmas tree worms (Spirobranchus giganteus)

Abstract  Hiding is a common anti-predatory behavior that many organisms utilize. This anti-predatory tactic is adjusted to minimize factors such as lost time spent foraging and reproducing. Variation in hiding exists to minimize these costs while optimizing the benefit of predator avoidance. The hiding behavior of Christmas tree worms, Spirobranchus giganteus, was observed using an artificial stimulus to assess how density (number of individuals cm$^{-2}$), existence within an aggregation or as a solitary individual, the nearest neighbor behavior, and body size affect the re-emergence time of the worms after hiding. This study also assessed the natural hiding responses of S. giganteus using videos. Individuals that were solitary had significantly longer re-emergence times than individuals that were part of an aggregation. These results emphasize the benefits of aggregated living in reducing the hiding time of Christmas tree worms. In aggregations, the re-emergence times of an indirectly stimulated individual increased with distance from the directly stimulated worm. These results could be indicative of a communication system within aggregations. Within these aggregations, re-emergence times were consistent regardless of size whereas solitary individuals had significantly longer re-emergence times as the size of the worm increased. Variations in hiding times illustrate the importance of refined behavioral decisions in animals. Hiding behaviors of aggregated individuals could be a useful tool in studying community dynamics, specifically the existence and mechanisms of communication between individuals.

Keywords  Predator avoidance • Aggregated living • Re-emergence

Introduction

Prey may alter foraging and mating behavior in their avoidance of predators (Cotton et al. 2004). The alteration of behavior indicates how trade-offs exist between the benefit of avoiding predators and the cost of spending less time foraging and mating. These trade-offs have led to the study of the benefits and costs of anti-predator decision-making (Lima 1998). An optimal balance is thought to exist between the costs and benefits of predator avoidance.

Hiding is a type of anti-predator behavior utilized by a variety of organisms such as barnacles, tubeworms, and turtles (Dill and Fraser 1997). Organisms withdraw into a protective structure while waiting for a predator to leave. Re-emergence is an obstacle that an animal must overcome due to the lack of ability to detect if the predator has left (Dill & Fraser 1997). As the time spent hiding by an organism increases, the risk of re-emergence decreases because predators search for prey elsewhere (Dill & Fraser 1997). Variation in hiding duration has been attributed to differences in the costs and benefits in responding to predators by prey. Hiding time has been considered as a trade-off between food acquisition and predator avoidance (Krivan 1996). Accurate predictions about an organism’s optimal hiding time are difficult to make because variation in hiding time is attributed to different factors (Jennions et al. 2003).
The costs and benefits of anti-predatory behavior often depend on factors such as body size and population density. Feeding rates and reproductive requirements vary depending on the body size of organisms as smaller individuals may have greater nutritional needs for growth than larger individuals. Therefore, the costs and benefits of hiding tend to be different between differently sized organisms (Jennions et al. 2003). Anti-predatory vigilance has been shown to decrease with increasing group size in many species of animals such as sandpipers and fiddler-crabs, reflecting risk management in a group through collective detection and predation risk dilution (Beauchamp & Ruxton 2007). Living in a group increases the odds that a predator will be detected by at least one member of the group. Group membership also reduces personal investment in vigilance at no increase risk to individuals while also reducing the odds that an individual will be preyed upon when a predator attacks (Beauchamp & Ruxton 2007). Studies have found that dilution (the greater amount of individuals per area) is an effective method of predator evasion but only if prey are selected randomly in a group (Beauchamp & Ruxton 2007). It has been predicted that vigilance decreases with group size but increases with greater spacing between individuals of semipalmated sandpipers (Beauchamp & Ruxton 2007). Aggregations may confer a benefit by shortening mean hiding times as hidden individuals may be able to detect when neighbors resume feeding as a signal that the predation risk has been lowered (Poloczanska et al. 2004). Much of the work on how distances to the nearest neighbor influence vigilance behavior has been conducted on meerkats (Furrer and Manster 2009). A high group density enables meerkats to profit from communal anti-predatory behavioral systems such as alarm calling (Furrer and Manster 2009).

It has also been shown in the serpulid *Serpula vermicularis* that different types of predatory stimuli (i.e. different species of predators) have different effects on hiding behavior (Poloczanska et al. 2004). There has been shown to be a hierarchy of stimuli to which prey respond differently. Time spent hiding by individuals has been shown to strongly correlate with the strength of the stimulus and the species triggering the reaction (Poloczanska et al. 2004).

Studies of the hiding response of organisms have been conducted on certain species within the marine worm family Serpulidae, such as *Serpula vermicularis*, but none have been conducted on *Spirobranchus giganteus*. The studies on serpulid hiding responses have been restricted to the re-emergence times in response to food availability yet none have evaluated how other factors might affect the re-emergence times of serpulids (Dill and Fraser 1997). The aim of this study was to investigate other factors that could affect the hiding behavior of *S. giganteus* such as size and density within aggregations. The re-emergence times of worms were measured in comparison to the different variables such as status (as a solitary individual or in a colony of individuals), colony, density, and size.

**H1:** Worms in higher density aggregations will have shorter re-emergence times than those in lower density aggregations

**H2:** Individuals living within an aggregated community will have shorter re-emergence times than solitary individuals

**H3:** Individuals that are indirectly stimulated will have longer re-emergence times at greater distances from a directly stimulated individual

**H4:** Individuals that are larger have longer re-emergence times than smaller individuals for both solitary individuals and individuals in aggregations
Materials and methods

Study site

All experimental work was conducted in front of the Yellow Submarine dive shop located on the leeward shore of Bonaire about 1 km away from the city of Kralendijk (Fig. 1). Bonaire is located in the southern Caribbean 65 km off the coast of Venezuela. The area utilized in the study was located within the Bonaire National Marine Park, which protects and monitors reef environments around Bonaire. Observational studies were conducted on solitary individuals and aggregations of S. giganteus between 3 m and 10 m of depth along the reef crest and on mooring blocks.

Fig. 1 Map of Bonaire, Dutch Caribbean. Black star indicates the study site in front of Yellow Submarine dive shop (12°09'36.47"N, 68°16'55.16"W)

Study organism

Spirobranchus giganteus of class Polychaeta and family Serpulidae are calcareous tube dwelling worms found on coral reefs in the western Atlantic Ocean from Florida to Brazil and in the Caribbean (Petitjean and Myers 2005). Spirobranchus giganteus is among the few known living serpulids that is found associated with living coral colonies in subtidal zones (Bailey-Brock 1976). The larval stages of S. giganteus burrows into scleractinian coral heads between polyps and forms a calcium carbonate tube (Smith 1985). The worm itself can range in length from a few millimeters to about 12 cm (Smith 1985). The tube is a permanent construction within the coral head. The lifespan of S. giganteus is largely determined by the lifespan of the coral, ranging from 10 to 20 years (Tapanila 2005). Spirobranchus giganteus, like other reef-dwelling serpulids, are extended almost continually, only retracting when stimulated by the close proximity to a predator (Evans 1969). The predators of S. giganteus are relatively unknown with no studies indicating the types of organisms that prey on the worms. Distribution of S. giganteus is shown to be non-random among coral with worm densities being higher on certain species of coral, mostly in the genera of Porites (Evans 1969). Clusters of S. giganteus occur with larger worms on more heavily colonized coral (Song 2006).

Effect of density on hiding

To study the anti-predatory response of S. giganteus, an artificial stimulus was used to trigger a hiding response of the worms. The artificial stimulus used was a 5-mL syringe filled to 3 mL at 5 cm away from the worm. Those circumstances were the minimal amount of water and maximum distance needed to trigger a hiding response by every worm during preliminary studies. A picture of each cluster of worms was taken using a SONY video camera in an Ocean Images camera housing. For each picture, a 10 cm measuring stick was placed in the camera frame to use as a scale during measurements on the computer software ImageJ. The data were taken while SCUBA diving once a week for 45 minutes from 8 October to 2 November 2014. The largest individual in each aggregation was chosen as the directly stimulated individual and the re-emergence time of that individual was recorded on a data sheet. Re-emergence time was defined as the total time from a retraction of the worm in the calcareous tube until the body of the worm was extended to its original fully extended body position. The photos taken were then used to measure the density of the aggregation (number of individuals cm\(^{-2}\)) which was defined as the area inside of the
outermost edge of the outside worms. A linear regression was used to examine the effect of aggregation density on re-emergence time.

**Benefits of aggregated versus solitary individuals**

A study was conducted to determine the effect status (being in an aggregation or as a solitary individual) has on the re-emergence times of *S. giganteus* and the ability of the worms to detect when neighbors re-emerge (the nearest neighbor behavior). The artificial stimulus of a water jet from a syringe as described in the previous section was used to initiate a hiding response of solitary individuals and aggregated individuals. The re-emergence times for solitary and aggregated individuals were recorded on a data sheet. Only groups of three or more individuals in an estimated 25 cm by 25 cm area were considered an aggregation. Photos were taken of solitary individuals and the aggregations with a 10-cm scale for measurements on ImageJ. The average re-emergence times of aggregated individuals were compared to the average re-emergence times of solitary individuals and analyzed using a t-test.

The behavior of *S. giganteus* in communication of individuals within aggregations was evaluated using the nearest neighbor behavior. An individual in an aggregation was directly stimulated. The worms surrounding the directly stimulated individual that also responded to the stimulus (i.e. that were indirectly stimulated) were observed. The re-emergence times of those individuals as well as the directly stimulated individual were recorded. Pictures of the aggregations were taken using a SONY camera in an Ocean Images camera housing to determine the distance of the indirectly stimulated worm from the focal individual using ImageJ. The distance was determined as the length between the two branchial crowns of the directly and indirectly stimulated worms. A linear regression analysis was conducted using the distance from the directly stimulated individual to the indirectly stimulated individuals and the re-emergence times of the indirectly stimulated individuals.

**Intersection of size and status**

The effect of both size and status (in a colony or as a solitary individual) were studied in relation to re-emergence times to determine how the two variables interact and affect the hiding behavior of the worms. Information from the aggregation study was used in addition to information on the size of those individuals measured on ImageJ. Size was measured as the longest distance from one brachial crown to the other on the bottom whorl of tentacles. The data were analyzed with an ANCOVA statistical test using size and status as explanatory variables and re-emergence time as the response variable.

**Natural stimuli study**

A natural study of *S. giganteus* was conducted to observe what types of organisms trigger a natural hiding response and record the duration of these hiding responses to natural stimuli. A Stingray G2 Light and Motion camera with a negatively buoyant stand was used to take videos of various clusters of worms. The videos were taken for 15-minute intervals while SCUBA diving. Four to five videos were taken once a week for five weeks giving 90 minutes of total footage. The types of organisms that triggered a hiding response of individual worms and the re-emergence times of the worms were recorded from footage on videos. The videos were analyzed on the computer software Picture Motion Browser (PMB), made by SONY. The re-emergence times were averaged for each type of stimuli (e.g. Yellowtail damselfish, unknown, Red-lipped blenny, etc.) and an ANOVA was used to compare the re-emergence times of the worms to the different stimulus types. The re-emergence times of all of the natural stimuli were then compared to the re-emergence times of artificially stimulated individuals in a colony. A t-test was conducted to determine if there was a significant difference between
pooled natural stimuli and the artificial stimulus used.

**Results**

Effect of density on hiding

Aggregations were defined as estimated areas of 25 cm by 25 cm in which three or more individuals were located. The re-emergence time and density were measured for aggregated individuals artificially stimulated to hide. There was a decrease in the re-emergence times of worms as the density of aggregated worms increased \((r=-0.2180)\), however, the density of aggregated individuals did not have a significant association with the hiding time of aggregated individuals \((df=1, F=1.5, p=0.231; \text{Fig. 2})\).

![Graph showing average re-emergence times (s) for individuals in aggregations of different densities. Dotted line indicates the linear trend-line for artificially stimulated individuals in aggregations (n=32).](image)

**Hiding times of aggregated versus solitary individuals**

The average re-emergence times were measured for aggregated individuals in comparison to solitary individuals. There was a statistically significant difference between the re-emergence times of aggregated individuals and solitary individuals \((t=3.15, p=0.002)\) with solitary individuals having significantly longer re-emergence times than individuals in aggregations \((\text{Fig. 3})\).

![Graph showing average re-emergence times for solitary (n=77) and in aggregated (n=60) individuals of *Spirobranchus giganteus* artificially stimulated to hide. Error bars indicate ± standard error of the mean. Data was taken from samples of 137 aggregated and solitary individuals.](image)

**Fig. 3** Average re-emergence times for solitary \((n=77)\) and in aggregated \((n=60)\) individuals of *Spirobranchus giganteus* artificially stimulated to hide. Error bars indicate ± standard error of the mean. Data was taken from samples of 137 aggregated and solitary individuals.

Effect of distance from nearest neighbor on hiding

When an individual was directly stimulated to hide, worms surrounding that individual were also indirectly stimulated to hide. The re-emergence times of these indirectly stimulated worms were recorded and the distance from the directly stimulated individual was measured. The re-emergence time of indirectly stimulated individuals increased when further away from the directly stimulated individual \((\text{Fig. 4})\). The directly stimulated individual had a faster re-emergence time than the surrounding individuals \((\text{Fig. 4})\). There was significant association between the distance of an indirectly stimulated individual from the directly stimulated individual and re-emergence time \((df=1, F=5.92, p=0.018)\).

The effect of size and status on hiding

The size and re-emergence times of *S. giganteus* were measured for solitary and aggregated individuals. There was a statistically significant difference in the re-emergence time between aggregated and solitary individuals \((df=123, F=2.97, p=0.024)\). There was also a significant association between the size and re-emergence time \((df=1, F=5.67, p=0.019)\). For solitary
Fig. 4 Re-emergence times of indirectly stimulated individuals (s) of different distances (cm) from the directly stimulated individual. The light grey point indicated the average re-emergence time of the directly stimulated individuals (46.87 ± 7.23 seconds) not included in the linear regression analysis.

individuals, there was a slight increase (r=0.295) in the re-emergence time with increasing size (Fig. 5). However, there was no correlation between re-emergence times and size in aggregated individuals (r=0.00759; Fig. 5). The statistical results of these variables indicated a significant interaction between the status and size in relation to re-emergence time (df=2, F=8.69, p=0.004).

Comparison of different natural stimuli and artificial stimuli

Several different types of fishes were identified during natural hiding response observations with a video camera. A total of 71 hiding events were observed for 112 individuals over 90 minutes of video footage. From the videos, there were 0.423 hiding events per individual per hour, however no predatory attempts were made during this time. The hiding time was very variable, ranging from 3-900 seconds with a mean of 105 seconds (SD±171.7). The majority of the hiding responses were initiated by water motion that resulted from the close proximity of fishes while some of the hiding responses were initiated by an unknown source. The fishes that stimulated the worms were mostly from the families Blennidae (blennies), Labridae (wrasses), and Pomacentridae (damselfish). The different types of stimuli that initiated a hiding response were Acanthemblemaria maria (Secretary blennies), Thalassoma bifasciatum (Blue-headed wrasse), Stegastes partitus (Bicolored

Fig. 5 Re-emergence times of different sized aggregated and solitary individual. Dotted line shows the linear trend-line of aggregated individuals. Solid line indicates the linear trend-line of solitary individuals.
damselfish), *Microspathodon chrysurus* (Yellowtail damselfish), *Elacatinus evelynae* (Sharknose goby), *Ophioblennius atlanticus* (Red-lipped blenny), *Pseudopeneus maculatus* (Spotted goatfish), *Coryphopterus glaucofraenum* (Bridled goby), *Halichoeres bivittatus* (Slippery dick), *Halichoeres garnotii* (Yellow-headed wrasse), and *Scarus iserti* (Striped parrotfish) (Fig. 6). Generally the longest hiding events of *S. giganteus* were triggered by *A. maria*, the unknown stimuli, and *T. bifasciatum*. The re-emergence time of *S. giganteus* was similar among different types of natural stimuli (df=11, F=1.18, p=0.322). However, the average re-emergence times of all natural stimuli was significantly higher than the average re-emergence times artificially stimulated worms in aggregations (t=2.06, p=0.0409).

### Discussion

#### Effect of density on hiding

Different variables such as density, aggregated or solitary status, nearest neighbor behavior, and size were studied to determine their effects on the hiding duration of *S. giganteus*. While it was hypothesized that there would be shorter re-emergence times with higher density aggregations, the results indicated that there was no significant association between the density and re-emergence times of aggregated individuals. Other studies have shown that dilution (i.e. a greater amount of individuals per a given area) is an effective tactic of predator evasion (Beauchamp and Ruxton 2008). Based on the results, this study did not find evidence of this phenomenon for *S. giganteus*. The densities of *S. giganteus* are highly variable and it was difficult to quantify where one aggregation ended and another started, or if an individual was included in an aggregation or not. It would be useful in future studies to re-define what an aggregation is in
terms of community dynamics using how far worms are away from each other as the defining factor.

Hiding times of aggregation versus solitary individuals

Data from this study has not been able to illustrate the effects of group density on worm behavior, but comparing worms living within aggregations to those existing as solitary individuals yields interesting results. There was a significant difference between the re-emergence times of aggregated and solitary individuals, with solitary individuals hiding for longer periods of time than aggregated individuals. These results were expected in this experiment and supported another study where it was shown that group membership reduces hiding time (Dill and Fraser 1997). In the temperate barnacle *Semibalanus balanoides*, the group living barnacles were found to emerge from hiding more rapidly than solitary individuals (Mauck and Harkless 2001). Mauck and Harkless (2001) suggest that the decrease in hiding times in a group affects the foraging efficiency in those barnacles. Similarly, the foraging behavior could be affected in *S. giganteus* by benefit of decreased hiding time attributed to living in a group.

Effect of distance from nearest neighbor on hiding

Another way that aggregations were assessed was by measuring the nearest neighbor behavior. In aggregations, the re-emergence times of an indirectly stimulated individual increased with distance from the directly stimulated worm. These results supported the hypothesis that individuals further away from a directly stimulated individual would have longer re-emergence times than closer individuals. This is based on the prediction that group-living individuals can detect when neighbors resume feeding (Poloczanska et al. 2004). This detection allows for lowered predation risk that is communicated throughout the group (Poloczanska et al. 2004). When one individual re-emerged first, typically the directly stimulated individual, the rest of the individuals in the group may be able to detect that it has re-emerged and follow suit. Based on this prediction, those closest to the directly stimulated individual would naturally have shorter re-emergence times because they are able to sense that the directly stimulated individual re-emerged first. Individuals further away from the directly stimulated individual may be unable to detect when the directly stimulated individual re-emerged and must wait until it can detect when other surrounding worms re-emerge, thus prolonging its hiding time.

These explanations for the behaviors of *S. giganteus* could be indicative of a communication system within aggregations. Communications systems have been shown to exist in a variety of different organisms. A study found that larger groups of spiders are better at perceiving danger from further away through vibrating signals (Uetz et al. 2002). Vibrating signals through the water could be utilized by *S. giganteus* as the means of communication. Communication networks in the former study were enhanced by interconnected colonies, which could extend to aggregations of individuals.

The effect of size and status as single or aggregated individuals on hiding

Aside from interactions within aggregations, size varies among individuals and contributes to differences in hiding behavior. In this study, a strong association with size and re-emergence time for solitary individuals was shown. Smaller individuals may have higher nutritional needs and therefore the cost associated with waiting to re-emerge is higher because the need to forage takes precedent. However, this size-associated trend did not appear in aggregated individuals. The re-emergence times of aggregated individuals stayed consistent regardless of size. While there was no difference between the re-emergence times of small individuals between the different statuses (solitary or aggregated),
the difference in re-emergence time between the statuses became more pronounced with size. These results show the foraging benefit of colonial living for larger individuals since larger individuals that were solitary had significantly longer re-emergence times than those in aggregation. Another study predicted that hiding time would vary among different sized individuals due to differences in nutritional and reproductive needs (Jennions et al. 2003). These results support this prediction, but only for solitary individuals. Among aggregations, the factor of size was less of a defining factor of re-emergence. It is possible that while variations in hiding differ between different individuals, there is a hierarchy of what determines hiding time.

Comparison of different natural stimuli and artificial stimuli

To have a more complete understanding of the hiding behavior of *S. giganteus*, it was important to observe how the worms behave in natural conditions. The types of stimuli, how often the worms hid in a certain time period, and how long the worms hid in response to different stimuli were observed in the natural environment of *S. giganteus*. This study showed that there was no difference between different species of stimuli on re-emergence times. It was hypothesized that there would be significant differences between the species that stimulated *S. giganteus* to hide based on the findings of Poloczanska et al. (2004). The findings in that study showed that stimuli generated by variations in size, proximity, and force of water motion by different marine species, influenced hiding times. Species that were larger and closer in distance to individuals resulted in longer hiding times in a previous study on serpulids, however the results of this study did not support those findings (Poloczanska et al. 2004). The natural stimuli that were observed may have been limited in number in comparison to the study by Poloczanska et al. (2004) that had far more variety in the species that stimulated serpulids to hide. Furthermore, the small sample size of the study yielded very little statistical power to detect differences between different stimuli.

To determine if the artificial stimulus had any substantial effect on the hiding behavior of *S. giganteus*, the re-emergence times from aggregated individuals that were artificially stimulated were compared to the re-emergences times of the naturally stimulated individuals. There was a significant difference between the natural stimuli and the artificial stimulus used, which was not expected. The artificial stimulus is inherently different from fish not only in size and shape, but also in the amount of water pressure that it puts on the worms, resulting in different re-emergence times from natural stimuli. Although the differences between the artificial stimulus and natural stimuli affected the re-emergence times, the general trends demonstrated by the use of the artificial stimuli could still apply to natural systems as it has supported evidence on hiding in at study by Uetz et al. (2002). This study used artificial predatory stimulations to study colonial spiders, showing how artificial stimulations are useful in studying behavior of colonial animals (Uetz et al. 2002).

The time prey spend hiding in response to an attack is an important determinant in a number of ecological and community patterns and processes. Hiding time is a behavior that can be studied to show the importance of refined behavioral decisions. Prey have been shown to alter hiding based on size, living in aggregations, density, and proximity to neighbors. These characteristics have implications on food intake, growth, and reproductive output. Further studies on the communication within aggregations of individuals could reveal the community dynamics of colonial organisms, contributing to further clarifications in anti-predatory behaviors. Overall this study contributes to the knowledge on the various factors that affect the fitness of an individual and have implications at the population and community levels.

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References

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Colonization and initial succession on artificial substrates over spatial and temporal gradients in a Caribbean coral reef ecosystem

Abstract  Human activity and higher frequencies of disturbance have increased coral reef degradation and created barren substrates with the opportunity for primary colonization. The pioneer microorganisms determine the subsequent community composition, as many organisms require specific substrates and environmental conditions to recruit. Considering that coral recruits more effectively on hard substrates (including crustose coralline algae, polychaete tubes, and other encrusted invertebrates), examining colonization on artificial substrates is increasingly important for coral recruitment. This study documented the pioneer species and early succession on introduced substrates on the fringing reef in Kralendijk, Bonaire, Dutch Caribbean. Ceramic tiles were used as the artificial substrate, and data was collected and analyzed weekly over a period of four weeks. Tiles were placed in three different environments in the reef ecosystem: the sand flat, reef crest, and reef slope. Tiles were analyzed in the laboratory using stereomicroscopy for organism colonization. Turf algae, and brown and green algal filaments were present on all tiles over the experimental time period, but there was no fleshy macroalgal growth. Strong increasing trends of invertebrate abundance and diversity occurred in all environments over the four-week research period. Microfauna including polychaetes, oligochaetes, bivalves, gastropods, crustaceans, bryozoans and foraminiferans were the main pioneer organisms observed. According to other studies, these organisms are also present on artificial substrates over the time period of two to five months. Therefore, these benthic invertebrates likely dominate substrates for several months before further succession occurs allowing organisms such as crustose coralline algae and coral recruits to settle.

Keywords  Pioneer organisms • Benthic invertebrates • Community composition

Introduction  Due to the recent escalation in coral reef degradation, there has been a heightened effort to create artificial reefs by adding introduced substrates to the environment (Perkol-Finkel and Benayahu 2005). In addition to artificial structures, reef disturbances create barren substrates for pioneer species to colonize and develop. The increase in exposed substrate availability generates an elevated interest in understanding the growth mechanisms of reef organisms and artificial substrate ecology (Perkol-Finkel and Benayahu 2005). To better understand these processes, it is important to study the pioneer species on these substrates and how the colonizer composition changes over time. All factors responsible for the decline in coral cover (including herbivore overfishing, climate change and disturbances) also affect the microorganisms that colonize and grow in reef ecosystems (Kriwy and Uthicke 2011). There is information on colonization and succession over timescales ranging from multiple months to multiple years, mostly focusing on coral cover and recruitment (Arnold and Robert 2011; Atilla et al. 2003; Borowitzka et al. 1978; Diaz-Castañeda and Almeda-Jauregui 1999; Trench
et al. 2014). Literature on microbial diversity and biofilms colonization exists on the time scale of less than a week (Kriwy and Uthicke 2011; Sawall et al. 2012; Witt et al. 2011b). However, to the best of my knowledge, no studies have been conducted on early microorganism colonization on bare reef substrates over the intermediate time period of several weeks. These microorganism colonizers are increasingly important to marine ecology with the rise of artificial hard substrates and bare surfaces from urbanization and human interference (Atilla et al. 2002).

Long-term settlement and growth is dependent on the initial colonization of substrates, both natural and artificial. Pioneer organisms provide the basis that determines the organism composition of each additional successional event. Biofilms are generally among the earliest colonizers and greatly influence what can live on a substrate by contributing to nutrient turnover and productivity (Sawall et al. 2012). Therefore, the presence and composition of biofilms is essential to the overall succession of a substrate. Coral can recruit most effectively on early successional substrates including biofilms, polychaete tubes, and coralline algae (Arnold and Robert 2011). However, the organisms forming these substrates can only settle in specific conditions. For example, coralline algae cannot grow if there are fleshy algae present on the substrate (Trench et al. 2014). The presence of sponges, ascidians, and bryozoans also disrupt the settlement of coralline algae and corals (Arnold and Robert 2011). Furthermore, the recent shift in reef systems from coral dominated to macroalgal-dominated results from the negative effects of macroalgae on coral recruitment both directly and by disrupting intermediate substrates (Arnold and Robert 2011). Determining specific colonizing organisms aside from algae may provide information pertinent to finding solutions to decrease coral overgrowth by macroalgae.

Just as colonization is essential to reef dynamics, understanding the succession of these benthic species is increasingly important due to the quickly changing environmental conditions of the oceans (Diaz-Castañeda and Almada-Jauregui 1999). Global rises in ocean temperature and acidification as well as local disturbances, such as nutrient enrichment and overfishing, are factors that affect resilience of benthic reef organisms (Anthony et al. 2011). Biofilms reflect changes in seawater chemistry; therefore these environmental stressors have a direct impact on biofilm composition (Witt et al. 2011a). In addition, ocean acidification has detrimental effects on skeleton formation of calcifying organisms including corals, coralline algae, molluscs, and foraminiferans (Kuffner et al. 2008). These organisms are all part of the early communities that colonize substrates. The fragility of these colonizers in an environment constantly changing emphasizes the importance of primary colonization, succession, and community composition.

This study aimed to measure colonization and succession on ceramic tiles as introduced substrates in a coral reef ecosystem within a four-week period. Since a time scale longer than several days and shorter than several months has not been studied thoroughly, this study provides novel findings on colonization and succession over this intermediate time period. Colonization was analyzed via organism composition (taxonomic identification), diversity (taxonomic group richness), and organism abundance (total number of organisms present). Rates of colonization were analyzed from three different environments within the reef: the sand flat, the reef crest and the reef slope. Each of these environments provide different conditions such as light exposure, nearby biotic composition, and amount of predation present (Pers. Obs.), which will affect the colonization community structure of the tiles (Witt et al. 2011b). The results of this research show the differences of colonizing and early successional organism among separate environments in the reef and over a time scale of four weeks.

It was expected that combinations of time elapsed and environment will affect the settlement and composition of colonizers (Borowitzka et al. 1978). Turf algae and
biofilms were expected to be the first to settle on the tiles, followed by small benthic organisms such as molluscs, arthropods, bryozoans, and polychaetes (Diaz-Castañeda and Almeda-Jauregui 1999).

H1: Respective to each environment, abundance of organisms present on the ceramic tiles will increase over the four-week experimental time period.

H2: Respective to each environment, taxonomic group diversity present will increase over the experimental time period.

H3: Respective to each environment, organism composition present on the tiles will change over the experimental time period.

H4: Organism composition will be significantly different between the three environments (sand flat, reef crest, and reef slope).

Materials and methods

Study site

This study was conducted on the fringing reef at Yellow Submarine dive site north of downtown Kralendijk, Bonaire, Dutch Caribbean (12°09’21.4” N, 68°16’45.3” W) (Fig. 1). At this site, three different environments and depths were compared. Experimental setups were placed on the sand flat, at a depth of 10 ft (3 m), at the reef crest with a depth of 30 feet (9 m), and on the reef slope with a depth of 45 feet (14 m).

Experimental setup design

To assess the rate of colonization over the research span of four weeks, ceramic tiles were introduced to the reef ecosystem as primary substrates. The 15 cm by 7.5 cm tiles were placed in the water mounted on 1-meter long PVC pipes. Six tiles (7.5 cm apart) were attached to each PVC pipe using zip ties (Fig. 2). Tiles were added to three separate environments in the reef ecosystem: the sand flat, reef crest, and reef slope. One tile per PVC was collected every week for organism composition and abundance analysis during the four-week experimental time span. The two extra tiles were attached to the PVC in case of damage or disturbance to one of the other tiles, affecting experimental results. In the sand flat, the PVC-tile setups were attached to the benthos with rebar poles. In the reef crest and slope weights were attached to the PVC using zip ties to ensure that the setups remained in place.

Fig. 1 Map of Bonaire. Study was conducted at Yellow Submarine dive site (12°09’21.4” N, 68°16’45.3” W; indicated with a star) in Bonaire, Dutch Caribbean.

Fig. 2 The experimental setup consisted of a total of six 15x7.5 cm tiles attached to a PVC pipe using zip ties. The structure was attached to the sea floor using weights or rebar poles.
Data collection

One tile from each PVC setup was retrieved per week and taken to the laboratory for analysis. The tiles were transported in sealable containers to prevent any of the colonizing organisms from getting damaged or lost on the way from the water to the laboratory. To account for data variability and increase data reliability, two apparatus replicates were placed in each environment (sand, reef crest, and reef slope); therefore two tiles from each environment were collected each week.

Laboratory work

In the laboratory, the tiles were examined using stereo and compound microscopes. Tiles were observed completely via the stereomicroscope and the compound microscope was used to identify organisms too small to observe with the stereomicroscope. Blue NIGHTSEA® light was used under the stereomicroscope to observe chlorophyll red fluorescence of algae. For microfauna, distinct species and number of determinable individuals were quantified and identified to the lowest taxonomic level possible. Additionally, since organisms became displaced from the tile and fell into the container during transfer to the laboratory, the water in the container was filtered. A 60 µm plankton filter was used for filtration, and organisms were quantified and added to the data from the tiles. Taxonomic group composition, abundance, and richness were compared between the three environments and over the four-week research period.

Statistics

Statistical analysis was used to determine significant temporal and spatial differences in data. Linear regressions and $R^2$ values were used to compare trends between each environment for organism abundance and richness over the four-week experimental period.

Results

Six phyla of benthic invertebrates were found on the ceramic tiles over the four-week experimental period. Most organisms found were nestled in the sand on the tiles, only a small number were encrusted on the tile surface. The invertebrates found and identified to taxonomic class were polychaetes (tubewelling and epibenthic), oligochaetes, bivalves, gastropods, copepods, decapods, and echinoids (Table 1). Bryozoans and foraminifera were also prominent invertebrates found on the tiles, but were not identified to a lower taxonomic group than phylum. Copepods, while not specifically benthic invertebrates, are included in the colonization data since they were present on the tile substrates and may have an effect on the overall community. Types of algae including brown and green algal filaments, turf algae and crustose coralline algae were also found.

Although not a clear trend in succession of invertebrate types, the most prominent taxa on the tiles of each environment seem to shift over time. Copepods and gastropods were the dominant invertebrates for all environments for the week one and week two, whereas bryozoans were the dominant invertebrates for all environments for week three. Bryozoans were also the dominant invertebrates for the sand flat and reef crest for week four, and foraminifera were dominant in the reef slope (Table 1). It is also important to note that tube-dwelling polychaetes were prevalent invertebrates in the reef crest for each week. Additionally, the size of the encrusted polychaete tubes increased over time.

Total organism abundance increased steadily over the four weeks for the three environments: sand flat, reef crest, and reef slope (Fig. 3). Invertebrate abundance in the sand flat increased from three organisms in week one to 45 in week four for replicate one (Fig. 3a). Replicate 2 increased from one organism after the first week to 31 organisms after week four. Both replicates show strong positive linear relationships between number of
Table 1 Invertebrate abundance observed on individual replicates (n=2) over time for sand flat, reef crest, and reef slope environments

<table>
<thead>
<tr>
<th></th>
<th>Sand</th>
<th>Crest</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalve</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bryozoa</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Copepod</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ctenoid Scale</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Decapod</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Echinoid</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Foraminifera</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Gastropod</td>
<td>1</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Oligochaete</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Polychaete (epibenthic)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Polychaete (tube-dwelling)</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

weeks after tile placement and total invertebrate abundance (replicate 1: R²=0.95, replicate 2: R²=0.79). The rate of colonization between the two replicates on the sand flat varied by 41%. Invertebrate abundance in the reef crest increased from eight to 36 (replicate 1) and four to 26 (replicate 2) from week one to week four (Fig. 3b). Both reef crest replicates also show strong positive linear relationships between number of weeks and total invertebrate abundance (replicate 1: R²=0.99, replicate 2: R²=0.90). The rate of colonization between the two replicates on the reef crest varied by 35%. Abundance on the reef slope increased from three (for both replicates) to 60 (replicate 1) and 31 (replicate 2) from week one to week four (Fig. 3c). Strong and positive linear trends are shown between number of weeks and invertebrate abundance (replicate 1: R²=0.95, replicate 2: R²=0.90). The rate of colonization between the two replicates on the reef slope varied by 67%.

Replicates of each environment were averaged and invertebrate abundance was compared between the three environments over the four-week period (Fig. 4). All environments showed strong, positive trends between number of weeks and average invertebrate abundance (sand: R²=0.94, crest: R²=0.98, slope: R²=0.95). The reef slope had the highest rate of colonization; 27% higher than the colonization rate of the sand flat and 47% higher than the colonization rate of the reef crest.

Taxonomic group richness also showed an increasing trend over time for the three environments (Fig. 5). Average invertebrate
richness between the two replicates for the sand flat ranged from two to eight types of invertebrates present from week one to week four. For the reef crest, average richness ranged from three to nine, and for the reef slope, richness ranged from two to seven over the experimental time period. All environments showed strong and positive trends between number of weeks and average invertebrate richness (sand: $R^2=0.97$, crest: $R^2=0.997$, slope: $R^2=0.79$).

![Graph showing average invertebrate abundance over the experimental period.](image)

**Fig. 4** Average invertebrate abundance over the four-week experimental period compared between the three environments: sand flat, reef crest, and reef slope (n=6)

![Graph showing average invertebrate richness over the experimental period.](image)

**Fig. 5** Average invertebrate richness over the four-week experimental period compared between the three environments (sand flat, reef crest, and reef slope). Averages between the two replicates for each environment were used to compare richness of invertebrate taxa over time (n=6)

Algae were present on tiles from all environments and over all weeks. Algae cover for turf algae, brown algal filaments, and green algal filaments did not have significant differences over time or between environments. However, crustose coralline algae were only present on one tile from the reef slope in week two and week three respectively, and one tile from the reef crest in week four.

**Discussion**

The results of this study show clear differences of organism abundance, composition, and diversity over temporal and spatial gradients. Hypotheses one, two, and three were supported by the results. As proposed in hypothesis one, there are clear increases in invertebrate abundance for the sand flat, reef crest, and reef slope environments over the four-week experimental time period. Additionally, as predicted by hypothesis two, taxonomic group diversity of invertebrates also increased in each environment over the four weeks. Hypothesis three, projecting that organism composition changed over time, was supported although organism composition within each environment gradually transitioned between the dominant invertebrates and did not experience drastic changes in community composition. There was a general pattern of shifting from molluscs and crustaceans as the dominant invertebrates to the colonial organisms of bryozoans and foraminifera, but no distinct successional changes were observed. Moreover, the dominant invertebrates found earlier in the study remained present in later weeks, but were outnumbered by other invertebrate groups. Hypothesis four, however, was not supported by the data of this study, as there were no clear differences in community composition on the tile substrates between the three environments. Although dominating invertebrates often differed between environments over time, the majority of each invertebrate group was present on tiles from all environments.

One major result of this study was that colonization occurred at a higher rate in the reef slope compared to the sand flat and reef crest. This result may be attributed to the different community dynamics in the surrounding reef, which has relatively more structural complexity. The reef has an increased diversity of neighboring organisms.
and intricate structure, due to the higher prevalence of coral, sponges, and algae in the environment. The variety in this community may be able to contribute directly to the rate of colonization on the tiles in this environment. With a higher diversity in the reef, there are more organisms and larvae with the possibility of recruiting to the tile substrates. The sand flat and reef crest have surrounding organisms to settle on the tile substrate also, but based on this data it is possible that there are fewer in these less structurally complex environments.

Previous studies of colonization in coral reef ecosystems, although measured over longer time scales, observed similar results for initial colonization on artificial substrates. Polychaetes, molluscs, crustaceans, bryozoans, hydrozoans, and sponges represent the majority of pioneer organisms found on artificial substrates (Diaz-Castañeda and Almeda-Jauregui 1999; Perkol-Finkel and Benayahu 2005). These organisms were found to be the primary colonizers of the substrates by both Diaz-Castañeda and Almeda-Jauregui (1999) and Perkol-Finkel and Benayahu (2005) in a time period between two and five months. Since these organisms were prevalent on the artificial substrates over the time period of this study (four weeks) as well as over two to five months, these benthic invertebrates likely dominate substrates for several months before succession occurs allowing organisms such as crustose coralline algae and coral recruits to settle.

Other colonization studies observed additional colonizing organisms including, sponges, tunicates, ascidians, coral recruits, gorgonians, and fleshy macroalgae. Large molluscs, echinoderms, and tube-dwelling polychaetes as well as an increased amount of sessile organisms were also settled on the substrates (Arnold and Robert 2011; Atilla et al. 2003; Diaz-Castañeda and Almeda-Jauregui 1999; Perkol-Finkel and Benayahu 2005; Trench et al. 2014). These large colonizing organisms were absent, while microfauna and larvae dominated the tiles due to the short time scale of this four-week study. On either large or micro scales, increases in species abundance and richness observed over time suggest an increase of biotic interactions that allow communities to become more complex (Diaz-Castañeda and Almeda-Jauregui 1999). Complex communities also provide the environmental conditions for continued settlement and succession.

Due to the degradation of coral reefs in recent years, it is important to consider substrates that allow for coral recruitment when studying colonization processes. Hard substrate conditions produced by organisms including crustose coralline algae, polychaete tubes, and encrusted bryozoans are ideal for coral recruitment (Arnold and Robert 2011). These organisms were present even within the timescale of this study, showing that early colonization is crucial to determining the community composition over time. Had the experimental time period of this study been extended, it is possible that coral recruits may have settled on the tiles since the substrate was formed by polychaete tubes, crustose coralline algae, and encrusted bryozoans, assuming the patterns of colonization over the first four weeks continued. However, had these organisms not settled in the early weeks of colonization, the community structure and substrate may not have allowed for coral recruitment.

The results of this study were specific to the experimental time and sample size. Extending the experimental time period, while still analyzing tiles every week could provide more information relevant to coral and sessile organism recruitment as well as on primary colonization. Additionally, increasing the number of replicates per environment could yield data with clearer trends of colonization over time and between environments. Determining statistical significance was not possible for only two replicates, however a larger sample size may provide more comprehensive data on the differences in colonization over temporal and spatial gradients. Another consideration for future research regarding initial colonization is that for this study the artificial substrates were placed in each environment directly onto the
seafloor, which caused sand to accumulate on the tiles. Therefore, the results of the study proved to assess benthic invertebrate colonization primarily nestled in the sand, and may have yielded different results and composition of colonial organisms had the tiles been placed above the substrate. This study, however, did provide essential insight to the initial colonization processes within a coral reef ecosystem, and could be extended in the future.

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References


Can the first few decades of coral colonization give insight to species interactions and ecological complexity? A case study on artificial coral-based structures

Abstract A greater understanding of coral-based communities must be achieved in order to maximize reef conservation efforts. While a multitude of studies have analyzed coral reef recovery, resilience of reefs, and artificial reefs versus natural reefs, few have dissected the complex ecological networks of coral-based ecosystems during the first few decades of colonization. The following is a quantitative study of invertebrate and fish communities around a series of offshore mooring blocks in Kralendijk, Bonaire (n=18). These blocks were deployed roughly twenty years ago, are the same size, and are exposed to similar physical conditions. It was hypothesized that there would be positive correlations between coral cover and fish species richness, coral cover and fish species diversity, rugosity and fish species richness, and rugosity and fish species diversity. Visual surveys, photo quadrats, and a slightly modified chain intercept transect method were used to assess fish communities, coral cover, and rugosity, respectively. The results supported the hypotheses with significant positive correlations (p<0.05). Likewise, it was found that fire coral cover displayed significant positive correlation with both rugosity (p=0.005) and fish species diversity (p=0.014), whereas brain coral cover did not show a significant correlation with these two variables. Though these outcomes may have been expected based on the findings of previous studies, the manifestation of such ecology in these relatively young mooring blocks is impressive when compared to the same trends in well-established reefs. While this study constituted only a small window of the intricate field of coral reef ecology, the findings offer manageable insight into the dynamics of young artificial structures.

Keywords Structural complexity • Species diversity • Coral cover

Introduction

The unsustainable practices of the ever-increasing human population are causing coral reef ecosystems to decline and the unfolding consequences are catastrophic. Our ecological and economic dependence on coral reefs cannot be understated (Wilkinson 1996). Despite the widespread degradation of these ecosystems, studies have shown that recovery is plausible (Graham et al. 2011). Additionally, investments in artificial reefs and the gradual rise in ocean level, which submerges landmass, are providing new substrate on which coral can colonize (Bohnsack and Sutherland 1985, Buddemeier and Smith 1988). In order to facilitate this recovery and promote successful colonization, however, mankind must gain a more intimate understanding of how these complex communities operate, particularly in the first few decades of colonization.

A series of offshore mooring blocks along Kralendijk’s downtown area (Bonaire, Dutch Caribbean) offer a unique opportunity to study colonization dynamics in coral reef ecosystems. Deployed between 1994 and 1996, these 1-m x 1-m x 1-m blocks lie in groups of three along the edge of the reef crest at a depth of about 6 m. Detailed studies have analyzed
the physical conditions of this area and have determined that depth, composition, distance from the reef slope, light intensity, local currents, substrate, and non-directional water movement do not differ significantly between the blocks in this offshore stretch (Filkovsky and Hoback 2014). The fish and invertebrate communities on these blocks have also been compared to those of other artificial structures and the adjacent natural reef at different depths, locations, and times of day (Jaco 2012, Jensen 2012, Thomas 2009). No studies exist, however, comparing the species interactions and ecological complexity among the sets of mooring blocks. Likewise, rugosity (a measure of structural complexity) of the blocks has not been evaluated. Since studies have shown that structural complexity increases fish species richness and abundance, rugosity is an important physical quality that could affect community composition of the blocks (Gratwicke and Speight 2005a, Gratwicke and Speight 2005b). This study aims to answer the following: Can the first few decades of coral colonization give insight into species interactions and ecological complexity?

Studies in this domain are uncommon, as most that analyze the biotic communities in the early decades of colonization focus on deeper and more complex artificial structures (Perkol-Finkel and Benayahu 2005, Burt et al. 2009). Likewise, such experiments are often approached with the intention of comparing artificial structures to natural reefs (Thomas 2009). Still, benthic dominance and seasonal variability of fish communities can change dramatically in the first few decades following deployment (Perkol-Finkel and Benayahu 2005, Burt et al. 2009). The coral-based ecosystems provided by the offshore mooring blocks may be miniscule compared to the neighboring natural reefs, but they are dynamic and offer an experimental window through which ecological relationships can be studied in greater detail. The hypotheses tested in this study were as follows:

**H1:** There is a positive correlation between coral cover and fish species richness

**H2:** There is a positive correlation between coral cover and fish species diversity

**H3:** There is a positive correlation between rugosity and fish species richness

**H4:** There is a positive correlation between rugosity and fish species diversity

### Materials and methods

#### Study site

The sets of mooring blocks that were surveyed (n=18) are situated between 50 m and 60 m from the shore and extend northwestward and southeastward from the Yellow Submarine dive site (12°09’36.3”N 68°16’55.2”W) on the island of Bonaire, Dutch Caribbean (Fig. 1). The area is a sandy reef flat that extends 60 m from the shore before transitioning to the reef crest. The adjacent reef slope is a coral dominated, gradual slope that ranges from seven to thirty-seven meters deep. Blocks are situated at a depth of approximately six meters. Each set contains three mooring blocks that are clustered together such that the space between the three blocks is small and inaccessible to SCUBA divers.

![Fig. 1 Map of Bonaire, Dutch Caribbean with emphasis on Yellow Submarine dive site (12°09’36.3”N 68°16’55.2”W). Each grey square signifies a mooring block. The bold line and the dashed line represent the shore and the beginning of the reef crest, respectively](image-url)
Data collection

**Sessile invertebrate community assessment**

Photo quadrats and subsequent analysis on ImageJ 64 computer software were used to determine coral cover and to survey the sessile invertebrate species. Rugosity of the three or four accessible faces on each block were measured using a slightly modified chain intercept transect method (Hill and Wilkinson 2004). Two divers contoured a tape measure in a straight line along the block faces. The tape was marked at the start of the measurement such that it was allowed to fall after a portion of the transect had been fitted. The rugosity of each face was measured a total of four times at four parallel horizontal lines set 0.2 m apart.

**Fish community assessment**

A visual census was conducted to study the fish communities around the sets of mooring blocks. Following the methods outlined by Jaco (2012), fishes were counted and identified only if they hid within the block structures when approached, stayed within a 0.5-m radius of the blocks, or if they returned directly to the blocks after initially retreating from a diver’s approach. This ensured that transient fish species were not included as individuals residing in the blocks’ structures. Fishes were surveyed by a single diver swimming around the blocks from a 3-m distance for four minutes in order to minimize impact of diver presence. Immediately following, the blocks were further surveyed at close proximity for four additional minutes to account for cryptic fishes. Records were taken with an underwater slate and a video camera was used to record unknown species for later identification. Care was taken not to count the same individual twice and juveniles were distinguished from adults as often as possible. Each set of mooring blocks was surveyed on three separate dates between the times of 9:00 and 12:00 hrs.

Data analysis

Percent coral cover and sessile invertebrate surveys were measured using ImageJ 64. Species diversity of the sessile invertebrates and fish communities were calculated using the Shannon Index (\(H=\sum p_i \ln(p_i)\), where \(p_i\) is the proportion of each species) in order to adequately account for species richness and evenness. Regression and correlation tests conducted using Minitab 17 software were used to determine if significant relationships existed between variables. Mean values are presented as mean ± standard deviation.

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**Results**

**Sessile invertebrate community assessment**

The most abundant species of coral present on the mooring blocks were the brain corals *Diploria labyrinthiformis* and *Colpophyllia natans* and the fire corals *Millepora complanata* and *Millepora alcicornis*. When combined, these four species accounted for over 92.9% of the total coral cover. The mean coral cover on the blocks was 21.4% ± 9.4% (SD). The mean rugosity of all the blocks was 1.085 ± 0.090 (SD).

A significant positive correlation was found between coral cover and rugosity (\(R^2=0.363\), \(p=0.008\)). Likewise, fire coral cover and rugosity displayed a significant positive correlation (\(R^2=0.404\), \(p=0.005\)) (Fig. 2). The correlation between brain coral cover and rugosity was positive though not statistically significant (\(R^2=0.212\), \(p=0.054\)). No correlation was found between fire coral cover and brain coral cover (data not shown).

**Fish community assessment**

The most abundant fish species were *Acanthemblemaria maria* (secretary blenny), *Chromis multilineata* (brown chromis), *Stegastes partitus* (bicolor damselfish), juvenile *Thalassoma bifasciatum* (blueheaded wrasse), *Abudefduf saxatilis* (sergeant major),...
Fig. 2 Correlation of (a) mean coral cover and mean rugosity and (b) mean fire coral cover and mean rugosity. Markers represent individual blocks (n=18). Data was collected from the offshore mooring blocks at Yellow Submarine dive site (12°09'36.3"N 68°16'55.2"W) on Kralendijk, Bonaire.

and Ophioblennius macclurei (redlip blenny). Mean fish species richness for each block set was 10.56 ± 1.92 (SD) and mean fish species diversity (calculated with the Shannon Index) was 1.607 ± 0.189 (SD). Juvenile fish constituted an average of 19.62% ± 5.37% (SD) of the fish populations at each set. Actively defended A. saxatillis egg patches were observed on 10 of the 18 blocks. Of these 10 blocks, A. saxatillis eggs covered an average of 2.57% ± 2.04% (SD) of the total area analyzed. No correlations were observed between A. saxatillis egg cover and fish species richness or fish species diversity (data not shown).

Correlations between sessile invertebrate and fish communities

Supporting the proposed hypotheses, significant positive correlations were found between coral cover and fish species richness (R²=0.365, p=0.008), coral cover and fish species diversity (R²=0.244, p=0.037), rugosity and fish species richness (R²=0.441, p=0.003), and rugosity and fish species diversity (R²=0.446, p=0.002) (Fig. 3). Brain coral cover and fish species richness displayed significant positive correlations (R²=0.327, p=0.013) (Fig. 4). There was no significant correlation between brain coral cover and fish species diversity (data not shown). Fire coral cover displayed positive correlations with both fish species richness (R²=0.232, p=0.043) and fish species diversity (R²=0.325, p=0.014) (Fig. 4).

Discussion

There was a strong correlation between coral cover and rugosity, indicating that coral cover is a primary component of structural complexity on the offshore mooring blocks in this study. Only four species of coral, however, were responsible for the majority of the cover. This trend follows a past study that found that the highest coral species diversity was associated with an intermediate level of reef structural complexity (Aronson and Precht 1995). Evidently, higher degrees of coral species diversity are not necessary to support high structural complexity. This could indicate that predicted declines in coral diversity will not necessarily onset an equal decline in structural complexity (Chadwick-Furman 1996). Fire coral cover was found to have a greater correlation to rugosity than brain coral cover, which seems logical when considering the branching and plate-like structures of fire coral relative to the less complex, round shape of brain corals.

The majority of the same fish species were exhibited at each set of mooring blocks. These species were mostly blennies, gobies, damselfish, chromis, and juvenile wrasses. This observed composition of individuals with relatively small body sizes may be attributed to the small scale of the blocks. These findings coincide with those of Thomas (2009) in which similar fish species were observed across the
Fig. 3 Correlation of (a) mean coral cover and mean fish species richness, (b) mean coral cover and mean fish species diversity, (c) mean rugosity and mean fish species richness, and (d) mean rugosity and mean fish species diversity. Markers represent individual blocks (n=18). Diversity was calculated using the Shannon Index. Data was collected from the offshore mooring blocks at Yellow Submarine dive site (12°09'36.3"N 68°16'55.2"W) on Kralendijk, Bonaire.

Fig. 4 Correlation of (a) mean brain coral cover and mean fish species richness, (b) mean fire coral cover and mean fish species richness, and (c) mean fire coral cover and mean fish species diversity. Markers represent individual blocks (n=18). Diversity was calculated using the Shannon Index. Data was collected from the offshore mooring blocks at Yellow Submarine dive site (12°09'36.3"N 68°16'55.2"W) on Kralendijk, Bonaire.
block sets but total species composition between them and the natural reefs differed significantly.

It was hypothesized that increased coral cover and rugosity on the mooring blocks would correspond with increased fish species richness and diversity. The results supported these hypotheses with significant correlations. This is not necessarily surprising, as reef fish are usually associated with coral and previous studies have highlighted the relationship between structural complexity and fish species richness and abundance (Gratwicke and Speight 2005a, Gratwicke and Speight 2005b). When considering the relatively small size and young age of the coral-based systems on these mooring blocks, however, it is impressive that these correlations can reflect those of much larger and well-established reefs.

Fire coral cover displayed strong correlations with both fish species richness and diversity. Brain coral cover, however, exhibited significant correlation with fish species richness but not with fish species diversity. The association between fire coral and greater structural complexity may explain this observation. While a wide range of coral types may provide habitat to support adequate fish species richness, a high degree of structural complexity may be required for maximum species diversity. This reasoning could be explained by the Hutchinsonian concept of a niche, which states that more resources lead to more niches, resulting in more coexistence and species evenness (Pulliam 2000). The main resource in this case is shelter within the reef and the increased structural complexity of fire corals compared to brain corals may lead to a greater availability of this resource and an increased evenness of fish species. Because diversity is a calculation involving species evenness, this could explain why a structurally complex reef system, like blocks with high fire coral cover, might contribute to a maximized species diversity.

In this study, seasonal variations in fish populations and development of sessile invertebrate communities preceding this study were not captured. Analyses of the fish and sessile invertebrate communities on the mooring blocks every few years over the coming decades in addition to seasonal studies would offer a tremendous window through which to observe community development on artificial structures.

Reefs are often seen as ancient structures that grew on an evolutionary timescale to achieve their complex ecology. However, coral cover and structural complexity appear to have strong effects on fish communities in coral-based systems after only 20 years. As coral reef conservation efforts proliferate, recovery of damaged areas and colonization on artificial structures will create an abundance of relatively young coral systems. Understanding the ecology of these systems, as well as the ecology of all ecosystems, is absolutely necessary if human beings are to ever diverge from the trajectory of environmental destruction and move towards a cohesive existence with nature.

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References
Antibacterial effects of three Caribbean sponges from areas of varying pollution

Abstract Sponges are sessile marine organisms that have developed efficient defense mechanisms against microbial pathogens. These organisms are one of the most promising sources of antibiotic pharmaceutical products derived from the ocean. As human infectious microorganisms evolve to become more resistant to our current antibacterial medications, the medical community has developed an increased interest in the use of sponges for novel medications. This study aims to provide a basis for the collection of sponges to be used for pharmaceutical purposes. Sponges have shown variation in antimicrobial compounds due to changes in their environment, such as increased temperature or depth. This study analyzed variation in antibacterial properties based on proximity to a pollution source. Samples of three Caribbean sponges, Pseudoceratina crassa, Aplysina archeri, and Holopsamma helwigi, were taken from areas of low relative pollution and high relative pollution, caused by the presence of an adjacent drainage ditch. Sponge extracts were used to create antibacterial assays to test the inhibition of each sponge species at each site toward bacteria derived from the human mouth. Two of the three species, P. crassa and A. archeri, were found to inhibit bacteria, while H. helwigi showed no inhibition. Pseudoceratina crassa and Aplysina archeri taken from an area of high pollution showed greater inhibition levels than samples from areas of low pollution. Pseudoceratina crassa from both sites inhibited significantly less bacteria than A. archeri. These results suggest that sponges from high-pollution areas might be more useful than those from low-pollution areas in the production of pharmaceutical products.

Keywords Phenotypic plasticity • Antibacterial assay • Pharmaceutical

Introduction

Sponges inhabit nearly every type of marine environment across the world. They often contain abundant microbial communities including bacteria, archaea, microalgae, and fungi. The microbial symbionts can comprise as much as 40% of the sponge volume and contribute significantly to the host metabolism (Vacelet J 1975). Sponges are continually exposed to water-borne pathogens and other microbes, yet they rarely exhibit bacterial infections (Newbold et al. 1999). The sponge class Demospongia is known to produce the largest number of secondary metabolites, organic compounds not directly involved in growth and development, of all marine organisms. The ecological functions of these metabolites are largely unknown, however it is estimated they are used for defense against infection (Newbold et al. 1999).

The amount of research being conducted on the antimicrobial properties of sponges has become increasingly important over the past few years, as the medical community has become interested in the ocean as a potential resource for new medications. Marinho et al. (2010) investigated the effects of sponge extracts against 44 different bacterial strains, including fourteen antibiotic resistant strains. Results showed varying levels of bacterial inhibition by all six of the sponge species used...
(Marinho et al. 2010). Kelly et al. (2003) tested the effects of 26 different sponge species on the bacteria *Vibrio harveyi* and found that 21 species caused a significant reduction in bacterial growth. The medications that could be developed from these organisms have the potential to provide treatment to a large group of people with various diseases. More than 5,300 different products are derived from sponges and their associated microorganisms, and more than 200 new metabolites from sponges are reported each year (Laport et al. 2009). Infectious bacteria continually evolve and develop resistance to existing pharmaceuticals, making it necessary to find new sources of medication. Sponges have provided the medical community with products that fight against bacterial, viral, fungal, and parasitic diseases. Many marine products have been selected for promising leads in further pre-clinical assessment, such as psammamplin A, which shows antibacterial activity (Laport et al. 2009).

It has also been proposed that sponges could act as bioindicators for metal contamination in polluted water because they accumulate metals and become sensitive to these pollutants after a short period of time. Growth rates, shape, and stress proteins seem to be unaffected in sponges subjected to metallic pollution, but filtration rates significantly decrease (Cebrian 2005). Less is known about the effects of microbial pollution on sponges, although they have been recognized as a useful tool for marine environmental bioremediation in areas polluted with bacteria (Zhang et al. 2009). Sponges have been reported to possess high efficiency in removing bacteria pollution from seawater (Zhang et al. 2009). Milanese et al. (2003) found that the Mediterranean sponge *Chondrilla nucula* can filter up to 14 liters of seawater per hour, retaining *Escherichia coli* at a rate of up to 7 x 1010 bacterial cells per hour in a one square meter patch of sponge. This uptake of bacteria, along with other organic matter found in polluted water, can be utilized by sponges as food (Zhang et al. 2009). However, some of that bacteria may be pathogenic. It is reported that sponges possess molecules similar in structure to those involved in the mammalian immune system in order to protect themselves from harmful infections that could be found in the polluted water they filter (Muller et al. 1999).

Sponges have shown variation in antimicrobial compounds in several circumstances. One study showed an increase in the synthesis of an antimicrobial compound after exposure to a specific endotoxin (Muller et al. 2004). Another study found that the sponge *Ircinia fusca* has selective control of the microorganisms on its surface. The sponge seemed to have the ability to promote the growth of antibacterial compounds that could help it control bacterial attachment to its surface in response to temporal variations in the habitat (Thakur 2004). In these instances, the chemical nature and amount of antibacterial compounds produced by sponges seem to be governed by environmental conditions.

No currently available studies have investigated the relative success of sponge antimicrobial compounds based on the sponge’s proximity to a bacterial pollution source. This study aims to provide information regarding the antimicrobial activity of certain Caribbean sponges residing in areas of relatively high water pollution versus those residing in low pollution areas. By examining the response of bacteria to various sponges, this analysis will investigate whether or not sponges in areas of higher pollution contain more effective antibacterial compounds. Polycyclic Aromatic Hydrocarbons (PAHs) are a known component of many pollution sources. The presence of PAHs can result in increased microbial abundance when in the presence of associated extractable humic substances (EHS), a category of substances associated with seawater (Rezaei Kalantary and Badkoubi 2005). Dissolved organic matter from marine sediments is the main component of the EHS (Mecozzi et al. 2008). They have been shown to provide a growth substrate that can cause an increase in bacterial populations (Rezaei Kalantary and Badkoubi 2005). This means sponges in contaminated areas with excessive
run-off, such as near a drainage ditch, may come in contact with greater amounts of bacteria due to the PAHs in the water. This could suggest higher levels of antibacterial compounds present in sponges due to the increased need to protect themselves from a greater concentration of bacteria than in less contaminated areas. In other words, these sponges may have adapted to better defend themselves from harmful pollutants to which they are subjected, and therefore could have developed better antibacterial mechanisms in response to their environment.

If a correlation such as this were discovered, this study will provide a basis for the collection of sponge-derived medications by providing data on where the most effective compounds may be found. If antibacterial compounds are more active in sponges grown in areas of higher pollution, then sponges collected for medical use should be taken from areas of high pollution. In addition, medical labs harvesting these compounds for pharmaceutical products should take this into consideration in order to obtain the best possible product.

H1: Sponges from areas of run-off pollution containing bacteria will inhibit more bacteria than sponges from areas of lower pollution

Materials and methods

Study site

This study took place on the western coast of Bonaire, Dutch Caribbean in the city of Kralendijk. Sponge samples were taken from the Yellow Sub dive site (12°09’36.5” N, 68°16’55.2” W) as well as in front of a small drainage site located adjacent to the Kas di Arte property (12°09’21.4” N 68°16’45.3” W; Fig. 1). The Kas di Arte site is known to have elevated run-off and polycyclic aromatic hydrocarbons (PAHs), a recognized component of run-off pollution (Mason 2013). This is suggestive of an enhanced bacterial community at this site. These two locations provided a comparison between the polluted, bacteria concentrated water found at the Kas di Arte site, and the lower levels found at Yellow Submarine.

Sponges for analysis

The sponges used for this study were selected using criteria based on research completed in a 2014 spring study on the bioaccumulation of Caribbean sponges. It was found that Pseudoceratina crassa (branching tube sponge) had the highest levels of bioaccumulation of pollutants among the three species sampled (Middleton 2014). The high bioaccumulation levels could potentially point toward a greater amount of antimicrobial compounds necessary to protect the sponge from what it is bioaccumulating. Therefore, this species of sponge was an ideal candidate for this study. Aplysina archeri (purple stove pipe sponge) is a species of similar structure to the branching tube sponge that was also tested. The third species used in this study, also tested in spring 2014, is Holopsamma helwigi (lumpy overgrowing sponge). It was chosen due to the large abundance present at both testing sites.
Sponge collection

Sponge samples of each of the tested species were collected from both Yellow Sub and the Kas di Arte site. A previous study showed very little variation in bacterial communities of Caribbean sponges at different depths (Olson and Gao 2013). Nevertheless, the depth of the sponge was controlled for by collecting samples of sponges between 9 and 12 m on the reef slope. Samples were collected by cutting off several small pieces and subsequently storing them in plastic containers with water from the collection site. Samples were labeled based on species and location. Immediately after collection the samples were frozen in a -20°C freezer until further testing could be conducted (Middleton 2014).

Bacterial plate and sponge extract preparation

Bacteria cultures were made using the bacteria fauna found in the human mouth, which harbors one of the most diverse microbiomes in the human body (Wade 2012). Around 1000 bacterial species have been found, with representatives from the phyla Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, Synergistetes, and Tenericutes (Wade 2012). Nutrient agar plates were made with sterile petri dishes and a human mouth was swabbed with a q-tip to be added to the plates.

Each sponge was thawed and cut into small pieces to be placed in a 10ml methanol solution until a total volume of 15ml was achieved. Protocol was based on established methods that utilize the Kelly et al. (2003) study, which recognized the effectiveness at which secondary metabolites are extracted via this solution. The sponges were then squeezed from the sponge tissue into a separate container. The solvent was allowed to evaporate at room temperature before re-dissolving the extract in 5 ml of a 4:1 methanol:water solution so that the volumetric concentration was that of the original tissue. This solution of sponge extract was then pipetted onto holes that had been punched out of filter paper and the solvent was allowed to evaporate. The resulting discs were added to agar plates at the time of bacterial inoculation to create an antibacterial assay. Plates were then incubated at 30°C for 72 hours. Twelve plates were made total, each containing discs for two of the three species of sponge used, from both high and low pollution sites, as well as two of three controls (Fig. 2). This resulted in eight replicates for each species of sponge.

![Fig. 2 Antibacterial assay distribution diagram on bacteria cultured nutrient agar plate. Six of twelve plates included a variable water control and six included a positive antibacterial dish soap control. All twelve plates included a methanol control and two sponge species from high pollution and low pollution locations](image-url)
All twelve plates contained a methanol solution control disc prepared with only a 4:1 solution of methanol:water. Six of the plates contained a filter paper control disc prepared with only water, while the other six plates contained a positive control disc prepared with Dove antibacterial dish soap.

**Bacterial inhibition analysis**

To determine the amount of bacteria inhibited by each set of antimicrobial compounds the diameter of the zone of inhibition was measured in centimeters for each assay containing sponge antibacterial extract. The zone of inhibition is defined as the ring around the sponge extract-containing disc in which no bacteria grew. The diameter included the disc itself (Fig. 3). The average zone of inhibition was calculated to provide a comparison of overall antibacterial effects at the Kas di Arte site versus the Yellow Submarine site for each sponge species. Antibacterial data was analyzed using t-tests.

**Results**

The three species of sponge *Pseudoceratina crassa*, *Aplysina archeri*, and *Holopsamma helwigi* were used in this study to test the antibacterial effects of sponges based on their proximity to a pollution source. Eight assays for each sponge species at both high and low relative pollution levels were placed on 12 nutrient agar plates, along with negative and positive controls. It was found that two of the three species of sponge, *P. crassa* and *A. archeri*, inhibited bacteria found in the human mouth, while *H. helwigi* had no inhibition for all eight trials. On average, *P. crassa* had less inhibition than *A. archeri* at both sites, with a significant difference of 0.9 cm between the two species at Kas di Arte (t= -2.62; p=0.0201) and 1.16 cm between the two species at Yellow Submarine (t= -2.49; p=0.0259). In other words, *A. archeri* inhibited 3.18 times more bacteria than *P. crassa* at Yellow Submarine, and 1.58 times more bacteria than *P. crassa* at Kas di Arte (Fig. 5).

*Pseudoceratina crassa* trials from the Kas di Arte site (greater pollution) inhibited a significantly greater amount of bacteria than trials from Yellow Submarine (low pollution; t= -3.35; p=0.00477; Fig. 4a and Fig. 5). *Aplysina archeri* trials from Kas di Arte also inhibited a greater amount of bacteria than *A. archeri* trials from Yellow Submarine, though this trend was insignificant (t= -1.58; p=0.135; Fig. 4b and Fig. 5). The average of all eight trials for each sponge species was calculated to give an average zone of inhibition diameter for each sponge at each site (Fig. 5). The averages for *H. helwigi* at high pollution and low pollution sites were both zero, as no inhibition was seen. *Pseudoceratina crassa* trials from an area of higher pollution exhibit an average zone of inhibition 2.94 times greater than trials of the same species taken from an area of lower pollution. *Aplysina archeri* taken from an area of higher pollution exhibit an average zone 1.46 times greater than that of samples taken from a lower area of pollution (p=0.342; Fig. 5).

**Discussion**

Two of the three sponge species used in this study, *P. crassa* and *A. archeri*, exhibited inhibition of bacteria found in the human mouth, while the third species, *H. helwigi*,
Fig. 4 Diameter of bacterial inhibition measured in centimeters on a nutrient agar plate due to (a) *Pseudoceratina crassa* (*Branching Tube sponge*) and (b) *Aplysina archeri* (*Purple Stove Pipe sponge*) antibacterial extract. Samples from both a low pollution site (Yellow Submarine) and a high pollution site (Kas di Arte) are shown. Eight measurements were taken for each site on eight different nutrient agar plates. Bacteria from the human mouth were used to test inhibition caused by sponge extracts.
showed no inhibition for all eight trials. Pseudoceratina crassa taken from an area of high pollution showed significantly greater inhibition levels than those samples taken from areas of low pollution. Aplysina archeri showed an increase in bacterial inhibition from low to high pollution levels, though it was not statistically significant. Pseudoceratina crassa from both sites inhibited significantly less bacteria than A. archeri from both sites.

These results indicate a significant difference in the ability of each sponge species to inhibit bacteria found in the human mouth. The findings of this study are congruent with those of other studies, such as Marinho et al. (2010), implying a mechanistic difference in the capability of sponges to inhibit different bacterial strains. Sponge species therefore may vary in their antimicrobial compounds and the intensity of those compounds. Certain species are more capable of inhibiting specific bacterial strains than others. Variability in bacterial inhibition can give a wide range of results between different species of sponge in regards to what type of bacteria is subjected to inhibition. When using sponges for the development of pharmaceutical products against a certain bacterial strain, it is therefore important to test the effects of a wide range of sponge species to find the most successful product.

The ability of marine sponges to inhibit human derived bacteria could stem from the proposed theory that sponges are more closely related to humans than once realized (Srivastava et al. 2010). A recent study suggested Amphimedon queenslandica, a demosponge from the Great Barrier Reef, has a genome remarkably similar to other animal genomes in content, structure, and organization (Srivastava et al. 2010). Sponges seem to share a range of features with humans, including stem cells, cell growth, and immunity (Srivastava et al. 2010). These similarities could be the reason sponges produce such a large array of compounds of interest to the
medical community, as well as the reason the sponges in this study were able to inhibit bacteria taken directly from a human mouth.

Results also indicated a significant increase in the bacterial inhibition of *P. crassa* samples taken from areas of high relative pollution in comparison to samples taken from an area of lower pollution, with those from highly polluted areas showing 3.18 times more inhibition. The same trend was observed with *A. archeri*, though it was not significant. However, in the case of *A. archeri*, if more samples had been taken a significant relationship may have been observed between samples taken from high and low areas of pollution.

The results collected based on the bacterial inhibition in this study also suggest that certain sponge species have the ability to adapt to waters with greater bacterial pollution by secreting more successful antibiotic compounds than they would in areas of lower pollution. This could be a phenotypic change in the sponge immune system, used for increased protection against the potentially pathogenic bacteria they encounter while filtering water. This phenotypic plasticity allows for a significant alteration in the properties of the sponge on a short ecological time scale.

Sponges are considered to have a high level of phenotypic plasticity in several circumstances (Bertolino et al. 2013). This characteristic allows them to live in a variety of different habitats by adhering to their environment. One study conducted in 2002 showed a morphological response to predation in which mechanical stimulation can serve as a cue to increase spicule concentration in sponges (Hill 2002). As previously mentioned, another study showed an increase in the synthesis of an antimicrobial compound after exposure to a specific endotoxin (Muller et al. 2004). In the Thakur (2004) study it was found that the sponge *Ircinia fusca* has selective control of the microorganisms on its surface. In this study the sponge seemed to have the ability to promote the growth of antibacterial compounds that could help it control bacterial attachment to its surface in response to temporal variations in the habitat (Thakur 2004). Most studies of environmental stress in sponges have examined the effects of elevated seawater temperature (Simister et al. 2012).

It has therefore been made clear on several occasions that sponges have the ability to alter their phenotypes in response to environmental stimuli, often times including their associated microbial community. This phenotypic plasticity allows them to survive in a variety of environments, including those that are highly polluted. The results found in this study are consistent with the findings of previous research, again demonstrating a phenotypic change in sponges residing in different environments. This could suggest that sponges inhabiting areas of high pollution have undergone adaptations to make them phenotypically more successful in their environment by providing a better immune system, leading to the increased bacterial inhibition level seen in this study.

This study provides a basis for the collection and harvest of sponges used for medical purposes. Antibacterial compounds derived from sponges to be used against a specific bacterial strain may be more successful coming from sponges that live in bacteria polluted waters. The variation in sponges based on environmental factors suggests that sponges living in polluted environments will harbor more effective antibacterial compounds, as seen in this study. Therefore, sponges collected for antibacterial purposes should be taken from areas of high pollution to ensure the most successful product. In addition, sponges that are harvested in medical labs should be done so in water with a high concentration of bacteria. This would allow for the growth of antibacterial compounds phenotypically suited for a polluted environment, giving them heightened inhibition.

To further understand the relationship between the success of antibacterial compounds and their environment, future research should be conducted to analyze the bacterial levels in water at which sponges begin to secrete greater levels of antibacterial compounds. This would better establish the
relationship between environmental bacterial conditions and sponge antibacterial activity. Studies such as this could also be used to test if a specific type of bacteria causes these changes in the sponge microbial community. Additionally, certain species of sponge may respond to this change better than others. Several different species of sponge should be sampled as done in this study to determine which species have the ability to increase their antibacterial properties, as well as how long it would take for a significant increase to be seen given a new pulse of bacteria.

Overall, this study offers a basis for the collection of sponge-derived medications by providing previously un-recognized data on where the most effective compounds may be found. As pathogenic bacterial strains infecting humans become increasingly resistant to our currently available medications, it is important that we discover effective alternative antibacterial products. Sponges have been shown to be an important resource for this reason, and this study has found that more successful antibacterial compounds reside in sponges in areas of higher pollution. The medical community should take this into consideration in order to obtain the best possible pharmaceutical product to fight against human bacterial infections.

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Increasing coral cover and decreasing coral species diversity with decreasing light availability at depth: a study of the future effects of sea level rise on fringing reef ecosystems

Abstract  Global climate change is having widespread effects on the world’s oceans. Particularly vulnerable to changing global conditions are coral reefs, which boast high biodiversity levels though they comprise a small percentage of the oceans. Sea level rise decreases light availability to corals and their symbiotic photosynthetic zooxanthellae, which are responsible for fixing the carbon that corals use to create their carbonate skeleton. Studies have shown how changing light availability with depth can be used as an indicator of coral species diversity. Species richness and diversity of corals is greatest at intermediate depths, with decreasing diversity at greater depths. This study investigated the relationship between coral species diversity and light intensity at depths of 12, 18, and 24 m on the fringing reef ecosystem off the western coast of Bonaire, Dutch Caribbean. Light intensity data were based on theoretical values provided by Beer-Lambert’s law, and video transects were conducted to determine composition of the substrata and to calculate coral species diversity and richness. Strong correlations were found between decreasing coral species diversity with depth (p = 0.041) and percent coral cover and decreasing percent cover of macroalgae and cyanobacteria with depth (p = 0.000, F = 37.60, df = 1). This indicates that while species diversity of corals decreases with lowered light intensity, corals are better able to outcompete macroalgae in environments with decreased light availability. This information is useful in understanding how reefs will respond to environmental changes brought on by sea level rise.

Keywords  Climate change • Coral reefs • Light intensity

Introduction

Sea level rise has become a topic of increasing concern in recent years. Carbon emissions from the burning of fossil fuels have been causing a “greenhouse effect”, in which infrared radiation from the sun is absorbed and trapped in the planet’s atmosphere instead of being reflected back into space (Buddemeier and Smith 1988). The retention of heat by the atmosphere has strong effects on the world’s oceans. There are two factors that contribute to sea level rise: (1) thermal expansion of water due to increased ocean temperatures and (2) increased water mass due to the melting of land ice (Nicholls and Cazenave 2010).

The combined effects of the factors contributing to sea level rise have resulted in average sea level increases of 2.3 mm yr\(^{-1}\) during the last 60 years (Buddemeier and Smith 1988). Using estimates of temperature rise between 1990 and 2100 (1.4 to 5.8°C) provided by the Intergovernmental Panel on Climate Change (IPCC), Rahmstorf (2007) has demonstrated that expected sea level rise will range from 55 and 125 cm during this period. This rise corresponds to annual rates of sea level rise between 3.2 and 3.5 mm per °C temperature rise (Rahmstorf 2007). These annual rates imply that sea levels could rise by up to 20 mm yr\(^{-1}\) before the end of the 21st century. Coral reef ecosystems are particularly susceptible to the effects of sea level rise since
coral reef platform growth occurs at much slower rates than that of sea level rise, with normal vertical accretion rates in the range of 1 to 10 mm yr\(^{-1}\) (Hamlyton et al. 2014). Since rates of sea level rise are expected to exceed common coral reef vertical accretion rates, corals are at risk of being drowned.

Corals grow by accretion of their calcium carbonate skeletons. It is this calcification process that builds the structure of the reef and creates microhabitats for an abundance of organisms, making coral reefs some of the most diverse ecosystems on the planet. Studies have been conducted showing trends of coral species diversity decline over the years. Bak and Nieuwland (1995) found that coral species richness decreased between 5 and 45 percent over twenty years on the Caribbean islands of Bonaire and Curação. It has also been shown that different types of corals display different rates of survivorship over time. In a study of coral species diversity revisited by Bak et al. (2005), it was found that hemispherical corals (e.g. Diploria strigosa, Colpophyllia natans) have been most affected by changing environmental conditions over time. This has resulted in a marked decrease in percent coral cover of these species, while species that have more variable morphologies have shown no decrease in relative coral cover over time. No correlations have been made between these observed trends and sea level rise, but the impact of light availability is mentioned as a significant factor in the biological function of shallow reef corals (Bak et al. 2005). These studies serve to illustrate the idea that not all coral species are equally affected by changing external conditions and that an analysis of trends of coral cover decline must be examined on a species level and not just for corals as a collective whole.

While many factors affect the diversity and richness of coral reefs, none are so evident within the scope of sea level rise as light availability. Since hermatypic (reef-building) corals utilize symbiotic photosynthetic zooxanthellae for most of the carbon that they fix in calcification, light intensity is intrinsic to the functioning of these photosynthetic organisms (Jackson 1991). It is through calcification that corals grow, and a decrease in light intensity at depth could result in a decline of photosynthetic activity and could lead to coral death. A study analyzing factors affecting coral species diversity found intensity of light to be the only factor that follows the trend of species richness of corals with depth; other biotic and abiotic factors such as competition for space, turbidity, and wave energy varied in frequency and intensity and thus were insufficiently correlative (Huston 1985). According to the author, decreasing light availability with depth is the only physical gradient that accurately explains the trend of coral species diversity levels on all coral reefs (Huston 1985).

Numerous studies have been conducted showing relationships of coral species richness and percent coral cover with depth. Huston (1985) concluded that the coral diversity gradient has a strong association with the changes in availability of light energy, increasing in diversity to 20 m with a decrease after this point. A twenty-year study conducted on the fringing reef ecosystems of Bonaire and Curação found that the greatest number of coral species was found at 20 m, the second highest at 10 m, and the fewest at 30 and 40 m (Bak and Nieuwland 1995). Corals at 10 and 20 m experienced the greatest reduction in coral cover over time, with decreases ranging from 20 to more than 70%. Percent coral cover at 30 and 40 m remained constant or even increased during this time period. Light was mentioned in their study as an important factor about which further study is needed. The apparent trend of greatest coral species richness at depths in the range of 20 m is supported further by a study that found that the number of coral species was greatest at intermediate depths (Jackson 1991). It was suggested that this trend is due to accordance with the intermediate disturbance hypothesis, which postulates that species diversity is greatest when disturbance from biotic and abiotic factors is not too low or too high (Jackson 1991).
This study provides information that will allow for a better understanding of the relationship between light availability and coral species diversity and the impacts that increasing sea levels could have on coral reef ecosystems by the end of the 21st century. It has been shown that the availability of light is at least correlational to coral species richness and diversity. It is anticipated that a similar trend will be present in this study on the western fringing reef near Kralendijk, Bonaire. This study builds on the literature providing information about the trends of coral species diversity changes over time, but it is the first that aims to find a direct relationship between light availability and coral species diversity and richness.

H1: Due to accordance with the intermediate disturbance hypothesis, coral species diversity increases with depth until approximately 20 m

H2: Due to accordance with the intermediate disturbance hypothesis, coral species richness increases with depth until approximately 20 m

H3: Coral species diversity decreases at depths greater than 20 m

H4: Coral species richness decreases at depths greater than 20 m

Materials and methods

Study site

The study was conducted at the Yellow Submarine dive site (12°09′36.42″ N, 68°16′54.84″ W), located on the western side of the island of Bonaire, Dutch Caribbean (Fig. 1). The site is defined by a sand flat that extends 50 m offshore and reaches a maximum depth of 5 m at the reef crest. The reef slopes downward at an angle of 45° to a depth of 28 m, where a second sand flat begins.

Fig. 1 Map of Bonaire, Dutch Caribbean showing the study site. Yellow Submarine dive site (12°09′36.42″ N, 68°16′54.84″ W) is located on the western side of the island is denoted by the black circle

Video transects

Two 30-m underwater video transects were conducted at depths of 12, 18, and 24 m. Underwater video cameras were used to record the composition of the substrate in 50-cm wide bands along the 30-m transects, resulting in 15-m² areas that were used in analyses of coral species richness and diversity. Video transects were taken with the tape measure running through the center of the transect. Methods used were a slight variation of those defined by Lam et al. (2006), with changes made only to transect length and width.

Light measurements

Light intensity measurements were gathered using theoretical values that follow Lambert-Beer’s law. The equation is as follows:

\[ I(D) = I_0 e^{-kD} \]

\(D\) represents depth in meters and \(k\) (0.035) represents the extinction coefficient of light in salt water (Mateus 2012).

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Data analysis

Non-overlapping frames were manually extracted from each video using Picture Motion Browser software. These images were imported into Coral Point Counter (CPCe) to calculate substrate composition. The entirety of each transect was split into frames and 20 sampling points were randomly selected from each frame for identification. Images were also used in measurements of species richness of corals. Calculations of species diversity were determined using Simpson’s Diversity Index:

\[ D = 1 - \frac{\sum n(n - 1)}{N(N - 1)} \]

\( N \) represents the number of organisms of all species and \( n \) represents the number of organisms of a single species.

Results

Increasing coral cover with depth

Comparisons of coral species cover with depth were made by examining video transects and identifying species from video frames using Coral Point Counter (CPCe) (Fig. 2). *Undaria agaricites* (UAGA), *Orbicella annularis* (OANN), and *Orbicella faveolata* (OFAV) were the three most common coral species. Percent cover of UAGA and OFAV show a significant increase with depth. At 24 m, UAGA and OFAV dominated the substrate, accounting for 18.15 (SE ± 1.62) and 13.07 (SE ± 1.57) percent cover, respectively. The percent cover of *Orbicella annularis* decreases greatly with depth, from 7.28 (SE ± 1.34) percent at 12 m to 0.69 (SE ± 0.27) percent at 24 m.

Comparison of cover of various substrate types

Percent cover of substrate types were identified using the same methods for identifying coral species cover, described in the previous section (Fig. 3). The four most common substrate types were identified (coral; macroalgae; cyanobacteria; and sand, pavement, and rubble), and a series of two-sample t-tests found that changes in coral species cover (\( p = 0.030 \)) and macroalgae cover (\( p = 0.015 \)) with depth were statistically significant. Changes in percent cover of cyanobacteria (\( p = 0.216 \)) and
sand, pavement, and rubble (SPR) \( (p = 0.184) \) with depth were not statistically significant. An ANCOVA test was run to determine the relationship between percent coral cover and percent cover of macroalgae and cyanobacteria, which were combined. Depth was used as a covariate. A strong association \( (p = 0.000, F = 37.60, df = 1) \) was found between percent coral cover and combined percent cover of macroalgae and cyanobacteria. A strong association \( (p = 0.000, F = 8.19, df = 2) \) was also found between percent coral cover and depth, but no association was found between all three factors.

Comparison of coral species diversity and light intensity

Coral species diversity was determined using Simpson’s Diversity Index. Light intensity measurements were calculated using Beer-Lambert’s law (Fig. 4). A statistically significant relationship \( (p = 0.041) \) was found between coral species diversity and light intensity. Both coral species diversity and light intensity decreased with depth.

Comparison of coral species richness and light intensity

Coral species richness was determined at each of the three depths. These values were displayed with the same light intensity values that are shown in the previous section (Fig. 5). Coral species richness remains relatively constant despite the gradually decreasing light intensity levels at depth and shows no significant relationship with light intensity.

Discussion

The results of this experiment show a clear trend of decreasing coral species diversity with decreasing light intensity at depth. No correlation was found between coral species richness and light intensity. The first and second hypotheses were rejected because the trends of coral species diversity and richness...
were not consistent with the intermediate disturbance hypothesis. The third hypothesis was accepted because coral species diversity is found to decrease more from 18 to 24 m than from 12 to 18 m. The fourth hypothesis was rejected because there was no clear relationship between coral species richness and light intensity. Coral species diversity decreased with each increase in depth, and coral species richness remained relatively constant across depths. It was found that increased percent coral cover correlated strongly with decreased macroalgae and cyanobacteria cover at depth.

The strength of the relationship between percent coral cover and percent cover of macroalgae and cyanobacteria cannot be understated. The percent increase of coral cover from 12 to 24 m almost identically matched the combined percent decrease of macroalgae and cyanobacteria over the same depth range. Tanner (1995) conducted a study that investigated the competitive interactions between scleractinian corals and macroalgae and found that coral cover increased three times faster in areas where algae had been removed compared to those areas where algae was present. This study also stated that encrusting coral species that grow outward are more likely to experience spatial competition with algae compared to branching corals that grow upward (Tanner 1995). The decrease in macroalgae and cyanobacteria cover at depth may decrease the spatial competition between corals and algae. This would allow corals to cover a larger percentage of the substrate in areas where light levels are too low to support significant algae growth but still high enough to support coral growth.

Though percent cover of corals increased with depth despite a decrease in light intensity, coral species diversity decreased with depth. This is likely due to the changing morphologies of corals like UAGA and OFAV, which flatten at depth to increase the amount of sunlight they receive. The other coral species that were identified in this study are not able to change their morphologies like UAGA and OFAV can (Humann and Deloach 2001). This provides an suggestion as to why coral species diversity decreased with depth. Studies have shown that some coral species can have highly variable morphologies depending on light levels at depth (Todd 2008). Decreased light levels at depth cause coral species like UAGA and OFAV to spread out in order to maintain optimum photosynthetic processes of their symbiotic zooxanthellae. Though these corals are able to spread, it has been shown that the amount of light absorbed per unit area of coral decreases with increasing colony size (Stambler and Dubinsky 2005). This suggests that spreading coral species are less productive at depth, which indicates decreased resilience of corals to environmental changes.

Based on the data collected in this study, predictions can be made about the effect that sea level rise will have on coral reef ecosystems. By measuring coral species diversity at depth, estimates of how coral species diversity will change in the next century can be inferred. Sea level rise will increase the depth at which corals sit, decreasing the light available for their growth. If the vertical accretion rate of corals cannot keep up with the rate of sea level rise, coral reefs will become increasingly submerged. This study shows that coral species diversity decreases with decreased light intensity at depth, but the extent to which this change affects the resilience of coral reefs is still unknown. Suggestions for future studies include conducting more in depth studies on the species diversity of coral reef ecosystems, including measuring species diversity of fishes and invertebrates. Whether a decrease in coral species diversity equates to a similar decrease in overall species diversity on coral reefs was not investigated in this study. Other suggestions include collecting light intensity measurements in situ instead of utilizing theoretical light attenuation levels. Conducting transects at more depths would result in a more complete understanding of the changing diversity of the reef ecosystem.

The increasing ability of corals to outcompete macroalgae and cyanobacteria at depth implies that in a scenario with raised sea levels, corals would be more likely to
outcompete algae in an area that is today being overtaken by algae. There are many other factors that affect coral reef ecosystem health, but when only sea level rise is considered, there is evidence to suggest that reefs will remain coral-dominated though the diversity of corals will decrease. If coral diversity is used as an indicator of reef health, sea level rise will negatively affect coral reefs since fewer species are able to cope with decreased light intensity levels.

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References

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Control of algae on coral reefs by large herbivorous fishes

Abstract Coral reefs harbor a vast amount of global diversity relative to their size, and are an important economic resource to coastal communities. Over the past few decades, many coral reefs have undergone a phase shift from a substrate dominated by coral to one dominated by algae, largely due to anthropogenic stress. Herbivorous fishes play a major role in top-down control of algal growth and composition; however, depletion of biomass due to overfishing and habitat degradation has threatened the top-down control of fish herbivory on algal composition. This experiment compared endolithic turf algae (TA) composition on coralline rubble under complete fish exclusion, large fish exclusion (>13 cm), and no fish exclusion treatments. There was a significant increase in growth, richness, and percent cover of TA in response to reduced herbivory. The greatest compositional shift occurred in complete fish exclusion treatments. Crustose coralline algae (CCA), important in coral recruitment and growth, significantly increased in cover under every treatment except complete fish exclusion. This illustrates the importance of large-bodied herbivorous fish in controlling TA growth and maintaining bare substrate to facilitate coral recruitment and growth. This study provided insight into how Caribbean reefs go through initial stages of a phase shift from a coral-dominated benthos to one dominated by taller, denser algae. Finally, it illustrates how Bonaire’s reef, currently regarded as one of the most intact in the Caribbean, could change in composition if large herbivorous fish are removed from the ecosystem.

Keywords Coral reefs • Herbivore size • Algal phase-shift

Introduction

Over the past century, many coral reefs worldwide have become severely degraded or destroyed due to increased anthropogenic stress (Hughes 1994; McClanahan et al. 2002). Coral reefs harbor a disproportionally large amount of global species diversity (Gray 1997; Pandolfi et al. 2011) and are an important economic resource, particularly for tourism and fishing industries (Hughes 1994; McManus 2000; van Beek 2007). Coral and algae compete for space in the benthos. Historically, coral has been the dominant substrate (Box and Mumby 2007; Ferrari et al. 2012). However, over the past few decades, many reefs have undergone a phase shift from a coral-dominated to an algae-dominated substrate (Hughes 1994; McCook 1999; McClanahan et al. 2002; Hughes et al. 2007; Steneck et al. 2007). Herbivorous macro fauna such as echinoderms, scarids, and acanthurids play a major role in the top-down control of algal growth and composition (Hughes et al. 2007; Lewis et al. 2014; McManus 2000). Overharvesting and habitat degradation of many herbivorous fish populations have reduced their biomass and threatened the control of algal composition (Hughes 1994; McCook 1999; McManus 2000; Hughes 2007). In 1983, there was a mass mortality event of the long-spine sea urchin Diadema antillarum throughout the Caribbean. The loss of this key herbivore compounded with already depleted herbivorous fish populations lead to a significant increase in algal cover (Hughes 1994; McManus 2000; Steneck et al. 2005; Weil 2005). Algae cover morphologically shifts from low-lying TA (<2 cm) to fleshy macroalgae
when released from grazing pressure (Huntly 1991; Lewis et al. 2014). Fleshy macroalgae limits coral recruitment and survivorship, thereby creating a positive feedback loop (Fig. 1) that reinforces the phase shift towards an algae-dominated reef (Box and Mumby 2007; Rasher et al. 2012).

**Fig. 1** Theoretical positive feedback loop of phase shift towards an algae-dominated substrate

CCA is a reef building algae that facilitates coral recruitment, but is generally outcompeted by fleshy macroalgae in the absence of herbivory (McManus 2000; Harrington et al. 2004). Therefore, larger-bodied scarids have a greater impact on algal composition through their grazing regime, and preference for endolithic, and highly dense algal substrate.

Bonaire has a desert landscape surrounded by fringing coral reefs. The Bonaire National Marine Park (BNMP) contracts Stichting Nationale Parken Bonaire, a local not-for-profit, non-governmental organization to manage and enforce regulations of the marine park (Arnold et al. 2011). The BNMP extends from the high water mark to a depth of 60 m, and is made up of 2,700 hectares of coral reef, seagrass and mangal ecosystems (Arnold et al. 2011). Bonaire’s economy relies heavily on revenue from tourism related to the marine park (Van Beek 2011; Shep et al. 2013). However, the reef is being threatened by coastal development, runoff, wastewater pollution, increased population levels, and fishing pressure (Van Beek 2011).

It is illegal to remove anything from the BNMP, with the exception of Bonairian natives using approved fishing methods (STINAPA 2010). However, it is unclear how well these laws are enforced. Bonaire has the opportunity to ameliorate the detrimental impacts on the reefs by enforcing current laws, and regulating development, and growth on the island.

The goal of this study was to evaluate the impact of depletion of large herbivorous fish on algal composition on coral reefs in Bonaire. The hypotheses are as follows:

H₁: Decrease in large herbivorous fish will lead to a shift in benthic composition towards fleshy macroalgal growth

H₀: Decrease in large herbivorous fish will have no effect on algae composition and successional stage

In this study, algal growth, percent coverage, and richness were measured over a four-week period under various treatments. In addition, abundance and size of herbivorous
fish species were surveyed at the study site. This provided evidence for the short-term successional changes algae undergoes when herbivorous fish of various sizes are excluded.

Materials and methods

Study site

This experiment was conducted immediately south of the Yellow Submarine dive site on the leeward side of Bonaire, Dutch Caribbean, Latitude 12°9’ 36.648” N, Longitude 68°16’ 55.578” W (Fig. 2). The site has a typical visibility of approximately 30 m and a mild northward prevailing current. The benthos is composed of a shallow sand terrace and coral rubble that extends approximately 30 m out to a reef crest; the crest begins at a depth of approximately 5 m. Yellow Submarine has a fringing reef with high scleractinian coral cover, including Orbicella spp., Diploria spp., Montastrea cavernosa, Colpophyllia natans, Undaria agaricia, Meandrina meandites, and Porites astroides. In addition, Yellow Submarine has abundant sponge cover, patchy turf algae (TA), CCA, and fleshy macroalgae (FMA) cover. Common herbivorous fishes include various members of the families Scaridae, Acanthuridae, and Pomacentridae. This site was chosen due to its relative proximity to the CIEE Bonaire Research Station facilities and relatively even reef crest composition.

Experimental design

Algal composition was monitored over a four-week period from 19 October to 9 November 2014. Twenty experimental plots along the reef crest were chosen on the basis of having small coralline rubble (i.e. hard coralline substrate with dimensions smaller than 38.7 cm x 34.9 cm) at an average depth range of 5-6 m. All plots had sparse endolithic turf algae growth, with little apparent variance in algal height or immediately observable variation in composition. These 20 plots were randomly partitioned amongst the four experimental treatments, with 5 replicate plots per treatment. The depth of each plot was recorded to ensure that there was not a large amount of variation in depth between the four treatments. Each plot was photographed with a ruler placed against the adjacent benthos using an Olympus Stylus Tough-8000 camera in underwater macro setting. Photos were analyzed using ImageJ 1.48 to calculate the circumference of each plot, again ensuring that there was not a large variation in circumference between the four treatments.

The four treatments were as follows: 1. cage to exclude fish herbivory, 2. cage to exclude larger fish (>13 cm width or height), 3. cage frame only (to control for effect of the cage itself), 4. no-cage plot. The cages were made from white Sterilite® letter carts with dimensions of 38.7cm length x 34.9cm width x 26.7cm height and were altered to suit each treatment. Sterilite® letter carts were selected...
because they are constructed from plastic which is relatively chemically inert and therefore provided negligible influence on algal growth and recruitment. Treatment one cages were covered with fine-mesh plastic mosquito netting to completely exclude fish herbivory. Treatment two cages were left unaltered, with a hole size of 13 by 13cm. Treatment three cages had all side slats removed so that only the cage frame remained. Cages were centered over plots, and secured to the benthos using weights. Treatment four sites were marked using a single L-shaped PVC pipe placed adjacent to the plot. Cages and markers were cleaned of algae twice per week over the course of the experiment.

Data collection and analysis

Quantifying biomass of herbivorous fish at Yellow Submarine

Methodology from the Atlantic Gulf Rapid Reef Assessment (AGRRA) version 5.4 Fish Survey protocol was used to conduct a visual survey of AGRRA fish species detailed in the FISH-UW-V5.4 datasheet (Lang et al. 2010) at Yellow Submarine. The surveys were conducted via SCUBA on 29 September 2014 between 1300 to 1400 hours, at a depth range 10.1 to 15.2 m. Size and abundance of AGRRA fish in a 2 m water column along 30 m long by 2 m wide transects were recorded. Eleven surveys were conducted (one transect per surveyor). In addition, surveyors recorded maximum reef relief, type of structure and species along the 30 m transect at 5, 10, 15, 20, 25, and 30 m. The survey site had an average reef relief of 0.7 m.

For each transect (n=11), mean length per species was calculated; fish biomass conversion equations (Marks and Klomp 2003) were used to convert mean length to mean weight. Surveys were analyzed to assess AGRRA herbivorous fish species biomass per 100 square meters. Damselfish (Pomacentridae) are not part of the AGRRA 5.4 fish survey protocol, nor are Caribbean sharpnose puffer fishes (Canthigaster rostrata); thus, no data was collected on the biomass of these herbivorous fish during this survey.

Initial and final data collection

After the first and last week of the experiment, algal species were destructively sampled from three randomly selected 1cm by 1cm points per plot. These samples were analyzed using a compound light microscope to identify species to the closest taxonomic level possible. References for algal identification were in Littler and Littler (2000), as well as Littler and Littler (2011). If identification was only possible to the phyletic level, then both phyla and functional morphology were recorded. Average richness of algae from each of the following groups was calculated per treatment: Rhodophyta, Phaeophyta, Chlorophyta, and CCA. Crustose coralline algae are part of the phyla Rhodophyta, order Corallinales; for the purposes of this experiment CCA was included as a separate group because of their reef-building properties. Average richness of each group was compared between the first and fourth week. Data were analyzed using a two-way Analysis of Variance (ANOVA) to evaluate the change in richness for each group (Rhodophyta, Phaeophyta, Chlorophyta, and CCA) in response to change from the initial to final week and the four treatments.

Weekly data collection

On a weekly basis, percent substrate composition was evaluated from underwater photos taken of each plot using an Olympus Stylus Tough-8000 camera in underwater macro setting. CPCe 4.1 software was used to randomly select ten points per plot, which were analyzed for substrate type (sand, FMA, TA, CCA, coral, or sponge). In this study, sand was defined as a thin covering over a relatively bare substrate. Data were analyzed using a one-way ANOVA to evaluate response of percent cover of each substrate type found (sand, TA, and CCA) between week one and week four, under each of the four treatments. Also on a weekly
basis, maximum algal height within each plot was measured in-situ using callipers. Data were analyzed using a two-way ANOVA to evaluate response of maximum algal height to the four different treatments over the course of four weeks.

**Results**

Biomass of herbivorous fish at Yellow Submarine

Analysis showed that scarids make up 92.8% of the biomass of herbivorous AGRRA fish at Yellow Submarine, while acanthurids make up 7.2%. Observed scarids ranged in length from 8.0cm to 30.5cm, which correlated to greater biomass; acanthurids had a smaller size ranging from 5.3cm to 17.2cm, which correlated to lower biomass (Fig. 3). The greatest biomass was made up of stoplight parrotfishes (*Sparisoma viride*), princess parrotfishes (*Scarus taeniopterus*), and striped (*Scarus iseri*). Damselfish (Pomacentridae) and Caribbean sharpnose puffer fishes (*Canthigaster rostrata*) are not included in the AGRRA 5.4 fish survey protocol; thus, no data was collected on the biomass of these herbivorous fish during this survey.

Change in richness of algae between treatments

Change in richness of total algae, as well as richness of the four individual algal groups (rhodophyta, phaeophyta, chlorophyta, CCA) was analyzed using a two-way ANOVA (Fig. 4). There was a significant increase in overall algae richness between the first and final week of the experiment (DF=1, F=7.90, p=0.008), but no significant difference among treatments (DF=3, F=0.33, p=0.806), or significant interaction between week and treatment (DF=2, F=0.22, p=0.798; Fig. 4). There was a significant increase in overall algae richness during the course of the experiment (DF=1, F=7.90, p=0.008), but there was no significant difference among treatments (DF=3, F=0.33, p=0.806), or significant interaction between week and treatment (DF=3, F=0.68, p=0.571; Fig. 4). Phaeophyta did not have a significant increase in response to change in weeks (DF=1, F=1.89, p=0.178), or treatment (DF=3, F=0.95, p=0.429), and there was no significant interaction between week and treatment (DF=3, F=0.94, p=0.432; Fig. 4). Chlorophyta did not have a significant increase due to change in time (DF=1, F=1.10, p=0.301) or treatment (DF=3, F=2.27, p=0.097), and there was no significant interaction between the week and treatment (DF=3, F=0.86, p=0.474; Fig. 4). CCA had a significant response to both change in weeks (DF=1, F=16.58, p=0.000) and change in treatment (DF=3, F=9.48, p=0.000), but there was no significant interaction between the week and treatment (DF=3, F=2.00, p=0.134; Fig. 4). Average CCA richness between the first and final week did not change in the no fish exclusion treatment; however, it increased in the large fish exclusion, cage frame, and no cage treatments by 0.4, 0.6, and 0.6, respectively (Fig. 4).

Changes in cover between treatments over time

**Percent CCA cover**

Percent CCA cover was greatest in week four in the cage frame and no cage treatments; the values were 8% and 12%, respectively (Fig. 5). CCA was never recorded in the complete fish exclusion plots, and never reached levels higher than 4% in the large fish exclusion plots. A two-way ANOVA showed there was a marginally significant response of percent CCA cover to treatments (DF=3, F=2.44, p=0.071), a significant response to change in time (DF=3, F=2.96, p=0.038), and no significant interaction between treatment and time (DF=9, F=1.52, p=0.159).
Fig. 3 Average biomass (g 100m$^{-2}$) of herbivorous Atlantic Gulf Rapid Reef Assessment (AGRRA) fish at Yellow Submarine. Data was collected using AGRRA fish survey protocol V5.4. The following species were included in the survey list, but not observed, nor included in the figure: Blue parrotfish (*Scarus coeruleus*), Greenblotch parrotfish (*Sparisoma atomarium*), Midnight parrotfish (*Scarus coelestinus*), Rainbow parrotfish (*Scarus guacamaia*), Yellowtail parrotfish (*Sparisoma rubripinne*). Error bars display SEM (n=11 surveys).

Fig. 4 Average richness of algae for each treatment, contrasted between week 1 and week 4. Figure legend applies to both weeks. Four algae groups were found: rhodophyta, phaeophyta, chlorophyta, and crustose coralline algae (CCA). Crustose coralline algae are a member of the phyla rhodophyta, but were included as a separate group due to their function as a reef building algae. Data was collected from *in situ* destructive samples that were later analyzed under a microscope. Error bars display SEM (n=5 plots)

Percent TA cover

TA cover increased by 25% in the complete fish exclusion treatment between the first and final week (Fig. 5). Cover decreased in the large fish exclusion treatment by 8%; in the cage frame and no cage treatments cover decreased by 26% and 24%, respectively. A two-way ANOVA showed there was a significant response of percent TA cover to treatment (DF=3, F=7.36, p=0.000), as well as time (DF=3, F=2.70, p=0.052); there was also a significant interaction between treatments and time (DF=9, F=2.05, p=0.048).

Percent sand cover

Sand cover decreased by 28% in the complete fish exclusion treatment between the first and
final week (Fig. 5). Cover increased in the large fish exclusion treatment by 4%; in the cage frame and no cage treatments cover decreased by 20% and 12%, respectively. A two-way ANOVA showed that there was a significant response of percent sand cover to treatment (DF=3, F=5.76, p=0.001) as well as time (DF=3, F=3.16, p=0.030), but no significant interaction between treatment and time (DF=9, F=1.71, p=0.104).

Maximum algal height between treatments over time

Over the course of the four-week experiment, there was a significant change in maximum algal height between the four treatments over time (Fig. 6). A two-way ANOVA showed a significant response of maximum algal height to treatment (DF=3, F=13.08, p=0.000), as well as time (DF=3, F=3.40, p=0.022); there was no significant interaction between treatment and time (DF=9, F=0.56, p=0.821). In week three, a maximum algae height of 4.4cm (Cladophora sp.) was recorded in a large fish exclusion plot; this was an outlier. At the end of the four week experiment, there was a 56% increase in maximum algae height for the cage frame (control) treatment, and a 45% increase in the large fish exclusion treatment; however height decreased by 3% in the cage frame (control), and 15% in the no cage treatment. These data suggest that the cage might have had a minor influence on growth rate of algae compared to the no cage treatment; however these differences were not significant.

Discussion

Response of algal composition and growth to reduced herbivory

Increase in overall growth and richness

In this study, there was a significant overall increase in algal richness due to change in time, but not to change in treatment. Rhodophyta richness significantly increased between the first and final week of the experiment. There was no significant response of phaeophyta or chlorophyta to temporal change or change in treatment.

Algae undergoes bottom-up control by limitation of resources such as nutrients, light, and space, though this typically has less influence on algae compared to top-down control by herbivory (McClanahan et al. 2002; Ferrari et al. 2012). There was an apparent increase in rainfall in the final 1.5 weeks of this study, which may have indirectly increased algal growth due to influx of nutrients into the water. Further study is needed to corroborate these observations.

Increased TA growth in response to decreased herbivory over time

Percent TA cover significantly increased in response to the four treatments, as well as temporal change. There was also a significant interaction in the response between treatment and time. TA cover had a significant overall increase in the complete fish exclusion cage, while it had a significant decrease in all other treatments. Since there is a compounding interaction between algal growth in response to both herbivory and temporal change, the difference in algal cover between reefs with low and high rates of herbivory will become increasingly apparent over time. These data support previous findings that percent algal cover has an inverse relationship to herbivory (McManus 2000; Hughes et al. 2007; Rasher et al. 2012; Fricke 2011). Furthermore, these data illustrate the particular importance of large herbivorous fish in controlling TA cover.

Over the course of the four-week experiment, there was a significant change in maximum algal height in response to both treatment and temporal change. However, there was no compounding interaction between the two variables. There was a marginally significant increase in algal height in both the complete fish exclusion and large fish exclusion treatments between the first and final weeks. However, there was not a significant change in algal height in the cage frame and no
Fig. 5 Average percent cover for each treatment, shown in a four-week series. The figure legend applies to all four weeks. The four treatments were as follows: 1. Complete fish exclusion, 2. Large fish exclusion (>13 cm), 3. Cage frame (control), 4. No cage, (n=5 plots). Within these plots, the following types of cover were found on the substrate: Crustose coralline algae (CCA), Turf algae (TA), and sand. Photos of each plot were taken on a weekly basis, and analyzed using CPCe4.1. Average percent cover per treatment over the course of four weeks was calculated from these data.

Fig. 6 Average maximum algal height for each treatment over four weeks. The four treatments were as follows: 1. Complete fish exclusion, 2. Large fish exclusion (>13 cm), 3. Cage frame (control), 4. No cage. Algal height was measured in situ at each plot on a weekly basis. Error bars display SEM (n=5 plots). The positive SEM for the large fish exclusion treatment in week 3 was 2.4.
cage treatments. Studies conducted over longer time periods have shown that in the absence of herbivory TA (>2 cm) will be replaced with upright FMA (Gaines and Lubchenco 1982; Williams et al. 2001; Hughes et al. 2007; Ferrari et al. 2012; Rasher et al. 2012) and directly compete with coral (Mumby 2006; Box et al. 2007).

Increase in CCA richness and percent cover in response to herbivory

There was a significant response in CCA growth between the first and final week of this experiment and difference among treatments. There was no CCA growth in the complete fish exclusion treatments over any of the weeks. However, it increased significantly in all other treatments, particularly cage frame and no cage plots.

Percent CCA cover showed a marginally significant response to change in treatment, and a significant response to temporal change; there was no interaction between these variables. CCA cover was greatest in no cage and cage frame treatments, least in the large fish exclusion treatment, and not present in the complete fish treatment. The increase in percent cover was likely due to temporal environmental changes, as CCA levels were low in all four treatments at the start of the experiment but increased in the un-manipulated, no cage plot, as well as cage frame and large fish exclusion plots. However, if this was solely due to temporal change, then CCA cover should have increased in the complete fish exclusion cages, which it did not. This corroborates previous findings that increased coverage of upright algae suppresses abundance of CCA (Lewis 1986; McManus 2000) and that herbivory indirectly helps facilitate the growth of CCA and maintain complexity of structure on the reef (Lewis 1986).

Decrease in bare substrate in response to decreased herbivory

There was a significant change in percent sand cover in response to temporal and treatment change. For the purposes of this study, sand was defined as a thin covering over a relatively bare substrate. The percent cover of this bare substrate decreased significantly in the complete fish exclusion treatment, and increased significantly in all other treatments. This increase was particularly dramatic in the cage frame and no cage treatments. The change in bare substrate cover was inversely proportional to change in TA cover. Coral recruits more frequently and has increased survivorship on bare or CCA covered substrate in comparison to TA covered substrate (McClanahan et al. 2002; Burkenpile and Hay 2010; Rasher et al. 2012). Large herbivorous fish indirectly increase coral recruitment and survivorship by increasing the amount of bare and CCA covered substrate on the reef. However, small herbivorous fish (>13 cm) were not shown to greatly maintain the cover of bare or CCA covered substrate.

Scarids as a keystone species in controlling reef algae

The major herbivorous fish in Bonaire are acanthurids, pomacentrids, and scarids. Of these three families, scarids tend to grow to the largest size. This study found that scarids make up 93% of the AGRRA herbivorous fish biomass at the Yellow Submarine dive site. These data support previous findings that scarids make up over 80% of the biomass of herbivorous fish in Bonaire, and are the dominant grazer in Caribbean Reefs (Steneck et al. 2005; Mumby 2006). Due to their large biomass and size relative to other herbivores, scarids play a vital role in maintaining the health of Caribbean reef through top-down control of algal composition (Steneck 1994; Mumby 2006).

Studies have shown that scarids have exponentially greater algae grazing impact with increase in body size for both scraping and excavating species (Bruggeman et al. 1996; Lokrantz et al. 2008). The greatest biomass of herbivorous fish at Yellow Submarine was
made up of stoplight parrotfishes (*Sparisoma viride*). Previous studies found that adult *S. viride* have significant preference for endolithic algae compared to CCA (Bruggeman et al. 1994). *Sparisoma viride* also have a significant preference for high- compared to low-density algal substrate in their initial, and terminal phases, but not in their juvenile phase (Bruggeman et al. 1994). The findings of this study provide further evidence into the influence of large-bodied scarids in suppressing growth of endolithic TA, and overall TA growth, as well as facilitating growth of CCA.

Fishing pressure has been shown to reduce the size of fish, both through direct selection and indirect genetic effects on fish populations (Law 2000; McManus 2000). Increased fishing pressure would thus indirectly decrease the top-down control of algal growth by large herbivorous fish. In more degraded reefs outside of Bonaire, overfishing of large carnivores has led to increased fishing of small predators and large herbivorous fish, including scarids (McManus 2000). There was a decline in herbivorous fish biomass in Bonaire from 2003-2011 (Steneck et al. 2005; Steneck et al. 2013). In response, the BNMP banned harvest of scarids in 2008 and began phasing out use of fish traps in 2010 (Steneck et al. 2013). Since 2010, there has been an increase in scarid biomass (Steneck et al. 2013).

In the absence of herbivorous fish, reefs have been shown to undergo a phase shift from a coral dominant reef with sparse TA, to a reef dominated by FMA (Lewis et al. 2014). Scarid grazing has a disproportionate impact on coral communities relative to other herbivorous fish (Hughes 1994; Hughes et al. 1997; Mumby 2006). This study provides further evidence about the importance of proactive measures the BNMP to protect populations of herbivorous fish in Bonaire.

Overarching effects of large herbivores on algal composition

This study provided evidence how Bonaire’s reefs could change if large herbivorous fish populations are reduced or removed altogether. Findings showed that overall TA cover increased and CCA cover decreased when large herbivores were removed from the reef. This provides evidence of the initial stages of a phase shift from a coral dominated system to one dominated by algae. Furthermore, it illustrates the importance of large-sized herbivores, particularly scarids, in maintaining top-down control of algae on the reef and allowing for healthy competition between coral and algae in the benthos.

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Hydrocarbons on coral reefs: An analysis of the presence of anthropogenic PAHs in Bonaire, Dutch Caribbean

Abstract Phototoxic polycyclic aromatic hydrocarbons (PAHs) have been well-documented as major marine pollutants. While PAHs are known to have negative ecological effects, the spread of point-source PAHs into coral reefs is poorly understood. This study focuses on a potential source of marine PAH contamination from a drain into a coral reef in Bonaire, Dutch Caribbean. PAHs were believed to be detected outside of the drain between October and November 2013, providing incentive for continued monitoring of PAH presence. Insight from this investigation is important not only to the general understanding of point-source marine pollution pathways, but holds implications for drain management strategies. Water samples from sites of varying distances from the drain were analyzed for UV-reactive toxicity using two Artemia sp. bioassays. Results from the bioassays indicated that PAH presence was undetectable, and that there was no relationship between distance from the drain and UV-reactive toxicity. It was concluded that sediment dispersion and marine organism bioaccumulation likely accounted for the apparent temporal discrepancy in PAH presence. Field observations displayed noticeable coral reef degradation, which was assumed to be largely caused by factors other than PAH pollution. Despite the lack of evidence for current PAH presence, observations of poor reef health outside of the drain suggest that further studies and management strategies be considered for the drain and cement trough.

Keywords Polycyclic aromatic hydrocarbons • Point-source pollution • Artemia bioassay

Introduction

Out of countless marine pollutants, anthropogenic polycyclic aromatic hydrocarbons (PAHs) are of particular concern because of their highly toxic, mutagenic, and carcinogenic nature. Polycyclic aromatic hydrocarbons accumulate in the environment and can cause considerable damage to exposed organisms (Samanta et al. 2002). In the marine environment, hydrophobic PAHs adsorb to particles in the water column and collect in sediments, where they are bioaccumulated by various marine organisms (Bouloubassi et al. 2012). Polycyclic aromatic hydrocarbons are primarily created by pyrolysis (incomplete combustion of organic material), although the chemicals can also be released into the environment by refinery products (e.g. crude oil, coal) and biogenic precursors (e.g. terpenes, pigments; Budzinski et al. 1997).

According to Bouloubassi et al. (2012), point sources are responsible for much of the PAH accumulation in marine sediments. The pathways that PAHs travel from their point source are complex and largely unknown, but shallow, near-shore ecosystems appear to be primary PAH sinks. This is highly relevant in tropical marine areas, which are likely to contain coral reefs and consequently high levels of biodiversity in near-shore shallow environments (Gray 1997). Thus, it is imperative to increase knowledge on the spread of PAHs from their point sources in tropical
regions, and determine the extent of their presence in benthic sediments.

The impacts of PAHs on coral reefs are especially critical considering the historically fragile nature of these ecosystems (Dubinsky and Stambler 1996). Coral stress is typically reported in terms of the oceanic effects of global climate change (ocean warming and acidification), but there are few studies on coral damage as a result of marine pollution (Dubinsky and Stambler 1996). While climate change may be the primary anthropogenic coral stressor, it has been shown that oceanic contaminants, as well as causing harm independently, can act synergistically with climate change symptoms to cause disproportionate damage to organisms (Schiedek et al. 2007; Whitehead 2013). For example, PAHs are known to exhibit phototoxicity (Arfsten et al. 1996), which is enhanced by depletion of the atmospheric ozone layer (Kerr and McElroy 1993). Furthermore, PAHs have been found in coral skeletons, indicating that these anthropogenic chemicals may infiltrate and accumulate in coral reefs (El-Sikaily et al. 2003). Due to the toxic nature of PAHs and their potential to harm vulnerable coral reef systems, further studies of PAH presence on coral reefs are warranted.

The coral reefs of Bonaire, Dutch Caribbean are relatively well-preserved (Sommer et al. 2011), but are still prone to degradation from pollutants such as PAHs. In a previous study by Mason (2013), PAHs were believed to have been detected 10 m outside of a specific drain near Bonaire’s capital city, Kralendijk. The presence of PAH contamination outside of the drain indicates that the drain may be a major source of anthropogenic runoff pollution. Well-documented existence of PAHs in the sediments could provide strong evidence for a mandated treatment program of the drain’s source (a cement trough). This study expands upon Mason’s research, and attempts to determine the range of detectable PAH presence in a limited area outside of the drain in Kralendijk. Two hypotheses were tested:

\[
H_1: \text{UV-reactive toxicity is detectable within the cement trough and 10 m outside of the drain}
\]

\[
H_2: \text{UV-reactive toxicity is detectable in decreasing levels with increasing distance from the drain}
\]

### Materials and methods

#### Study site

The study took place on a sand flat and coral reef outside of a specific drain in downtown Kralendijk, Bonaire, Dutch Caribbean (Fig. 1). The drain provides an outlet for a large water-filled cement trough, which is surrounded by limited vegetation or barriers to prevent runoff. Although the exact sources of the trough water are unknown, the trough likely collects primarily rainwater and street runoff. In recent months, land next to the trough has been frequented by construction equipment working on renovations for an adjacent restaurant. The study site is located next to the dive site Kas di Arte (12°09’21.4”N, 68°16’45.3”W), and within an area of high boat, swimmer, and diver traffic. From the shore, a sandy flat extends approximately 100 m out to the reef crest, ranging from a depth of 1 m to 8 m.

![Fig. 1 Map of study area. The study was completed in Bonaire, Dutch Caribbean next to the Kas di Arte dive site (12°09’21.4”N, 68°16’45.3”W; denoted by star)](image)
Field observations

Initial observations of the study site were made to assess the observable level of human impacts in the area. Activity near the cement trough was monitored, and conditions inside the trough were observed on days of sampling. Benthic composition was noted directly outside the drain and at the reef crest. On the reef crest, observations were focused on apparent coral health. Weather conditions on the day of sampling were recorded. Rainfall trends were monitored throughout the course of the study, as frequent heavy rain may increase contaminated sediment load outside of the drain. Ocean current speed, current direction, and visibility were also recorded.

Water sampling

Water for analysis was sampled from the epibenthos at seven sites outside of the drain, and once from inside the cement trough (Fig. 2). Water was sampled at 10 m outside of the drain and at four more sites extending from the drain to the reef crest at 20-m intervals. Samples were also taken at two 10-m intervals straight out from the reef crest down the reef slope (eight total samples). All distances were measured by transect. Water samples of 30 mL were taken using screened syringes from the epibenthos at each site weekly for a period of two weeks. In the lab, samples were transferred to glass bottles and placed in a refrigerator until analysis.

![Map of water sampling site locations](image)

**Fig. 2** Map of water sampling site locations. Water samples were taken inside the cement trough, and at seven sandy flat and reef locations extending from the trough’s drain to the reef slope.

UV-reactivity analysis

Two *Artemia* sp. bioassays were used to determine potential PAH presence in the sampled water (Lu et al. 2012; Arfsten et al. 1996). *Artemia* is a well-known test organism for studying water quality, and is commonly selected for its high tolerance to natural environmental stress, but low relative tolerance to toxicity (Lu et al. 2012). For both bioassays, *Artemia* were hatched and grown for 48 hours prior to the bioassay (Lu et al. 2012). Glass bottles containing the water samples were removed from the refrigerator and allowed to warm to room temperature before they were transferred to petri dishes (Sorgeloos et al. 1978). To ensure adequate oxygen supply for *Artemia*, samples were aerated for 30 minutes (Lu et al. 2012). Approximately 50 *Artemia* were placed in each petri dish with 10 mL sampled seawater for each of the eight sample sites. One negative control using filtered seawater and one positive control using filtered seawater with approximately ten drops of motor oil were used. Each of the petri dish solutions were replicated and one set was exposed to standing ultraviolet (UV) light for one hour (Arfsten et al. 1996), while the other set was covered with glass to block UV light exposure. After UV exposure, all *Artemia* were covered with glass and dark plastic overnight to block all light before analysis. The next day, petri dishes were observed under stereomicroscope and *Artemia* mortality rate was calculated (Ostrander 2005). Since PAHs are UV-reactive, it was assumed that higher levels of *Artemia* mortality in the UV treatment correlated with higher levels of PAHs (Arfsten et al. 1996). It should be noted, however, that the bioassay tested for UV-reactive hydrocarbons in general rather than PAHs specifically. Higher levels of *Artemia* mortality in UV treatment would not confirm, but instead would provide evidence for the presence of PAHs.
Data analysis

For both Artemia tests, UV-reactive toxicity of the water samples was measured using Abbott’s formula:

\[
\% \text{ effect} = \frac{100 \times (\% \text{ effect in sample} - \% \text{ effect in control})}{100 - \% \text{ effect in control}}
\]

where \% effect in sample is the mortality rate for UV-treated Artemia, and \% effect in control is the mortality rate for UV-blocked Artemia (Ostrander 2005). In this case, Abbot’s formula was used to calculate Artemia mortality rates adjusted for the control (UV-blocked) for each water sample. Adjusted Artemia mortality rates were equal to UV-reactive toxicity levels. For each bioassay, distance of the sampling site from the drain was plotted against the site’s measured percent UV-reactive toxicity. A linear regression was used to analyze predictability of toxicity levels with increasing distance from the drain.

Results

Field observations

Little human activity was observed around the cement trough and drain throughout the course of the study. During the two-week water-sampling period, no construction equipment was seen around the trough. A cement abutment separated the trough from the adjacent restaurant, and is believed to be a new construction since October–November 2013. There were, however, no other noteworthy manmade structures or vegetation to prevent runoff into the trough. Inside the trough, water (approximately 0.5 m in depth) was highly turbid. The bottom of the trough was overgrown with macroalgae, including abundant Padina sp. Dimensions of the trough were approximately 10 m by 30 m.

Weather during water-sampling times was mostly clear-skied, and no precipitation occurred on days of sampling. According to data from the Flamingo International Airport in Kralendijk, average precipitation is 10.54 cm in October, and 15.34 cm in November (increasing from 6.81 cm in September). Although the study was conducted during Bonaire’s “rainy season” and it rained at points throughout the data collection weeks, consistently heavy rain did not begin until after completion of data collection. Surface current was typically mild and ran from south to north.

Water sampling sites were located at 10 m (approximate depth = 1 m), 30 m (1.5 m), 50 m (3 m), 70 m (4.5 m), 90 m (9 m), 100 m (12 m), and 110 m (18 m) from the drain. The first 10 m extending outwards from the drain were consistently highly turbid. Substrate in this range was primarily dead coral pieces covered in macroalgae and sediment, although a few live corals were observed. Visibility extending outward from the drain to the reef crest was consistently 15-20 m. The reef crest itself appeared highly degraded relative to the reef crest in other areas of the island. Many dead corals were observed along the crest, and most live corals were relatively small in size (<30 cm in length and width). Sedimentation was visible on nearly every sessile organism. During initial site observations, SCUBA divers were seen handling corals on the reef crest.

UV-reactivity analysis

Percent UV-reactive toxicity of each water-sampling site was determined using two Artemia bioassays. Adjusted Artemia mortality rates, and consequently percent UV-reactive toxicity, were 28.309% for the positive control using a motor oil solution, and -3.714% for the negative control using filtered water. Due to changes in experimental design, these control values represent solely results from bioassay 2.

UV-reactivity analysis of water samples in bioassay 1 (Fig. 3a) and bioassay 2 (Fig. 3b) revealed no discernable trends. In bioassay 1, percent UV-reactive toxicity was relatively constant between 0% and 10% throughout all distances from the drain. Exceptions include 0 m from the drain (sample from the cement trough) and 70 m from the drain, with -4.608% and -30.956% toxicity respectively. Toxicity percentages less than or equal to zero implied
no detectable phototoxicity. Mean toxicity over all sampling sites was -0.402%. Linear regression analysis indicated little predictability of percent UV-reactive toxicity by distance from the drain (F=0.00, p=0.947, R²=0.00079).

Mean toxicity from both bioassays was also calculated. Linear regressions are shown on each plot.

In bioassay 2, percent UV-reactive toxicity was more variable, with values ranging between 10% and -15%. The sampling site at 70 m from the drain again had the lowest toxicity (-15.313%). Mean toxicity over all sampling sites was 0.422%. Linear regression analysis similarly indicated little predictability of percent UV-reactive toxicity by distance from the drain (F=1.42, p=0.278, R²=0.19164). Mean UV-reactive toxicity data over both bioassays (Fig. 3c) displayed comparable variability throughout all distances from the drain (F=0.19, p=0.675, R²=0.03132).

Discussion

According to the results of the UV-reactivity analysis, little evidence exists in support of either proposed hypothesis. Both *Artemia* bioassays produced UV-reactive toxicity levels indicating no detectable PAH presence at any of the seven sampling sites outside of the drain, or at the sampling site inside the cement trough. Calculated toxicity levels for all sites were minimal compared to that of the positive control, and were often negative. Maximum mean toxicity over all sampling sites was 8.252%, indicating that overall, little toxicity was present in the area sampled. The large discrepancy between positive and negative controls confirms that substantial UV-reactive toxicity likely would have been detected in water samples. Furthermore, results of linear regression analysis showed that there is no significant relationship between distance from the drain and toxicity levels. This is likely due to the low toxicity levels throughout.

During the course of this study, it was unlikely that large amounts of PAHs were actively being supplied into the trough. According to Mason (2013), major disturbances (construction activity, heavy precipitation) were frequently followed by a visible thick black plume in the water outside of the drain. Neither the listed major disturbances nor the black plume were observed during this study. Since motor vehicle emissions such as unburned fuel and lubricating oil contain PAHs (Marr et al. 1999), it is likely that construction equipment accounted for a portion of detected PAHs in 2013. Additionally, PAHs released by other anthropogenic activities can be washed into coastal waters from urban areas by heavy
precipitation (Hoffman et al. 1984). Runoff into the cement trough may have also been limited by the recent (Summer 2014) erection of a cement abutment between the trough and adjacent restaurant. These two potential sources of PAHs into the cement trough were largely absent during this study, likely accounting for lack of the black plume outside of the drain. Consequently, it is reasonable to assume that low levels of PAHs were introduced into the trough and outside of the drain during the course of the study. This could explain the lack of detected PAHs.

Existing PAHs from previous activity, shown to be undetectable outside of the drain with Artemia bioassays, were likely dispersed into the ecosystem such that concentrations were considerably lower than in October–November 2013. After point-source input, PAH concentrations depend primarily on rates of evaporation, microbial degradation, sedimentation, and photochemical oxidation (Lee et al. 1978). Bonaire, located near the equator, has a relatively hot climate (average year-round temperature of 28.89°C according to data from Flamingo International Airport) and evaporation could be one primary dispersal pathway. In the case of this specific drain, outside of which there was high turbidity, sedimentation is another key factor. PAHs tend to associate with fine sediments (Bouloubassi et al. 2012), and are thus subject to dispersal by moving water currents and wind reversals that cause high wave disturbance on the leeward (western) coast of Bonaire. Flemming (1981) found that waves and currents heavily influence sediment dispersal in southeast Africa, and these factors may have similar effects in the southern Caribbean. It is likely that PAHs released from the drain prior to the initiation of this study were distributed across fine sediments to concentrations undetectable at all sampling sites by Artemia bioassays.

Additionally, PAHs can be removed from point-source areas by bioaccumulation into marine organisms. Due to their hydrophobic tendencies, PAHs are often taken up and collected in lipids inside organisms’ tissues (Samanta et al. 2002). Exposure to PAH contaminated waters can cause accumulation in essentially all marine organisms, with levels depending on ambient concentration, time of exposure, and individual species’ metabolic rates (Meador et al. 1995). After introducing PAHs into controlled environments with marine fauna, D’Adamo et al. (1997) found that PAHs accumulated in mollusc and fish tissues. These groups of organisms are commonly found in the area outside the drain, and it is possible that they and other groups (e.g. plankton) played a major role in removing detectable PAHs via bioaccumulation (Lee et al. 1978). Future studies should examine levels of PAH accumulation in reef fauna dwelling outside of the drain.

Despite the apparent dispersion of PAH contamination and subsequent undetectable levels of UV-reactive toxicity, it is clear from field observations that the benthic community outside of the drain has undergone considerable degradation. It is quite probable that point-source PAHs have had measurable impacts on various aspects of the reef ecosystem and its communities, yet unlikely that they were the primary stressor. Other potential drain-sourced pollutants likely to have negative effects on the reef are nutrients and sediments. Terrestrial runoff commonly includes excess macronutrients, such as nitrogen and phosphorous, which in high concentrations can result in coral mortality and macroalgae infestation on coral reefs (Fabricius 2005). Nutrient pollution may account for observed abundant macroalgae inside the cement trough and directly outside the drain.

Furthermore, high loads of sediments have been shown to reduce light availability to reef corals, thus slowing growth rates and causing mortality (Rogers 1990). Water directly outside of the drain was observed as highly turbid, and it is clear that the cement trough and drain release sediment pollution. Observed sedimentation of reef crest corals is perhaps the outcome of sediment load from the drain, but careless recreational SCUBA divers are also likely contributors. Divers often disturb benthic sediments on coral reefs, causing sediments to settle on corals, and resulting in significant
Due to previous detection of UV-reactive toxicity outside of the drain (Mason 2013), further investigation of PAH presence is warranted regardless of the results of this study. More precise water sampling, PAH recovery, and PAH determination methods should be carried out in future studies outside of the drain. The most commonly used marine PAH detection methods, gas chromatography and high-pressure liquid chromatography, are prime candidates for methods on which to base future studies (Manoli and Samara 1999).

The results and conclusions from this study provide incentive for increased scrutiny on, and possible management of the cement trough and drain in Kralendijk, Bonaire. Historical PAH detection was likely caused by anthropogenic activity, and although PAHs are presently undetectable, the effects of the past-sourced PAHs are unknown. The drain also presents dangers to the coral reef ecosystem regardless of any PAH impacts with other anthropogenic pollution. Since the drain is currently unregulated, it is imperative that further investigation is conducted on the drain’s effects and protocols are considered for its management.

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Photoacclimation of *Ventricaria ventricosa* to a change in available light’s wavelength

**Abstract** A prominent process of photoacclimation accounts for the change in pigment concentration to improve photosynthetic performance. It occurs in algae due to their inherent characteristic of living underwater and receiving limited wavelength and irradiance of light from the sun. This process was investigated on the macroalga *Ventricaria ventricosa*, one of the largest known unicellular organisms. It contains a central liquid-filled vacuole that pushes the organelles towards the cell membrane. Upon perforation, the inner cell structure disassembles, facilitating pigment concentration analysis. On a first experimental stage, individuals at 8-10 m and 16-18 m deep were collected and analyzed for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) concentrations via spectrophotometry. The overall pigment concentration was not significantly different (ranging from 0.54-14.74 μg ml⁻¹ Chl *a*, 0.36-19.29 μg ml⁻¹ Chl *b*, 0.19-4.09 μg ml⁻¹ Car). The second stage included covering alga from the same depth belts with a green or transparent (control) filter cage for one week to then analyze pigment concentration. Control cages had pigment concentration values considerably lower from those of the first experimental stage (ranging from 0.02-3.38 μg ml⁻¹ Chl *a*, 0.02-2.20 μg ml⁻¹ Chl *b*, 0.01-1.44 μg ml⁻¹ Car). Membrane perforation upon detachment from the reef, decreased water flow, and the cells’ size may have influenced this outcome. It is concluded that the depth gap used was not large enough to promote photoacclimation. It is also suggested that *V. ventricosa* prioritizes healing over photoacclimation, resulting in loss of pigment concentration while reestablishing turgor pressure.

**Keywords** Irradiance • Pigment concentration • Chlorophyll

**Introduction**

Photosynthesis is the process in which plants or bacteria convert light energy (photons) and carbon dioxide into cellular energy (glucose) and oxygen in their chloroplasts, which contain the most important light-sensitive molecules: chlorophyll (Chl) and carotenoid pigments. The most abundant in green plants is Chl *a*, followed by Chl *b* in an average ratio of 3:1 (Boyer 1990). Chlorophylls *a* and *b* absorb light in the violet/blue (400-470 nm) and orange/red (630-700 nm) regions of the visible spectrum. Carotenoids (Car) absorb most of the light between 400 nm and 500 nm for both photosynthesis and photoprotection of the other pigments. The water absorbs light differently than air because it is 800 times denser. Colors with larger wavelength (red and orange) are absorbed at approximately 10 m deep, while colors with smaller wavelength (violet/blue) still persist at more than 60 m underwater. Underwater plants are affected by this phenomenon, which must condition their pigment concentration. It should increase with poor light conditions (little irradiation and smaller wavelengths available), following the classical view of acclimation (Falkowski and LaRoche 1991). The phenomenon happens in seagrass that can survive in both salty and fresh water bodies by increasing the number of
chloroplasts, Chl a, and Chl b in the riverine, murkier environments (Kahn and Durako 2009). It also occurs in unicellular organisms such as zooxanthellae: the Chl concentrations of the coral Stylophora pistillata holobiont increase with depth, regardless of the zooxanthellae’s surface area density (Mass et al. 2010). Macroalgae is no exception, and it may also undergo photoacclimation by increasing the Chl concentrations with depth, regardless of the zooxanthellae’s surface area density (Mass et al. 2010). Carotenoids responsible for photoprotection decrease with depth, while the ones with photosynthetic functions should increase (Colombo-Pallota et al. 2006), however, the overall Car concentration decreases with more irradiance (Henley and Ramus 1989).

Ventricaria ventricosa (previously under the genus Valonia and commonly known as sailor’s eye or sea pearl) is one of the largest cells known, reaching diameters up to 10 cm (Shepherd et al. 2004). An irregularly massive vacuole presses the cell’s organelles towards the plasma membrane with a 3.2 atm turgor pressure (Shihira-Ishikawa and Nawata 1992).

The interior pressure is instantaneously lost upon wound (pers obs), and all the organelles get disorganized. However, due to its coenocytic property (i.e. undergoes division, but no cytokinesis) (Shepherd et al. 2004), it can be repaired within hours (Sims 2013). In the reef slope, the alga is likely to be found in cracks and crevices on hard substrates, and has a silvery green appearance with the presence of epiphytic fauna (Littler & Littler 2000; pers obs). Its detachment from the substrate often deflates the cell due to the back-and-forth force applied (pers obs).

While the second stage of the experiment will aim to suggest the following:

H₃: V. ventricosa can adapt to the change of light’s wavelength by altering their pigment concentration

Materials and methods

Study site

Ventricaria ventricosa was collected from the dive site Yellow Submarine, in the leeward side of Bonaire, Dutch Caribbean (12°9’33”N 68°16’55”W). The back reef is mostly composed of sand or rubble, with small patches of brain corals on the mooring blocks present. These are at approximately seven meters deep,
and close to the reef crest. The initial part of the reef slope displays poor coral cover (mostly Orbicella spp.), which increases with depth (Undaria agaricites being the most common). The water visibility was clear throughout the experiment. The cells were mostly found on U. agaricites at depth, and inside the crevices of Orbicella annularis at shallow sites.

Stage 1

For the initial stage of the experiment, V. ventricosa was extracted at two different depth belts: 8-10 and 16-18 meters. The collected individuals were analyzed in the laboratory immediately upon exiting the water at a dim light to avoid photobleaching of the extremely light-sensitive pigments (Boyer 1990). The algae attached to the outer layer of V. ventricosa were carefully removed with tweezers to not interfere with the further analysis of pigment concentration. The cells were weighed and their volume was measured in a graduated cylinder, based on the Archimedes principle of displacement. The cell was cut open into 10 ml of 90% acetone, and filled up to 20 ml with the same solvent after removing the cell membrane. Cells that were greater than 10 ml were not taken into account, to allow the action of acetone (for the exception of one under the green filter condition at 16-18 m). The amount of 15 ml was transferred to a centrifuge tube, and it was centrifuged for 10 min at 3500 rotations per minute (rpm), as suggested by Kumar et al. (2010). The supernatant was pipetted into a 3 ml glass cuvette to be tested for absorbance of light. It was tested at the wavelengths at which Chl a, Chl b and Car fluoresce (661.6 nm, 644.8 nm and 470 nm) in a SpectroQuest-4802 Double Beam UV/Vis Spectrophotometer.

Stage 2

In the second part of the experiment, V. ventricosa was exposed only to green light to test its photoacclimation capabilities. The choice of the color green is based on the non-absorbing region of Chl a and Chl b. The individuals were collected and tested at the same depth belts of stage 1, for the sake of maintaining the same variables. They were placed on a PVC shelf inside a wire cage attached to limestone, either completely covered in green or transparent (control) cellophane paper. The time allowed for photoacclimation was of one week, before recollection and laboratory analysis (same as stage 1).

Data analysis

Pigment concentration

The pigment concentration (μg.ml$^{-1}$) provided the basis for comparison between each cell. The Chl:Car concentration ratio was calculated to verify the photoprotective role of Car. The concentration of each pigment in acetone was calculated according to the formula used by Boyer (1990):

$$C_a = 11.24A_{661.6} - 2.04A_{664.8}$$
$$C_b = 20.13A_{644.8} - 4.19A_{661.6}$$
$$C_{a+b} = 7.05A_{661.6} + 18.09A_{664.8}$$
$$C_{x+c} = (1000A_{470} - 1.90C_a - 63.14C_b)214^{-1}$$

Where: C is the concentration in micrograms per milliliter (μg.ml$^{-1}$) of Chl a (a), Chl b (b), both of them (a+b), and Car (x+c). $A_y$ is the absorbance of light at the wavelength y.

Results

Pigment concentration on Ventricaria ventricosa is not significantly different with depth

To verify the effects of depth on the pigment concentration of V. ventricosa, concentrations of Chl a, Chl b and Car were compared at different depth belts (8-10 m and 16-18 m). There were higher concentration averages of Chl a (5.33 μg ml$^{-1}$, SE = 1.11) and Chl b (6.47 μg ml$^{-1}$, SE = 1.61) at the 8-10 m depth belt (n = 17) than the concentration averages of Chl a (4.00 μg ml$^{-1}$, SE = 0.86) and Chl b (4.32 μg.
ml⁻¹, SE = 1.61) at the 16-18 m depth belt (n = 17). The average Car concentration was found to be slightly higher at the deeper collection belt (1.70 μg ml⁻¹, SE = 0.28) than at the shallower one (1.6 μg ml⁻¹, SE = 0.20) (Fig. 1). The Chl:Car concentration ratio was lower on the alga found deeper (4.58, SE = 0.80), rather than shallower on the reef slope (7.85, SE = 2.05). Furthermore, there was no significant difference with the concentration of any pigment and the Chl:Car ratio across the depth belts (tChl a = 0.35, tChl b = 0.38, tCar = 0.81, tChl:Car = 0.15). These results suggest that V. ventricosa does not alter pigment concentration from 8-10 m to 16-18 m as part of the photoacclimation process. Due to natural variation and availability of V. ventricosa in the field, there was a difference in size (measured as volume) of the individuals collected at the different belts. The average volume of V. ventricosa collected from the deeper belt was greater (3.8 ml, SE = 0.76) than the shallower one (2.67 ml, SE = 0.65).

Individuals placed in green cages had higher pigment concentration and lower survival than controls.

To understand the pigment process of photoacclimation on V. ventricosa, pigment concentration was measured after exposing individuals to stressful photo-events. Upon detachment, V. ventricosa cells were placed in cages with a green (n8-10 m = 4, n16-18 m = 5) or transparent (n8-10 m = 5, n16-18 m = 4) filter for a 7-day period and then analyzed for their pigment concentration. Only the larger individuals under the latter condition did not deflate at both depths (10 ml, 3.7 ml, n8-10 m = 2; 19.5 ml, n16-18 m = 1). The survival of individuals placed in the transparent cages was greater, and they were smaller on average (1.04 ml, n8-10 m = 5; 0.90 ml, n16-18 m = 3). There was a generally larger pigment concentration on the alga covered by the green filter across most depths and pigments (Fig. 2). The only exception occurs with Chl a concentration at 16-18 m, in which the green filter condition (1.12 μg ml⁻¹, n=1) is slightly lower than the control (1.16 μg ml⁻¹, SE = 1.11, n=3). The greatest concentration difference between both conditions happens at the shallower collection point with Car (Fig. 2), in which the green filter condition (1.35 μg ml⁻¹, n = 2) is roughly 13 times higher than the control (0.10 μg ml⁻¹, SE = 0.15). Finally, the transparent filter condition did not serve as a control, because it did not yield the same pigment concentration as the first stage (Fig. 2). This data suggests that 1) detachment affects pigment concentration and that 2) there is a change in pigment concentration upon a photo-stressful event in V. ventricosa.

Discussion

Even though the Chl concentrations were higher and Car concentration was lower on the V. ventricosa collected at 8-10 m, there was no statistically significant difference between any of the pigments analyzed at both depth belts, contrary to the proposed hypotheses. This suggests that the alga does not change the pigment concentration within this depth gap. The Chl:Car concentration ratio was lower in cells found deeper in the reef slope, but with no significant difference as well, which does not reflect the photoprotective role of Car proposed by Siefermann-Harms (1985). When the alga
was detached and exposed to the green filter for a 7-day period, only the larger individuals survived. The pigment concentration was greater compared to the control individuals, where most cells survived. The transparent filter did not serve as a control, because it displayed lower pigment concentrations compared to the individuals that were collected and analyzed on the first experimental stage. Finally, the average volume of the alga found deeper in the reef slope was larger than the shallower one.

There may not be any significant difference in the Chl concentrations from 8-10 m to 16-18 m because it is too small of a depth increment for the cell to photoacclimate. As it was previously mentioned, macroalgae do increase the Chl concentrations with depth, but the necessary increment across depths varies in different species. On one hand, Ramus et al. (1976) found significant difference between the Chl concentrations from different macroalgae at 1 m and 10 m deep. On the other hand, the Chl a concentration in *Macrocystis pyrifera* only presents a big change from 6 m to 9 m deep, remaining fairly equal until reaching 18 m deep (Colombo-Pallota 2006). Pérez-Lloréns et al. (1996) suggest low Chl concentrations on *Ulva curvata* and *Ulva rotundata* on both very low and high levels of irradiation, leaving a wide irradiance range in between with fairly constant concentrations. It is possible that the chosen depths for this study are within that range that *V. ventricosa* does not yet require photoacclimation. Mass et al. (2010) suggest photoacclimation in zooxanthellae of *Stylophora pistillata*, comparing the unicellular organisms from branches collected at 3 m and 40 m deep, which is a considerably larger depth gap. It would be important to test *V. ventricosa* from more depths, preferentially deeper, to determine the threshold at which the
algae photoacclimates, if at all, in nature. Another factor to keep in mind is the amount of light irradiance at each depth, as well as the presence and effect of epiphytes. Performing the experiment in a laboratory setting may lack the \textit{in situ} natural variability, but it would enhance the control of variables.

The same photoacclimation depth threshold may apply to the Car concentration, because both the total concentration and the Chl:Car ratio presented lower values in the belt at greater depth, but with no significant difference. As seen in Henley and Ramus (1989), the general Car concentration and the Chl:Car ratio decrease with more irradiance (for this case it represented by the shallower depth for the sake of comparison), which suggests that Chl concentrations decreases more rapidly than Car concentration. The effort to reduce photodamage is thought to entail a decrease in photosynthetic Chl and Car, allowing the photoprotective Car to remain and most likely increase. Future research should take into account the different carotenoids, their functions, and the appropriate depth gaps to verify their photoprotective role as a process of photoacclimation.

The size of \textit{V. ventricosa}, detachment from the reef, as well as less nutrient flow may have influenced the second stage of the experiment. This suggests that \textit{V. ventricosa} prioritizes healing over photoacclimation, losing pigment concentration while reestablishing the turgor pressure. Firstly, there may be micro lesions that disrupt the turgor pressure and the consequent organelles’ organization (Shihira-Ishikawa and Nawata 1992) inside the cell upon detachment, including the chloroplasts. Secondly, \textit{V. ventricosa} requires calcium (stored in vacuole and taken from the surrounding) to heal its wounds (Sugiyama et al. 2000), and the placement in completely covered cages could have influenced the intake of calcium by decreasing the water and nutrient flow. Sims (2013) also detached \textit{V. ventricosa}, but they were placed in a laboratory setting in which the seawater was renewed every four days and the light intensity was constant. Finally, only the larger \textit{V. ventricosa} individuals did not deflate under the green filter, perhaps because the already existent calcium in the vacuole was enough to heal the wounds, channeling the energy to increase pigment concentration. The smaller cells under the green filter may not have had enough energy to intake calcium from the already decreased surrounding levels of calcium, and sequentially photoacclimate to a harsh photosynthetic condition. The control individuals may have undergone the same healing process, which led to the decrease in pigment concentration. However, since they did not require photoacclimation, the little pigment concentration was enough to photosynthesize sufficient energy for survival. To verify the priority system of \textit{V. ventricosa}, it is advised to perform the same experiment without detaching the cell from the reef slope, and maintain the water flow by simply shading the area. Another possibility would be to collect the cells in the cages at different time periods, to determine if small cells can survive in the green filter condition in less days, or if control individuals can regain their original pigment concentration.

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“I hope for your help to explore and protect the wild ocean in ways that will restore the health and, in so doing, secure hope for humankind. Health to the ocean means health for us.”

– Silvia Earle