

The cover features a vibrant underwater scene. A large, colorful angelfish with a yellow body and blue stripes is the central focus, swimming towards the bottom right. To its left is a large, textured brown coral structure. In the upper left, a smaller, spotted fish is visible near some orange coral. The background is a clear, bright blue water with some light scattering.

PHYSIS

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PHYSIS

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Journal of Marine Science

CIEE Research Station Bonaire

Tropical Marine Ecology and Conservation Program

Vol XII, Fall 2012



“From birth, man carries the weight of gravity on his shoulders. He is bolted to earth. But man has only to sink beneath the surface and he is free.”

- Jacques Cousteau

Physis is a Greek word meaning “nature.” Since even the word “nature” is a broadly and poorly defined representative for the awesome beauty we see in the world around us, we interpret Physis as having a much more subtle meaning. We apply the beauty and grace we see in nature and use that to inform our definition.

Firstly, we see Physis as embodying growth and change. Homer used Physis to describe the way a plant grows. We apply this not only to individual plants, but to communities of organisms. We see underwater cities of coral, calcium carbonate structures grown up from algae and little polyps, and we realize that no city made by humans is as alive and growing as these natural ones are. We accept that nature has some sort of inventive style that we cannot yet replicate or even completely comprehend. We appreciate it anyway.

Secondly, we see Physis as a romanticization of natural healing. We see it as nature’s “bounce-back” ability in the face of adversity. We see hurricanes pass through a reef and cut down the branching coral, and we marvel that nature can somehow survive this. Maybe the branching corals do not return, but something else will always take its place. We are awed by this perpetuation.

As a facet of this, we mourn the damage that humans have already inflicted upon the environment. We realize that as much as we see nature always healing and growing, we have never seen nature recover from destruction of the extent that we’ve caused in the past several years. Coral reefs are reeling from the effects of physical and chemical pollution, overfishing, changing climates, and physical breaking to make way for human development. The more we realize that these damages are occurring all over the world, the more concerned we become that maybe nature is more fragile than we originally thought. Maybe we’ve already lost parts of our world.

In the face of our newfound awareness of nature’s fragility, we feel even more compelled to learn about Physis. We quote the Senegalese environmentalist Baba Dioum, who famously said, “In the end we will conserve only what we love, we will love only what we understand, and we will understand only what we are taught.” We want Physis to be conserved, and so we strive to learn as much as possible.

Finally, we view Physis as the reality that, as much as we learn about nature, we as humans will never be able to replace it. Even science is only our attempt to build a model describing our world. It will only ever be our best estimation, never truly representing reality. The more details we add, the more accurate our estimation becomes. Every experiment takes little steps to build our perception of the larger picture. Every experiment brings our model closer to describing the true way the world works, even though we believe that we will never have all the answers. Through this journal, we hope to add our experiments, our observations, and our thoughts to the world’s understanding of nature. Someday we will be closer to seeing what Physis really means, how nature really works, but we will still be just far enough away to keep looking.

Thus, we present Physis.



FOREWORD

The Council on International Educational Exchange (CIEE) is an American non-profit organization with over 150 study abroad programs in 45 countries around the world. Since 1947, CIEE has been guided by its mission:

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

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

The student research was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Dutch Caribbean. The research this semester was conducted on the leeward side of Bonaire where most of the population of Bonaire is concentrated. Students presented their findings in a public forum on the 21st and the 22nd of November 2012 at the research station for the general public.

The proceedings of this journal are the result of each student’s Independent Research project. The advisors for the projects published in this journal were Rita Peachey, Ph.D. Enrique Arboleda, Ph.D., and Catherine Jadot, Ph.D. In addition to faculty advisors, each student had CIEE Interns that were directly involved in logistics, weekly meetings and editing student papers.



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Dr. Rita Peachey is the Resident Director of the CIEE program in Bonaire and the Director of the CIEE Research Station Bonaire. She received her B.S. in Biology and M.S. in Zoology from the University of South Florida and her Ph.D. in Marine Sciences from the University of South Alabama. She teaches Independent Research and Cultural and Environmental History of Bonaire. Dr. Peachey has conducted research on larval ecology of corals and other invertebrate species in Hawaii, Australia, the mainland US, and the Caribbean. Her research interests include the effects of ultraviolet radiation on marine invertebrate larvae, environmental analysis, fish behavior, invertebrate taxonomy and reproduction in lionfish.



Dr. Enrique Arboleda is the Coral Reef Ecology Faculty for CIEE and co-teaches Independent Research and Marine Ecology Field Methods. He has been with the research station since August 2012. He is a marine biologist from the Jorge Tadeo Lozano University (Colombia), holds a specialization on Biodiversity and Evolutionary Biology from the University of Valencia (Spain) and obtained his 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. Catherine Jadot is the Tropical Marine Conservation Biology Faculty since August 2012. She holds a Ph.D. in Eco-Ethology, a M.S.c. in Oceanography and a M.S.c. in Zoology. She has worked for various universities and agencies in Belgium, France, the Azores, Dubai, Trinidad, the Cayman Islands, the Bahamas and Turks and Caicos. Her research interests are marine resources management, near-shore habitat enhancement and restoration. Catherine has authored and co-authored numerous scientific papers and technical reports on issues related mainly to eco-ethology, ecological restoration, fisheries management and coral reef ecosystems.

FACULTY



Professor Caren Eckrich is the Dive Safety Officer for CIEE, Instructor for Advanced Scuba, and co-instructor for Marine Ecology Field Research Methods. Prof. Eckrich holds a B.S. in Wildlife and Fisheries from Texas A&M University and a M.S. in Biological Oceanography at the University of Puerto Rico in Mayaguez. Her research interests include fish behavior, seagrass and algal ecology, sea turtle ecology, and coral disease. She is currently working on a study of crustose algae and other benthic invertebrates in Lac Bay.



Anouschka van de Ven is the Assistant Resident Director for CIEE. She is a PADI Dive Instructor and she assists with teaching Advanced Scuba. She has a B.A. and First Class Honours Degree in Communications Studies from the London Metropolitan University and worked in television and advertising in Amsterdam before moving to Bonaire. Anouschka provides administrative support for the research station and is responsible for the website and public relations. She is a volunteer operator at Bonaire's



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



Graham Epstein is the Lab Analyst at CIEE Bonaire. He has a background in Genetics with a B.S.c. in Biological Sciences from University of Edinburgh, with an M.S.c. in Marine Ecology and Environmental Management from Queen Mary, University of London. He has been a PADI and BSAC dive instructor for five years working in both tropical and temperate climates. His specific research interest is biogenic reef systems with research on Scottish coralline algae beds and tropical coral reefs. He is currently conducting coral recruitment research and is working on long-term projects at CIEE.

INTERNS



Franziska Elmer is an intern this Fall 2012. She has just finished her Masters degree in Environmental Science at the Swiss Federal Technical Institute in Zurich with collaboration with the NOVA Southeastern University in Florida. Additionally, Franziska worked as a Marine Park Intern for the St. Eustatius Marine Park and as a Divemaster in Mallorca, Spain.



Abigail Hills is an intern this Fall 2012. She recently graduated with a masters in Environmental Science from Miami University (OH) where she specialized in Conservation Biology and Coral Reef Ecology. Since 2009, Abby has been engaged with research on coral reef health assessments, focusing on methodology efficiency and ecological impacts.



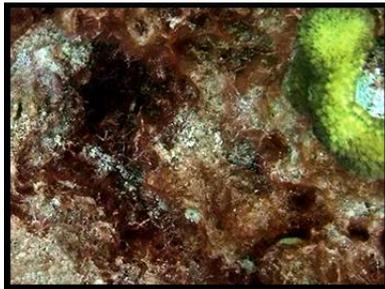
Rachael Wright has been an intern at CIEE for seven months. This semester she assisted with Cultural and Environmental History of Bonaire and Independent Research classes. She has an M.S.c. in Marine Ecology and Environmental Management from Queen Mary, University of London and a B.S.c. in Geography with Ocean Science from the University of Plymouth, UK. While on Bonaire she has been conducting a study into micro-plastics in tropical marine sediments focusing on the Lac Bay area.

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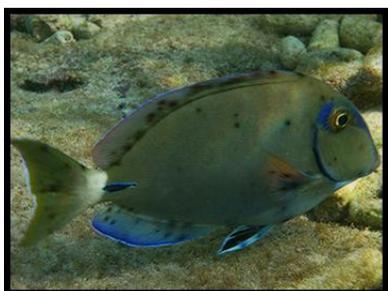
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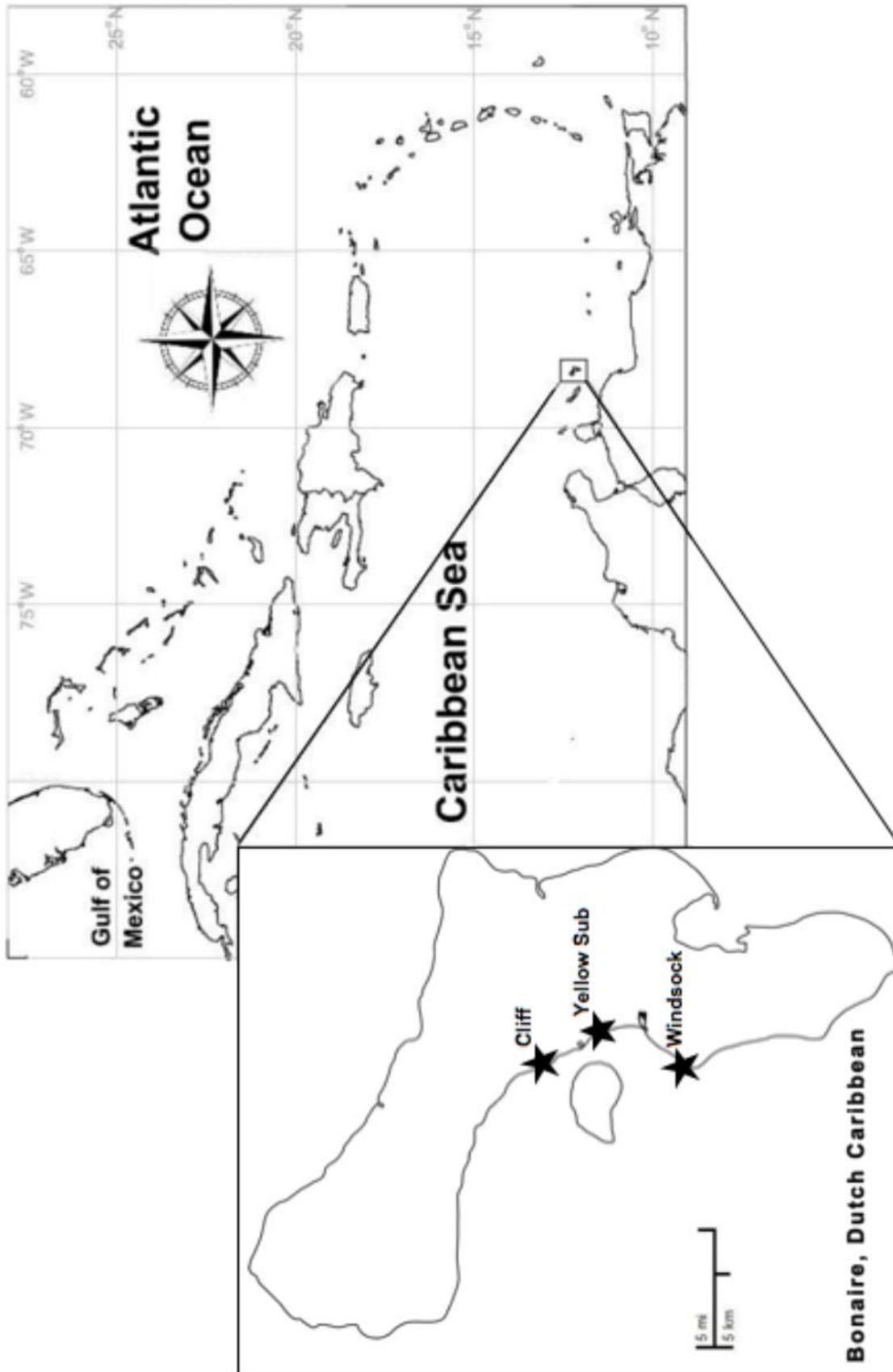
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Predation and habitat depth affect coral reef fish recruitment

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Abstract

Reef fish have a pelagic larval stage and settle onto the reef before transitioning to their juvenile or adult morphologies. Settlement can be dangerous for new recruits and mortality is highest during the first one to two days after settlement. Experiments were conducted to determine the effects of predation and habitat depth on reef fish recruitment. Standard habitat units (SHUs) were created from pieces of *Millepora* skeleton. Two treatments were created using SHUs. The first contained an SHU placed on the substrate that was open to predation (NC). The second treatment contained an SHU in a wire cage to exclude predators (FC). Two replicates of each treatment were placed in two meters of water and at six meters. New recruits were surveyed twice a week for six weeks (n=11 surveys). SHUs were cleared of recruits and algal growth was removed after each census. Overall recruitment was greater in two meters than six meters of water. Recruitment was also greater in FC treatments than in NC treatments at both two and six meters. Trends in recruit density should not have been observed since recruit censuses were taken as replicates. However, recruitment increased over the course of the experiment, which coincided with the lunar cycle. Seven species of fish were observed over the course of the experiment but only two of these species, the wrasse, *Halichoeres bivittatus*, and the razorfish, *Xyrichtys splendens*, were observed at six meters. One species, the pufferfish *Canthigaster rostrata*, was only observed once.

Introduction

Most reef fish spend time in a pelagic larval stage of life (Montgomery et al. 2001). The length of time spent in this larval stage varies between species. Some species such as the damselfish *Stegastes partitus* may spend only a few weeks in the plankton stage, while other species such as the surgeonfish *Acanthurus bahianus* may remain pelagic for a few months (Sponaugle and Cowen 1996). Most larvae remain offshore during the day (Leis et al. 1996) but return to the reef at night to settle onto the reef (Stobutzki and Bellwood 1998); however, there is evidence that some settlement occurs during the day as well (Leis and Carson-Ewart 1999). Settlement at night may be due to a higher risk of predation near the reef during the day when there are many predators active (Leis and Carson-Ewart 1999). Recruitment can be described as the change when pelagic larvae join the benthic reef community, usually accompanied by morphological changes. Mortality rates for new recruits are highest

in the first one to two days of settlement from predators (Almany and Webster 2006). Doherty et al. (2004) found that 61% of potential settling surgeonfish, *Naso unicornis*, were lost overnight and that mortality ranged from 9 to 20% per day after settlement and was density dependent.

Larval supply is essential for maintaining populations of reef fish (Sponaugle and Cowen 1996) and can affect recruitment patterns. Pulses of large amounts of late-stage larvae near reefs tend to occur on a monthly basis, suggesting that they use the lunar cycle as a cue for settlement (Robertson 1992). Corresponding rises in recruitment have been shown to occur during larval pulses (Milicich et al. 1992; Sponaugle and Cowen 1996). Once larvae settle onto the reef, many species go through a metamorphosis process. The larval fish are specialized for the pelagic environment and must adapt to the new benthic reef environment. This can take anywhere from a couple hours to days to occur and includes changes in coloring, body length, and fin

length (McCormick et al. 2002). Larval supply can differ between islands and even between sites on one island (Sponaugle and Cowen 1996). Each reef in the Caribbean may have its own slightly different patterns of recruitment and few studies have been done on Bonaire to assess local settlement patterns. Not all reef fish reproduce year round (Luckhurst and Luckhurst 1977), so certain species may be more or less abundant or absent in a recruitment study during the fall, such as this research, than at other times of the year. Particular species may also show a preference in recruiting to a particular type of habitat. The gray snapper, *Lutjanus griseus* settles in seagrass beds (Lindeman et al. 1998) while the rainbow parrotfish, *Scarus guacamaia* settles and grows only in mangroves before migrating to the reef as adults (Mumby et al. 2004).

Little research has been done on whether larvae show a preference of depth in where they choose to settle. This study addressed this gap in knowledge and assessed the differences in predation pressure at two different depths. A previous study by Shulman (1985) examined settlement and the effects of predation as a function of distance from the reef. Using different methods, this study investigated the effects of predation on recruit density and examined the effects of depth which implies distance from the reef as well. Various treatments using artificial habitats and predator exclusion cages were used to measure reef fish recruitment. The following hypotheses were tested:

- H₁: There are more new recruits found in six meters of water, near the reef crest, than in two meters of water.
- H₂: Predation pressure is found at all depths.
- H₃: Predation pressure is greatest in the shallows.
- H₄: Species richness is the same at each depth.

Materials and methods

Study site

Data collection took place in Bonaire, Dutch Caribbean, located in the Southern Caribbean (Fig. 1). The study site Yellow Sub comprised of a fringing reef structure with the reef crest approximately 50 meters from shore and 6 meters deep. From the intertidal zone to the reef crest the substrate was composed of sand flats with few corals or other habitat.

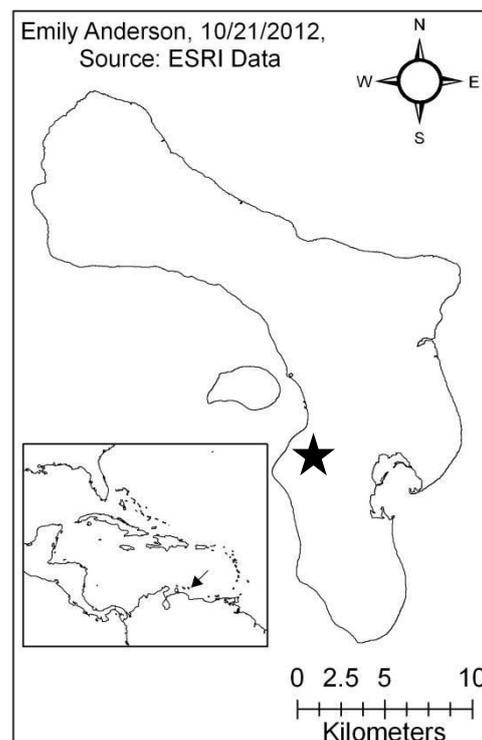


Fig. 1 Map of Bonaire, Dutch Caribbean located in the Southern Caribbean. The study site Yellow Sub (12°09'36.47"N, 68°16'55.16"W) is indicated with a black star. Yellow Sub is comprised of a fringing reef structure with the reef crest approximately 50

Standard Habitat Units

Previous recruitment studies have used a wide variety of sampling techniques. Several larval recruitment studies have used small live coral habitats to perform experiments (Sweatman 1985; Forrester 1990; Schmitt and Holbrook 2000; Stier and Osenberg 2010). Others have used shells from the

queen conch *Strombus gigas* (Shulman 1985) or artificial materials such as terra cotta bricks (Robertson et al. 1988) to create artificial reefs. Standardized habitats are successful in recruitment studies and were utilized in this experiment as well. Experiments were done with “standard habitat units” (SHUs) after Stier and Osenberg (2010). The SHU’s consisted of pieces of *Millepora* coral skeleton. Two different treatments were created (Fig. 2). The first treatment contained an SHU without a cage or a no-cage (NC) SHU. A full cage containing an SHU comprised the second treatment (FC SHU).

In order to evaluate any possible interference of the wire cages with recruitment success two additional treatments were created as a control. The first control treatment contained an SHU with a wire cage bottom (CB SHU). The same gauge wire grating was used to create an open cage containing an SHU for the second control treatment (OC SHU). The open cage left the two opposite sides of the cage open (Fig. 2). Replicates of each treatment were placed at two and six meters depth. Treatments were placed two meters apart and at least two meters away from any coral or other habitat (Fig. 3).

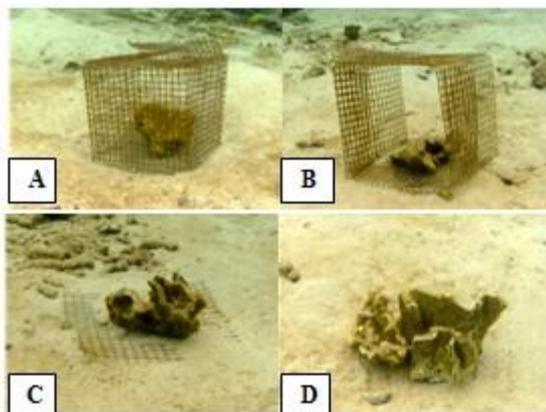


Fig. 2 A-D Standard habitat units (SHUs) built from pieces of *Millepora* skeleton. A) Full cage (FC) SHU. B) Open cage (OC) SHU. C) Cage bottom (CB) SHU. D) No cage (NC) SHU

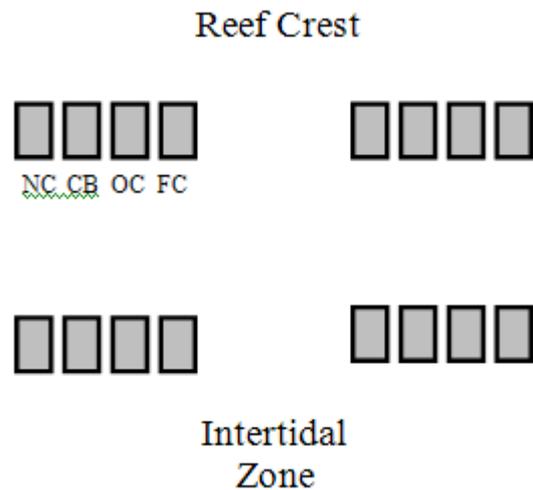


Fig. 3 Placement of standard habitat units (SHUs) at the study site Yellow Sub, Bonaire in the Dutch Caribbean. Two stations of four treatments were placed near the reef crest in six meters of water and two stations were placed near the intertidal zone in two meters of water. NC: No cage, CB: Cage bottom, OC: Open cage, FC: Full cage

Data collection

A visual census of all the recruits on each SHU using SCUBA was taken every Wednesday and Sunday at 08:30 for six weeks ($n = 11$) during the months of September to October of 2012. Pictures were taken of any juvenile fish that was not immediately identified to determine the species. Since juvenile and adult fish can affect the number and species of fish that settle on a habitat (Sweatman 1985), new recruits were forced several meters away after each census and every SHU was cleaned using a wire brush to remove algal growth and moved a short distance.

Data analysis

Statistical analysis was completed using the Mann-Whitney U test (Avery 2007). To assess whether the use of a cage affected recruitment, the FC and OC SHUs were compared at each depth. The two treatments were found to be significantly different in density of new recruits so only data from the FC treatments were used in the final

analyses. The CB and NC SHUs were also compared and found to have no significant difference in recruit density. However, only data from the NC SHUs were used in the final analyses.

In the final analyses the mean total abundance of new recruits at deep and shallow stations were compared. Predation pressure was determined by comparing the mean abundance of recruits in caged treatments and open treatments. The statistical significance of predation at each depth was analyzed by comparing the FC SHU and NC SHU at two and six meters. Number of species observed at each depth was used to compare species richness.

Each census was taken as a complete replicate so no trends should have been observed in fish recruitment over time. However a trend of increasing recruitment was recorded at both 2 m and 6 m. A linear regression was plotted to analyze the increasing recruitment over time.

Results

Control treatments

Two of the four treatments (OC and CB) were used as controls to assess the effects of the cage used to exclude predators on recruitment of reef fish. The Mann-Whitney U test (Avery 2007) was used to determine if recruitment densities observed at each treatment were significantly different from each other (2m $p=0.783$, 6m $p=0.129$). FC and OC treatments had the highest recruitment of the treatments (Fig. 4). The FC treatments had significantly greater recruitment than the OC treatments at both two meters and six meters showing that the complete cage did not deter recruitment. Recruitment was low in the CB and NC treatments at both depths and showed no significant differences in recruit density showing that the presence of the wire cage bottom did not affect recruitment on the CB treatments. At the two meter stations OC treatments had significantly higher recruitment than NC treatments but at six

meters OC treatments did not have significantly higher recruitment from NC treatments (Table 1). Since the OC and CB treatments were used to assess whether the presence of the cage would deter recruitment they were excluded from the final analyses.

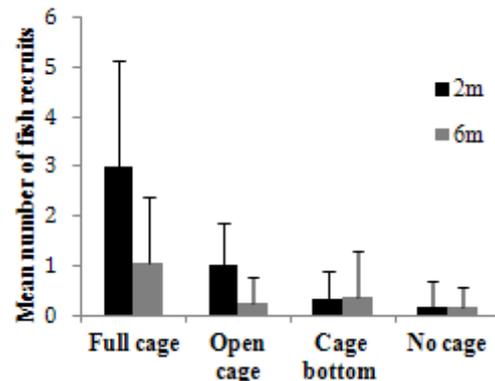


Fig. 4 Mean number of fish recruits (\pm SD) observed at each of four treatments in 2 m (black) and 6 m (grey) of water over $n=11$ fish recruit surveys at Yellow Sub, Bonaire in the Dutch Caribbean.

Table 1 Mann-Whitney U test p-values comparing Standard Habitat Unit (SHU) treatments at each depth at Yellow Sub, Bonaire in the Dutch Caribbean. Open-cage (OC) and cage-bottom (CB) were used as control treatments for full-cage (FC) and no-cage (NC) treatments.

| Depth | FC vs OC | OC vs NC | CB vs NC |
|-------|----------|----------|----------|
| 2 m | 1.00E-04 | 0.001 | 0.45 |
| 6 m | 0.041 | 0.963 | 0.906 |

Recruitment

The effects of predation were assessed by recording fish recruitment to open control habitats and habitats within a predator exclusion cage. The stations at both two meters and six meters showed higher recruitment in treatments that excluded predators than the open treatments (Fig. 4). Mean fish recruitment was significantly greater in the FC treatments than in NC treatments ($p=1.598E-7$). Recruitment to open treatments were similar at both depths; NC treatments did not show significantly different recruitment between two meters

and six meters depth ($p=0.833$). However, treatments that excluded predators at two meters had higher recruitment than replicate treatments at six meters. FC treatments were significantly different between two meters and six meters ($p=4.908E-4$). Overall recruitment was highest on FC treatments and in the shallow stations (Fig. 4).

Each recruit census was taken as a replicate sample and therefore no trends in recruitment should have been observed. However a trend of increasing recruitment over the course of the experiment was observed at both two meters and six meters (Fig. 5). The sampling period coincided with two full moon periods and a new moon period which appears to correlate with the recruitment density observed over time at both stations.

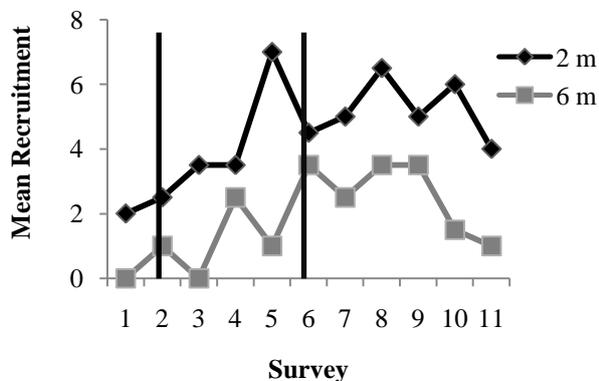


Fig. 5 Mean fish recruitment during surveys at Yellow Sub, Bonaire in the Dutch Caribbean (n=11 surveys). Surveys were performed every Wednesday and Sunday for six weeks. The first and last vertical lines indicate full moon periods and the middle line indicates the occurrence of a new moon

Species richness

Species richness was greater at two meters than at six meters (Fig. 6). Seven species were observed at the two meter stations while only two of those species were observed at six meters (Table 2). The wrasse *Halichoeres bivittatus* and the razorfish *Xyrichtys splendens* were the only species recorded at six m. *H. bivittatus* was common

at this depth while *X. splendens* was only observed twice during the experiment. *H. bivittatus* and *X. splendens* were the most common recruits observed at the two meters stations. Of the seven species observed at two meters the pufferfish *Canthigaster rostrata* was the most uncommon. *C. rostrata* was only observed once during the course of the experiment.

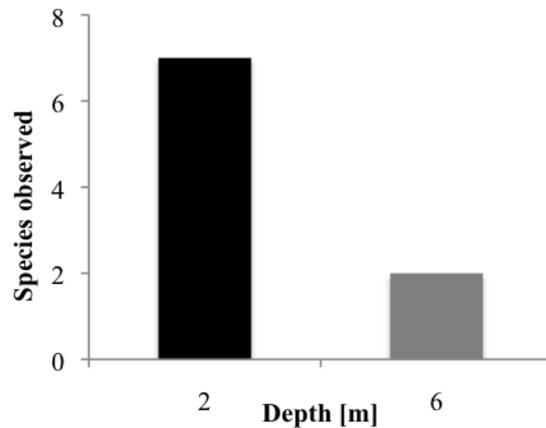


Fig. 6 Total number of fish species observed during recruitment surveys at two m (black) and six m (grey) depth at Yellow Sub, Bonaire in the Dutch Caribbean (n=11 surveys)

Discussion

Control treatments

Two SHU treatments were created to assess predation on recruit density (FC and NC). Predators were excluded from the SHUs by a cage in the FC treatment while the NC treatment was open to predators. Two additional treatments were created to test for any effects of the cage used in the FC treatment (OC and CB). Like the NC treatment, the CB treatment was completely open to predators but had a wire grate attached to the bottom. NC and CB treatments had very similar recruit density at two meters and six meters showing that the wire bottom did not affect recruitment. The second control treatment (OC) contained an open cage which limited predator access to the SHU. OC treatments in six meters of water did not have significantly different recruitment than NC treatments showing that

the presence of the cage did not deter larval settlement. However in two meters of water the OC treatments had greater recruitment than the NC treatment showing not only that the cage did not diminish recruitment but that the open cage may also have reduced predation. FC SHUs at both depths had significantly higher recruitment than OC treatments showing that the full predator cage did not reduce recruitment compared to the open cage but also demonstrating the significance of predation present between partial and full cages.

Table 2 Species of juvenile fish observed during fish recruit surveys on Standard Habitat Units (SHUs) at Yellow Submarine, Bonaire in the Dutch Caribbean (n=11 surveys). Seven species of fish were observed in 2 m of water but only two of those species were also observed in 6 m of water

| 2 m | 6 m |
|-------------------------------|-------------------------------|
| <i>Halichoeres bivittatus</i> | <i>Halichoeres bivittatus</i> |
| <i>Xyrichtys splendens</i> | <i>Xyrichtys splendens</i> |
| <i>Stegastes partitus</i> | |
| <i>Stegastes diencaeus</i> | |
| <i>Canthigaster rostrata</i> | |
| <i>Ocyurus chrysurus</i> | |
| <i>Lutjanus mahogoni</i> | |

Recruitment

One of the primary objectives of this study was to determine if recruitment was greater in shallow water near the intertidal zone or in deeper water near the reef crest. Recruitment was hypothesized to be greater near the reef crest (H_1) since the post-settlement larvae would be close to a large source of habitat and food. New recruits would have less distance to travel as settling larvae swimming in and as juveniles or adults migrating back to the reef. However the risk of predation may be much greater near the reef crest due to reef associated predators. Shulman (1985) demonstrated that predation was significantly greater at the reef crest than 20 m away from the reef.

This study found that recruitment was significantly greater at two meters than at six meters but only in the FC treatments. FC treatments at two meters had significantly higher recruitment than at six meters ($p=4.908E-4$) while NC treatments had similar recruit densities ($p=0.833$). Excluding the effects of predation, recruitment is significantly higher in shallow regions. However, when the effects of predation are included recruitment is equal between depths. Therefore recruitment was not greater at the reef crest. A previous study using experimental habitat plots also found increasing recruitment as distance from the reef increased (Shulman 1985).

Predation was hypothesized to be found at both depths tested (H_2). At both two meter and six meter FC treatments had significantly higher recruit density than NC treatments. Use of predator exclusion cages was successful in showing the effects of predation on reef fish recruits. Predation was also hypothesized to be greater at two meters than at six meters (H_3). This study found recruitment was equal in NC treatments, which allowed for predation, demonstrating that predation was equal at both depths. Further research is needed to determine if predation is higher in shallow water far from the reef or in deep water near the reef crest.

Each recruit census was taken as a complete replicate and no trends should have appeared in recruit density in the results. However recruit density increased over time. As these results were unexpected a linear regression of the trend was created. The linear regression showed a weak positive correlation of recruitment over time at both two meters ($R^2 = 0.377$) and six meters ($R^2 = 0.221$). Surveys began shortly before a full moon and ended shortly after the next full moon. When the last three surveys were excluded from the regression so that samples included began at the full moon and ended at the waxing quarter moon the correlation at two meters ($R^2 = 0.676$) and six meters ($R^2 = 0.690$) were much stronger. Recruit density appears to correlate with the lunar cycle, specifically with how much light is present

from the moon. Settlement of reef fish often follows the lunar cycle with large numbers of larvae settling during the new and quarter moons, the darkest times of the cycle (Robertson 1992). A longer experimental time frame would be needed to confirm or reject lunar effects on recruitment at this site. If settlement shows a correlation with the lunar cycle, recruitment patterns at Yellow Sub would be similar to patterns from several other studies that have shown settlement following the lunar cycle (Robertson et al. 1988, Robertson 1992, Sponaugle and Cowen 1996).

Species richness

Species richness was hypothesized to be very similar between treatments at two and six meters (H_4). However five more species of reef fish recruits were observed at two meters than at six meters. These five species consisted of two species of damselfish, two species of snapper, and one species of puffer. Two species, *X. splendens* and *H. bivittatus* were observed at both depths but only *H. bivittatus* was observed regularly at both sites. *X. splendens* was common at the shallow stations but only observed twice in deeper water.

Note that only the Labridae species were observed at both depths. Labridae species are fast swimmers (Fulton et al. 2005) perhaps enabling them to evade predators better than the other recruit species observed. However this does not explain the absence of other species from FC treatments where predators were excluded. Since SHUs were identical at two meters and six meters a preference for habitat type cannot explain the difference in species settlement. Since SHUs were either in six meters of water near the reef crest, or at two meters which was much farther from the reef, depth or distance from the reef may then be a factor for where certain species choose to settle.

Certain species prefer particular habitats to settle in. The rainbow parrotfish (*S. guacamaia*) exclusively settles in mangrove forests (Mumby et al. 2004) while the

yellowtail snapper (*Ocyurus chrysurus*) settles in seagrass beds and mangroves (Pollux et al. 2007). Where certain habitats are not present, or may have been removed by human activity, some species may adapt to settle to different substrates. *O. chrysurus* recruits were observed on FC and OC SHU treatments showing that this species is not limited to settlement in mangroves and seagrass. The main settlement substrate of another species of snapper *Lutjanus mahogoni* is seagrass beds (Nagelkerken 2007) although *L. mahogoni* was also recorded on SHU treatments.

The pufferfish *C. rostrata* was only observed once during the course of the experiment and little is known about this species settlement preferences. A settlement and transition study by Kaufman et al. (1992) only observed one *C. rostrata* settlement event and another settlement study reported *C. rostrata* as a rarely observed species (Shulman 1985). As juveniles of this species are rarely seen, recording their settlement preferences may be difficult. Little research has been done on why species richness may vary with depth or distance from the reef.

Limitations

This experiment had a few limitations that future experiments should take into account. SHUs were placed in the water clean and accumulation of small algae and other biofilm may have caused a change in habitat over time meaning that each sample was not a true replicate despite cleaning the SHUs and cages of algae after each census. In the future, SHUs should be placed in water for a period of time for biofilm to accumulate so that the habitats do not change over time.

After each census, recruits were forced away from the SHUs. However, it is possible that the juvenile fish may have returned to the SHUs and were surveyed in the next census. Since the presence of resident juvenile fish on a habitat can affect the number and species of new recruits

(Sweatman 1985), it is essential that future experiments completely remove recruits after sampling to ensure the same juvenile fish are not counted repeatedly or affect the settlement of new recruits.

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Large scale, medium scale, and small scale patterns of benthic cyanobacteria & the possibility of groundwater association on a coral reef

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Abstract

Cyanobacteria presence in the marine ecosystem is biologically significant due to its versatile nature. This phylum is responsible for destructive red tides and black band disease as well as building up the limestone in reefs. *Nodularia cf. spumigena* has been an indicator of groundwater off of the coast of Bahamas and may provide insight into where Bonaire's groundwater enters the marine ecosystem. Bonaire is a tropical oceanic island with a fringing coral reef located in the south Caribbean Sea and, although the population of the island is small (~16,000), it has developed a population center of residential and commercial use in Kralendijk, a coastal city. To compare how the benthic cyanobacteria mats off of Bonaire relate to this growing population, a three-fold study was conducted. A large scale pattern of cyanobacteria was studied at nine sites on the leeward side, a medium scale pattern was studied at four sites off the coast of Kralendijk in between two known nutrient outputs, and tests of mean levels of *Escherichia coli* and total coliforms inside and outside the cyanobacteria mats were completed. Video transects were used to determine percent cover of the large and medium scale patterns and IDEXX technology was used to test pore water inside and outside the mats for two types of bacteria associated with human waste. No definite patterns of groundwater or population center were directly linked to benthic cyanobacteria cover. The large scale did show a higher average cyanobacteria cover throughout the three-year study, indicating that there may be a relationship between the hydrology of the island and nutrient circulation. The medium scale showed an inverse relationship between turf algae and cyanobacteria.

Introduction

Cyanobacteria, previously called blue-green algae, are one of the oldest living organisms with fossils dating back 3500 million years ago (Veron 1995). This organism can be destructive by creating red tides in nutrient-rich waters (Ng et al. 2012) and are also responsible for black band disease in coral. Other species of this phylum can aid in building up the limestone in coral reefs, while others deteriorate the structure, making it hard to classify the contribution of cyanobacteria to an ecosystem as a whole (Schneider and Le Campion-Alsumard 1999). Cyanobacteria can form ecological relationships with other organisms. For example, the *Synechococcus* and *Prochlorococcus* species are known to

inhabit sponges and create symbiotic relationships within sponges and provide protection from microbial infection (Kobayashi and Ishibashi 1993). Macroalgae on coral reefs is harmful to these ecosystems by outcompeting corals for space or overgrowing corals (Arnold et al. 2010) and by producing toxic secondary metabolites that can deter grazers (Kobayashi and Ishibashi 1993). Cyanobacteria often share these characteristics as well but are less recognized (Kuffner and Paul 2004). Since there is an increase of nutrients due to anthropogenic interactions (Umezawa et al. 2002, Blanco et al. 2011) and a decrease in herbivores (Hughes 1994), the cyanobacteria and algae are becoming more prevalent in benthic cover in the coral reef ecosystem (Littler et al. 2006a).

Another threat to coral reef ecosystem health is the nutrient input from submarine groundwater from adjacent coastal cities. In a study on Ishigaki Island, Japan measured dissolved inorganic nitrogen in two areas that were subjected to different levels of groundwater on a fringing reef. Levels of nitrogen were 4- or 5.5-fold higher in the Shiraho watershed, used extensively for agricultural practices, versus the Kabira watershed which was 80% forested and had a population that was 20% less than that of Shiraho (Umezawa et al. 2002). In the Bahamas, the cyanobacteria *Nodularia cf. spumigena* was linked to the presence of polluted groundwater that was seeping out of the sand (Littler et al. 2006b). In Bonaire, it is still largely unknown where groundwater enters the marine ecosystem. If the species of cyanobacteria that reside in Bonaire has a similar role, the mats of cyanobacteria could be indicator species of high levels of nutrients and be indicating the areas where the groundwater is seeping into the marine ecosystem. This information would be useful, since it is still unknown where the groundwater leaches into the marine ecosystem.

Although Bonaire does not have an agricultural industry that would cause groundwater contamination, sewage leaches into the groundwater from bottomless septic tanks and septic pits that are currently in use on the island. Testing for dissolved inorganic nitrogen might be a useful indicator of groundwater presence (Umezawa et al. 2002). However, nitrogen levels may be lower under microbial mats, since cyanobacteria may utilize nitrogen for growth (Lapointe 2004). Instead, the presence or absence of *Escherichia coli* and total coliforms (TC) would give a clear indication of contaminated groundwater as *E. coli* is a bacteria found in sewage waste from humans. Although *E. coli* starves in natural saltwater, it has been discovered that the bacteria colonies will survive longer when saltwater is combined with wastewater (Munro et al. 1987).

The purpose of this study is threefold: 1) the large scale pattern of percent cover of cyanobacteria (20 km scale) along the leeward coast of Bonaire was plotted using long term research data from CIEE, 2) medium small scale differences in percent cover of cyanobacteria (2 km) were studied along residential/commercial area of the waterfront north of Kralendijk, and 3) a field study of the relationship between levels of bacteria, *E. coli* and TC, and cyanobacterial mats was conducted.

- H₁: On a scale of 20 km, benthic cyanobacteria levels are higher near Kralendijk due to the number of residents and commercialization, while the regions further North and South have lower percent coverage.
- H₂: On a scale of 2 km, benthic cyanobacteria cover is higher at the sites with large inputs of runoff during storms, such as the study sites Kas and Something Special, than it is at less impacted sites.

Using IDEXX technology, determination of TC and *E. coli* will be made on pore water samples to test the following hypothesis:

- H₃: The levels of TC and *E. coli* will be higher in pore water taken from under cyanobacteria mats than in pore water from adjacent areas without mats.

Materials and Methods

Study site

Bonaire is a tropical oceanic island located in the South of the Caribbean sea (85 km North of Venezuela) with a fringing coral reef that lies close to the coastline (Fig. 1). The leeward side of Bonaire is most developed near the capital of Kralendijk, where most of the population and resorts are located. Further away from the capital there are fewer residents and less potential for ground groundwater contamination.

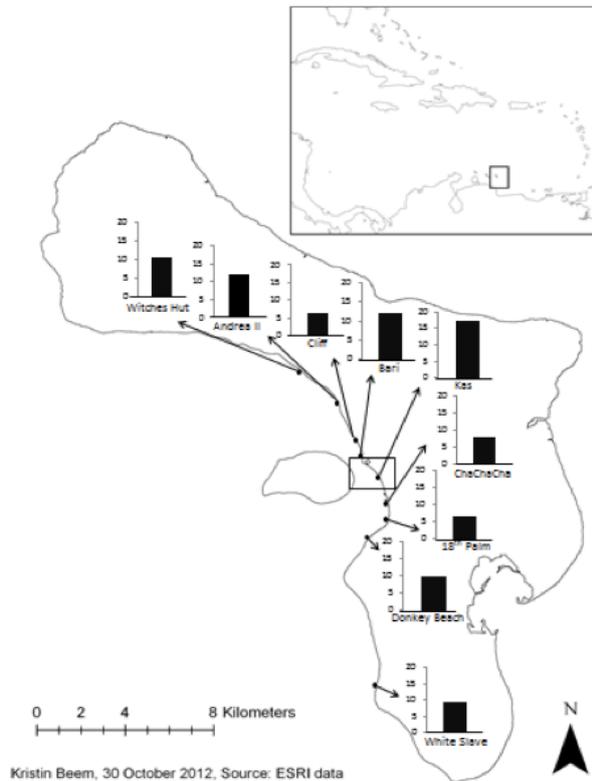


Fig. 1 Map of Bonaire, Dutch Caribbean, in the context of the Caribbean Sea, insert above. Location of the 9 study sites utilized for examining large scale patterns of cyanobacteria. Graphs show the mean percent cover for each location from spring 2009-fall 2011 from CIEE's long term research program (n=6). The open box around Kas indicates the study area utilized for the medium scale benthic cover shown in Fig. 2

To examine this large scale distribution long term CIEE benthic cover data was utilized. This data was collected from nine

sites on the leeward coast over a period of three years. The sites where data was collected for the medium scale study were Something Special and Kas. Both of these dive sites are located adjacent to areas known to have a large amount of runoff during storms. Something Special is just south of a marina, while Kas is just north of a drain that serves most of the city. The Yellow Submarine study site is located in the middle of these two dive sites and should have less exposure to runoff that may contain nutrients or other pollutants (Fig. 2). To study the association of cyanobacteria on a small scale, pore water samples were taken ~200 m north of Yellow Submarine at a cyanobacteria mat on the reef crest.

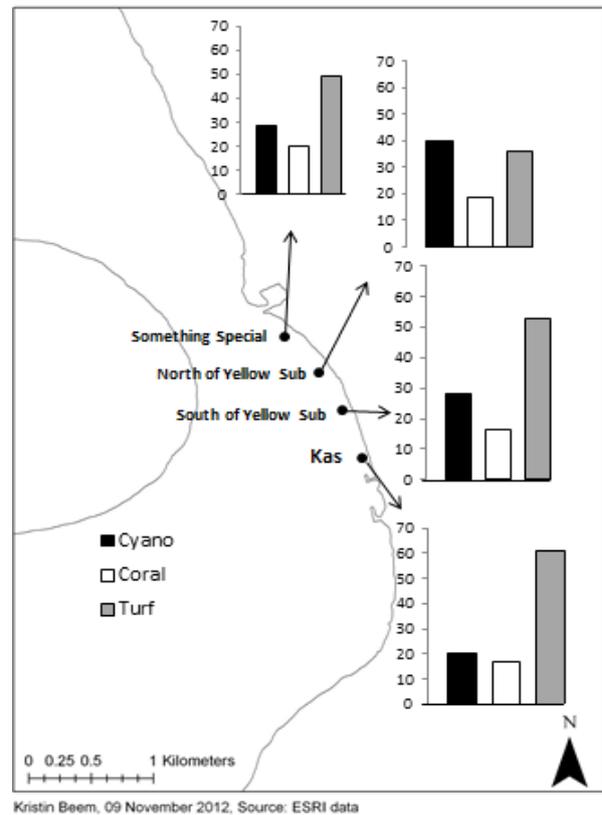


Fig. 2 Location of medium scale study area along with percent cover of cyanobacteria, turf algae, and coral cover are shown above and indicated by an open box in Fig 1

Large scale patterns of cyanobacteria

For the long term research, video transects (100 m) were used to determine the percent cover of cyanobacteria at nine sites along the west coast of Bonaire from White Slave, in the south, to Witches Hut, in the north. Permanent transects were marked at 12 m depth with rebar to facilitate long term monitoring of the sites. A 50 cm wand was attached to the underwater housing to ensure a set distance from the reef and correct positioning of the digital video camera. The videographer moved at a pace of about 3 min m⁻¹ during filming. To estimate percent cover of cyanobacteria, non-overlapping frames of the 100 m transect were analyzed, using 15 random points for each frame, using Coral Point Count software (CPC, version 4.0).

Medium scale patterns of cyanobacteria

Four transects of 30 m in length were surveyed using video to determine the percent cover of cyanobacteria at the medium scale study sites (Fig. 2). Each transect was filmed using an underwater digital video camera at the depth of 12 m and field methods were the same as in the large scale study as described above. CPC software was also used to analyze the medium scale benthic cover. Fifty random frames were chosen using Microsoft Excel. By generating 75 random numbers, then reducing the number to 50 after removing duplicates and numbers that were within 2 sec of each other, 15 random points were overlaid. The categories used in the program to determine benthic cover were coral, turf, unknown sponge, cyanobacteria, and old dead coral. Old dead coral was only used when corallites were visible in the frame; otherwise it was assumed that turf algae were covering the substrate.

Association between groundwater contamination and cyanobacteria

For testing the presence of groundwater, samples of pore water were collected ~200 m north of Yellow Submarine dive site near the reef crest from a cyanobacteria mat located near cement mooring blocks. The cyanobacteria mat ranged from 4.5 m to 7.5 m in depth and the benthic mat was measured in each dive to record changes in mat size. Samples were taken across a span of two weeks using SCUBA to collect pore water. Each sample was taken 1 m inside from the edge of the cyanobacteria mat and the paired sample was taken 5 m away from the edge of the mat (a control). The water was collected 8 cm below the surface of the sand, in a sterilized 60 ml syringe using 250 μ m mesh secured over the tip with a rubber band to avoid collecting debris in the sample. In total, nine sets of samples were taken.

Laboratory

To determine the content of bacteria in the water, the Colilert system was used to estimate the mean probable number (MPN) of TC and *E. coli* tests (IDEXX technology) using IDEXX protocol (www.idexx.com).

Data analysis

For the large scale study, percent cover of benthic cyanobacteria were averaged from Spring 2009 to Fall 2011 and overlaid on a map of Bonaire (Fig. 1). Patterns were then discussed in relation to the coastal populations. For the medium scale study, graphs were overlaid on a map of the study area to compare benthic cover among two sites that are impacted by runoff and two sites that were further from the source of runoff to visually compare the sites.

To determine the association of cyanobacteria mats and ground water from the pore water samples, the amount of squares that changed color on the IDEXX trays were entered into the IDEXX MPN Generator 3.2 to generate a MPN of TC. The same was done to generate an *E. coli* MPN after examining each tray under a black light. Paired t-tests ($\alpha=0.05$) were then used to compare the amount of TC and *E. coli* in pore water inside and outside of cyanobacteria mats.

Results

From the large scale study, the percent cover of cyanobacteria ranged from 6.5-17.2% (Fig. 1). The lowest cyanobacteria benthic cover was at the Cliff study site (6.5%) and the peak was at the Kas study site (17.2%). The study sites in the north, Kas to Witches Hut, were slightly higher with an average of 11.7%, than the study sites in the south, Chachacha to White Slave, with an average of 8.5%.

From the medium scale study, the percent cover of cyanobacteria were higher at the two transects in the middle than the two transects on the north and south of Fig.

2. North of Yellow Submarine had the highest counts of cyanobacteria (40.13%) and Kas had the lowest (19.95%). The percent cover of algae was high in all four transects. Turf algae were found to have the lowest percent cover north of Yellow Submarine (36.11%) and the highest at Kas (60.64%). Percent cover of coral remained around 17.9% for all four study sites (Fig. 2). The mean MPN of *E. coli* ranged from 0.0 to 613.1 cfu 100 ml⁻¹ with a higher mean inside the cyanobacteria mat (226.3 ± 307.3) than outside the mat (114.7 ± 177.0) (Fig. 3).

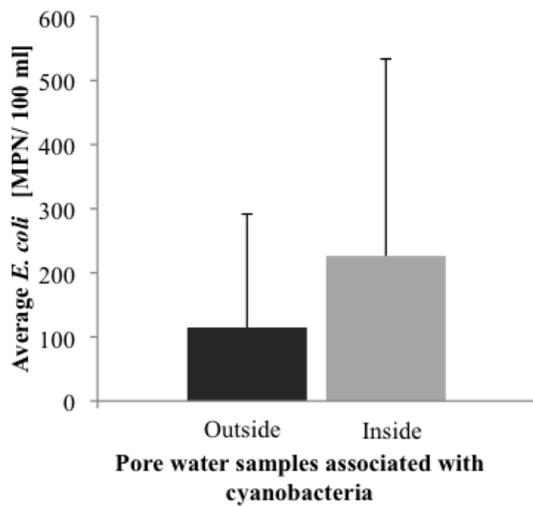


Fig. 3 Mean most probable number (MPN) of *Escherichia coli* present in pore water samples inside (grey) and outside (black) a cyanobacteria mat (± SD) collected at a mooring block ~200 m north of Yellow Submarine study site near Kralendijk, Bonaire. The depth ranged from 4.5 m to 7.5 m and samples were collected at the same location over the course of four weeks

The TC MPN ranged from 0 to 2419.6 with a lower mean of 1063.1 ± 917.5 inside the cyanobacteria mat and a higher mean of 1142.5 ± 919.2 outside the cyanobacteria mat (Fig. 4). A paired t-test showed that there was no significant difference between the mean CT MPN and the mean *E. coli* MPN for the pore water inside the mats and outside the mats (p>0.05). While processing samples, a blank was run and tested negative, showing that the shared lab equipment was not a source of contamination.

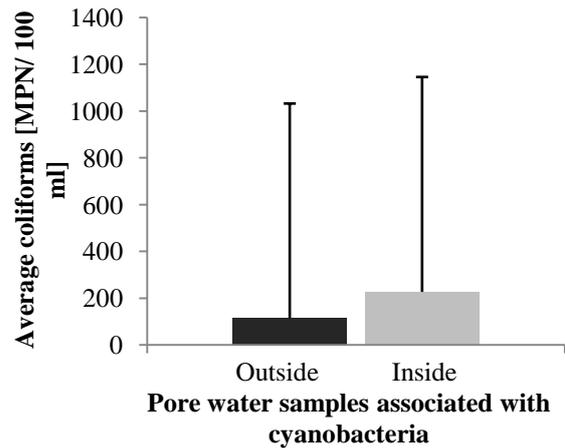


Fig. 4 Mean most probable number (MPN) of coliforms present in pore water samples inside (grey) and outside (black) a cyanobacteria mat (± SD) collected at a mooring block ~200 m north of Yellow Submarine study site near Kralendijk, Bonaire. The depth ranged from 4.5 m to 7.5 m and samples were collected at the same location over the course of four weeks

Discussion

From the large scale study, the hypothesis that benthic cyanobacteria levels would be higher near Kralendijk than areas further north and south was not supported. Even though there is a population center and more commercial activity in the capital city, there was no pattern in percent cover of cyanobacteria to suggest that there is a positive correlation between higher populations and higher levels of benthic cyanobacteria. The highest mean percent cover was at the Kas study site, which is slightly north or the drainage ditch that drains from northern Kralendijk. ChaChaCha and 18 palms study sites are also near large resorts and nutrient outputs, but have the lowest levels of cyanobacteria. A probable explanation for this distribution could be that the levels of cyanobacteria varied greatly between each year and season and taking the means might not give an accurate representation of what is really happening with the benthic cover of cyanobacteria. The hydrology of the island could also have an influence of the higher

cyanobacteria percentages in the north. Since the current usually flows from the south, the nutrients might be eddying off the northern coast keeping the water eutrophic and creating an ideal habitat for cyanobacteria. Another hypothesis could be that cyanobacteria might not be responding to large scale patterns but to smaller scale influences.

For the medium scale distribution, my hypothesis that there would be higher levels of cyanobacteria at the Something Special and Kas than at Yellow Submarine was shown to be incorrect. The pattern observed was completely opposite of the proposed hypothesis. High percent cover of turf algae were found at the sites of nutrient outflow, with very low levels of benthic cyanobacteria. Several other hypotheses could explain this observed phenomenon: 1) the time frame during which this study was done there was very little rainfall and there was not much input of nutrients from Kas and Something Special 2) the turf may respond to these runoff sources while cyanobacteria may be responding to another source, such as groundwater, or 3) there might be multiple factors that all contribute to this pattern. More research needs to be done to find the exact cause of this pattern.

For the small scale study of cyanobacteria patterns, the association between groundwater contamination and cyanobacteria was investigated to test the third hypothesis that higher mean levels of TC and *E. coli* would occur underneath the cyanobacteria mats than similar areas outside the mats, which was not supported by this study. It is not to say that the benthic cyanobacteria mats are not caused by groundwater seepage, but new methods need to be developed to collect the pore water.

Further research could be conducted at other sites along the leeward coast of Bonaire to examine the large scale pattern of cyanobacteria in relationship to the flow of nutrients. It would also benefit future studies to examine the currents on the east coast of Bonaire to see if the water is able to flow out to larger ocean currents. For proceeding

medium scale research, studies should analyze all possible nutrient input into the marine ecosystem. To determine the correlation between groundwater and cyanobacteria benthic mats, a different field method may be needed for the collection of the pore water. This study topic is important for the future of the coral reefs on Bonaire, since determining the location of where the groundwater is entering the marine ecosystem would aid in stricter regulations on waste management.

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Parrotfish abundance and corallivory at the Yellow Sub dive site in Bonaire, Dutch Caribbean

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Abstract

Parrotfish are some of the most widely recognized reef fish in the world. They occupy almost every tropical reef on the planet, and through their eating behavior, dramatically transform ecosystems. Parrotfish mainly consume macroalgae, but have been known to consume coral and therefore have a potentially negative impact on coral fitness. Although parrotfish corallivory is a well-known behavior, little is known about why it occurs, and how severe the effects are on marine ecosystems. The purpose of this experiment was to quantify the amount of coral colonies that parrotfish feed on at the Yellow Sub dive site in Bonaire Dutch Caribbean. By collecting data on the extent of parrotfish corallivory, there will be a larger body of knowledge from which questions about parrotfish grazing can be answered. To carry out this experiment, 30 meter transect tapes placed at depths of nine and 15 meters were used to first catalogue all of the coral by species, size, and if it had been bitten by parrotfish. Next three transect swims were used to categorize all parrotfish by size, species, and any special behavior (*i.e.* coral consumption). Results showed a higher abundance of parrotfish in transects at nine meters than at 15 meters. Parrotfish preferred the coral species *Montastraea annularis*, but there was no relationship between the presence of parrotfish and the percentage of coral bitten.

Introduction

Reef growth is accomplished mainly by the carbonate production of Scleractinians (*i.e.* stony corals) and coralline algae, while reef destruction is driven by physical or biological erosion (Ong and Holland 2010). Physical erosion, (*e.g.* coral fracture) can be caused by turbulent waters, while biological erosion is due to chemical dissolution or abrasion cause by corallivorous organisms such as parrotfish (Ong and Holland 2010). Both types of erosion are harmful to the reef in different ways; physical erosion typically occurs sporadically, and biological, continuously. While bioerosion can facilitate the transfer of nutrients through reefs, if not properly monitored can lead to a reduction in coral fitness and an overall decline in coral biodiversity (Ong and Holland 2010).

Although parrotfish play an important role in reef ecosystems by consuming algae that would otherwise outcompete many scleractinians, they also consume corals. Studies have shown that *M. annularis* has

the highest amounts of parrotfish biting. (Roff et al. 2011).

Coral consumption has even shaped the morphology of parrotfish (Price et al. 2010). This uncommon behavior negatively impacts coral fitness, but the extent of the coral consumption is unclear (Bonaldo and Bellwood 2009). Caribbean coral reefs have seen an increase in corallivory, and subsequently, a shift in coral populations (Roff et al. 2011). These changes have led to research pertaining to coral demographics and selective corallivory among different species of fish. Parrotfish corallivory is a well-documented behavior, but little research has been done to answer why this fish, that predominantly eats macroalgae, eats coral as well (Jayewardene 2009). Rotjan and Lewis (2009) showed that parrotfish selectively graze on coral polyps that have high reproductive effort such as gamete and egg size, and although they didn't obtain conclusive results, they offered a viable hypothesis.

Bonaldo et al. (2006) showed that there is a relationship between the species of parrotfish and its preference for macroalgae. By looking at the species of parrotfish within a belt transect and comparing those values to the species and amount of coral bitten, a preference for coral species can be correlated. The objective of this research was to answer specific questions about parrotfish corallivory. Do parrotfish prefer a species of coral? Do parrotfish prefer deeper or shallower depths? And how are parrotfish density and abundance of bite marks correlated? The hypotheses for these questions were as follows:

- H₁: Parrotfish prefer to consume *Montastraea annularis* more than other coral species.
- H₂: There is a higher abundance of parrotfish at shallower depths. Preliminary research indicated that algal abundance is greater at shallower depths, and therefore closer to the coral that parrotfish feed on.
- H₃: The abundance of parrotfish and abundance of biting are correlated. With a larger amount of parrotfish, there may be more competition for food, which may lead to higher rates of corallivory.

Materials and Methods

Data was collected over five weeks at the Yellow Sub dive site (12°09'36.47"N 68°16'55.16"W) in Bonaire (Dutch Caribbean), during the months of October and November of 2012. This location was chosen for research due to its close proximity to the CIEE research station and convenience of use. Data collection was carried out with one transect tape, two T-bars, a stopwatch, and data tables. A transect tape of 30 meters was laid at five locations along the Yellow Sub dive site at depths of 30 feet (9.14 meters) and 50 feet (15.24 meters). The five locations, each with data

collected at the two depths, making 10 transects in total, were determined by distance measured between mooring blocks. These blocks are identified as black dots shown in Fig. 1.

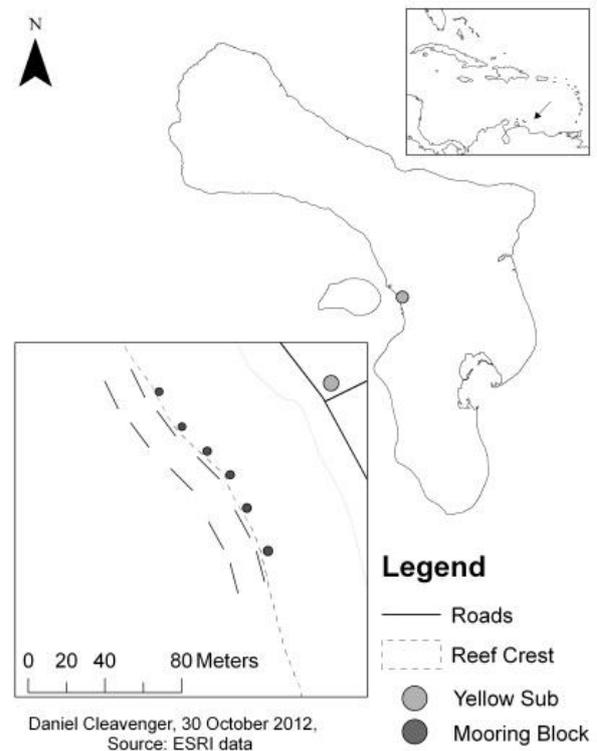


Fig. 1 Map of Bonaire, Dutch Caribbean (12°09'36.47"N 68°16'55.16"W). Small lines represent 30 m transects, and the black dots near them represent mooring blocks

The southern-most transects were placed haphazardly below the sixth mooring buoy south of the Yellow Sub dock. Every other pair of transects were placed three mooring blocks north of the last: the first was six blocks south, the second was three blocks south, the third was directly in front of the Yellow Sub dock, the fourth was three blocks north, and the fifth was six blocks north (Fig. 1). The placement of transects allowed for an even covering of the entire dive site. At each depth in the five locations, 30 meters of transect tape were laid out. For the first pass, T-bars were used to ensure that all corals within one meter of the tape were categorized by surface area in one of three sizes (<10 cm by 10 cm, between 10 cm by 10 cm and 40 cm by 40 cm, and >40 cm by

40 cm). The species of coral chosen were the most commonly seen on the reef: *Agaricia* sp. *Montastraea annularis*, *Porites astreoides*, *Montastraea annularis*, *Eusmilia* sp. *Meandrina* sp. *Millepora* sp. *Siderastrea* sp. *Diploria labyrinthiformis*, *Porites porites*, *Diploria strigosa*, *Colpophyllia* sp. and *Mycetophyllia* sp. Coral isolates that were farther than 30 cm apart were counted as two different colonies. Once completed, and all corals within the 2 by 30 meter belt had been categorized by size, the next three passes were for counting parrotfish. Each of these passes was separated by a three-minute wait in order to minimize the chance of disturbing the parrotfish and subsequently recording fewer fish per pass. The species of parrotfish recorded were *Scarus vetula*, *Sparisoma viride*, *Scarus taeniopterus*, *Scarus iserti* and *Sparisoma aurofrenatum*. All fish were recorded by size in one of three categories (<10 cm, between 10 cm and 30 cm, and >30 cm).

To analyze data, bar graphs were used to show the difference in percent coral abundance, and total coral abundance per depth. When determining if the abundance of parrotfish was positively correlated with biting, a bar graph for each depth displays the number of parrotfish graphed with the number of bites. To determine parrotfish selectivity for each coral species, Ivlev's Electivity Index was calculated (Rotjan and Lewis 2006):

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

Where r_i is the proportion of bitten corals of i th coral species, and n_i is the proportional abundance of the i th coral species. Total unbitten and bitten coral counts were summed for each depth to yield electivities based on coral species and depth. Electivity varies from -1.0 to +1.0, with the negative values indicating avoidance, and positive values indicating preference. Coral species grazed in direct proportion to their abundance have electivities of zero.

In order to determine a relationship between parrotfish abundance and percentage of bitten coral, regression analysis was used. Ivlev's Electivity Index as well as abundance values for unbitten and bitten coral were taken into account to compare two species of coral that were both in higher abundance than most coral species, and bitten to a relatively high degree. Each plotted point indicated the total amount of parrotfish and the percentage of the coral species bitten on each transect.

Results

Parrotfish abundance

There were marked differences in parrotfish abundance at each depth. The total amount of all parrotfish observed at a depth of nine meters was 210. At 15 meters the total amount of parrotfish was 155. All but one species, *S. aurofrenatum*, were found in greater abundance in the shallower part of the reef (Fig. 2).

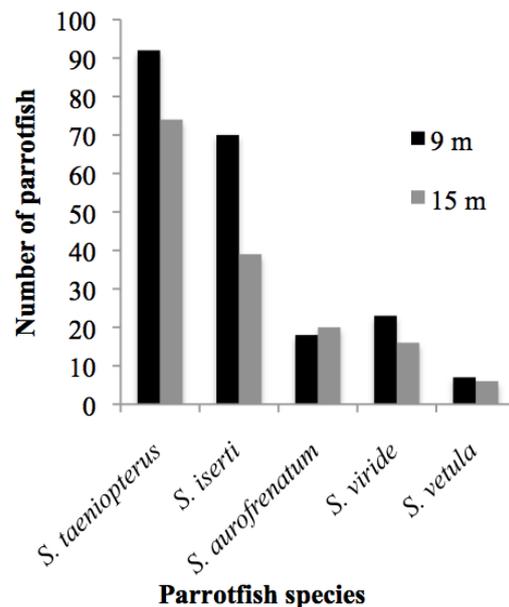


Fig. 2 Number of parrotfish sightings per species. Parrotfish were observed at 9 m (black) and 15 m (grey) at Yellow Sub study site in Bonaire, Dutch Caribbean (n=365)

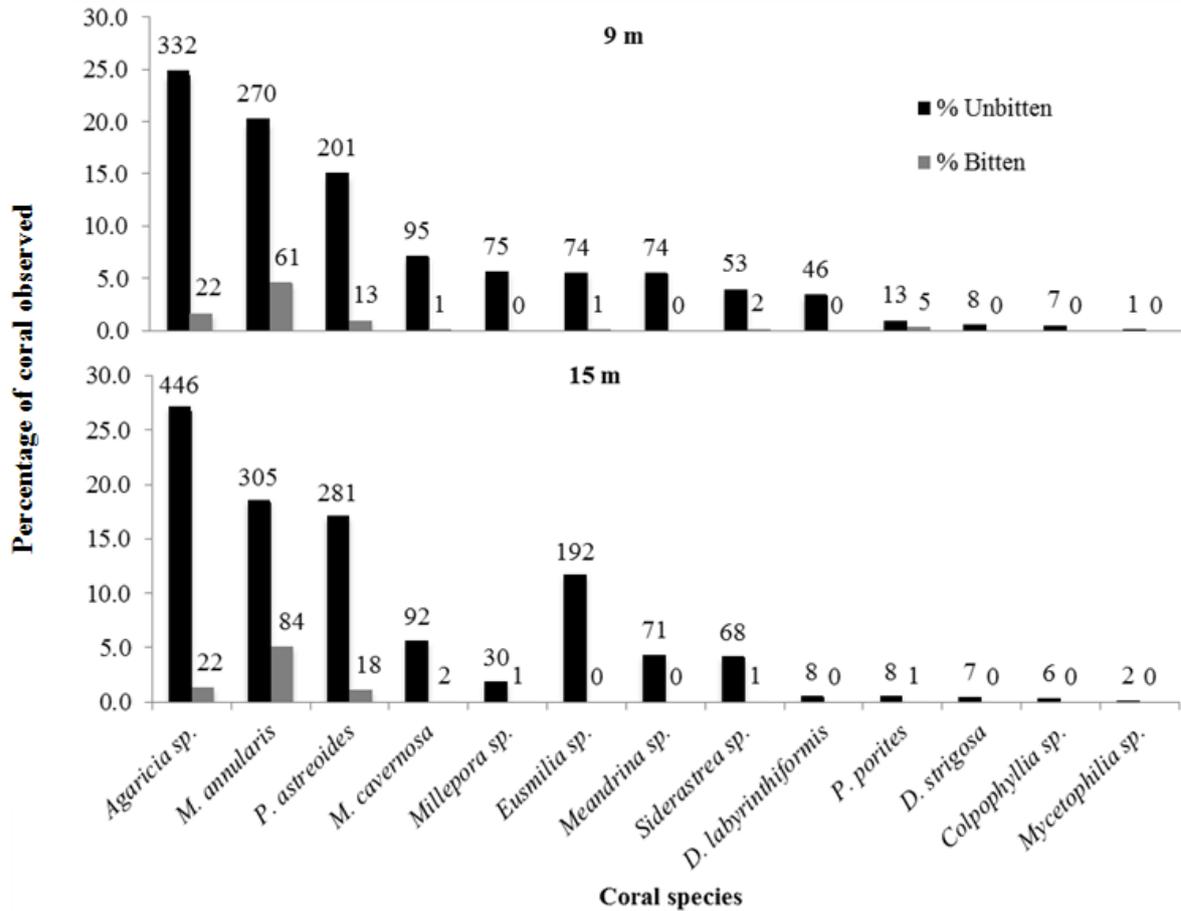


Fig. 3 Graphs represent percentage of unbidden (black) and bitten (grey) coral per species of all transects at 9 m (top) and 15 m (bottom). Values above columns represent absolute values of respective categories. Total amount of corals observed was 1354 at 9 m and 1545 at 15 m

The most common species at both depths was *S. taeniopterus* with abundances of 92 among transects at a depth of nine meters, and 74 at those of 15 meters. The least common species at both depths of nine and 15 meters was *S. vetula* with total amounts of seven and six respectively.

Coral biting and preference values

Percentages of unbidden and bitten corals were similar between depths. The proportion of unbidden coral to the total amount of coral observed was 92.2% at nine meters and 92.16% at 15 meters. At both depths, the most abundant coral was *Agaricia sp.* with a total of 354 or 24.52% observed at a depth of nine meters, and 468 or 27.11% observed at a depth of 15 meters (Fig. 3). The least abundant coral in both cases was *Mycetophilia sp.* with only one colony observed among all transects at nine

meters, and two among all transects at 15 meters. *M. annularis* had the highest percentage of bitten colonies at both depths, 4.51% among transects at nine meters and 5.11% for those at 15 meters. *M. annularis* also comprised a considerable amount of the coral at both depths, with 24.45% at nine meters and 23.65% at 15 meters. Ivlev's Electivity index indicated with negative values that all corals were avoided, with the strongest avoidance as a value of negative one (corals that were never observed as being bitten). These species were *Colpophyllia sp.*, *D. labyrinthiformis*, *Diploria sp.*, *Eusmilia sp.*, *Meandrina sp.* and *Mycetophilia sp.* The coral that was avoided least was *M. annularis* with similar electivity index value for both depths of nine and 15 meters of -0.59 and -0.58 respectively (Table 1).

Table 1 Ivlev's Electivity Index values listed with the corresponding species of coral

| 9 m | | 15 m | |
|----------------------------|--------------------------|----------------------------|--------------------------|
| Coral Species | Ivlev's Electivity Index | Coral Species | Ivlev's Electivity Index |
| <i>Agaricia sp.</i> | -0.86 | <i>Agaricia sp.</i> | -0.93 |
| <i>Colpophyllia sp.</i> | -1.00 | <i>Colpophyllia sp.</i> | -1.00 |
| <i>D. labyrinthiformis</i> | -1.00 | <i>D. labyrinthiformis</i> | -1.00 |
| <i>D. strigosa</i> | -1.00 | <i>D. strigosa</i> | -1.00 |
| <i>Eusmilia sp.</i> | -0.96 | <i>Eusmilia sp.</i> | -1.00 |
| <i>M. annularis</i> | -0.59 | <i>M. annularis</i> | -0.58 |
| <i>M. cavernosa</i> | -0.98 | <i>M. cavernosa</i> | -0.97 |
| <i>Meandrina sp.</i> | -1.00 | <i>Meandrina sp.</i> | -1.00 |
| <i>Millepora sp.</i> | -1.00 | <i>Millepora sp.</i> | -0.99 |
| <i>Mycetophilia sp.</i> | -1.00 | <i>Mycetophilia sp.</i> | -1.00 |
| <i>P. astreoides</i> | -0.86 | <i>P. astreoides</i> | -0.86 |
| <i>P. porites</i> | -0.59 | <i>P. porites</i> | -0.83 |
| <i>Siderastrea sp.</i> | -0.99 | <i>Siderastrea sp.</i> | -0.91 |

Regression Analysis

Percentage of bitten *M. annularis* and *P. astreoides* was graphed against abundance of parrotfish. Ten points per coral were used to represent the percentage of bitten coral among all transects regardless of depth, for both of the chosen species. Results show little statistical significance with an R^2 value of 0.011 for *M. annularis*, and 0.072 for *P. astreoides* (Fig. 4).

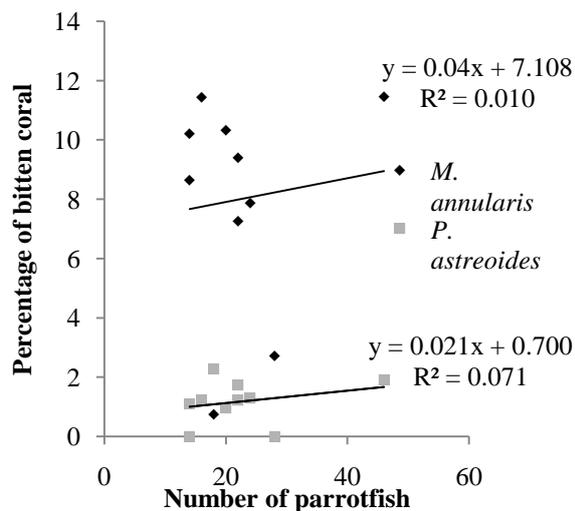


Fig. 4 Percentage of bitten *Montastraea annularis* and *Porites astreoides* compared to number of parrotfish sighted. Regression lines are shown

Discussion

The results of this study demonstrate some important things about the coral reef at

the Yellow Sub dive site. The most bitten coral in absolute value and in terms of percentage of total counts for a single species was *M. annularis*. This finding is congruent with those from research done by Rotjan and Lewis (2006) and validates the first hypothesis presented here. Although *Agaricia sp.* was the most common coral on the reef, perhaps due to a shift from brooding corals to spawning corals, it was still bitten far less than *M. annularis*, with only 1.62% bitten on transects nine meters deep, and 1.34% bitten on transects at a depth of 15 meters. This indicates that with some species of coral, abundance of a colony has little to do with the grazing habits of parrotfish on the reef. These grazing habits were further explored using the Ivlev's Electivity Index, which accounts for the ratio of abundance to proportion bitten. A high avoidance (-0.93) was calculated for *Agaricia sp.* The converse may be true for other species of coral though. *P. astreoides* was found to have 0.96% biting out of all corals in transects at nine meters, and 1.09% out of all transects at 15 meters. However, *P. astreoides* only accounted for 15.81% of all corals at shallow transects, and 18.18% at the deeper transects, compared to *Agaricia sp.* average of 27.30% among shallow and deep and *M. annularis*' average of 24.05%. Also it was observed that among certain transects, parrotfish preferred biting the smallest category (10 cm by 10 cm) of *P.*

astreoides. This was uncharacteristic, as the medium and large size coral colonies were bitten more frequently. More data should be collected to verify the size of coral colony preferred by parrotfish. If smaller coral colonies were bitten most, this could suggest that the percentage of biting would increase faster than percentage of coral cover. This also could have occurred as a result of more preferred colonies being absent in those areas.

Parrotfish sightings were highest at the shallower transects for all species but *S. aurofrenatum*. 18 were observed at transects that were nine meters deep and 20 were observed among the 15 meter deep transects. Parrotfish that were unusually active (ie. moving faster or biting more) were recounted if they reentered transects after approximately five meters. Perhaps this could account for why *S. aurofrenatum* was the only species that was more abundant at deeper depths. An anomaly such as increased competition among other parrotfish or a larger amount of territorial damselfish may have caused them to move faster and thus be counted more than once. The higher abundances of parrotfish at the shallower transects is most likely due to the algal densities at those depths (Bonaldo et al. 2006). Algae is more common at a depth of nine meters than 15 meters and the abundance levels suggest that parrotfish seen in the nine meter deep transects may be moving from one shallow foraging location to another (Jayewardene 2009).

Regression analysis was used to see if there was a relationship between the amounts of parrotfish sighted, and the percentage of bitten coral observed. To test this, parrotfish in the smallest size category (<10 cm) were excluded because no parrotfish observed of that size had a mouth large enough to leave a bite mark that was counted as corallivory. Next, the sum of all other sizes of parrotfish, regardless of species, was obtained for each transect. These values were plotted with the corresponding percentage of coral bitten. The two corals examined were *M. annularis*,

and *P. astreoides*. These species were chosen because of their electivity index values of -0.59 averaged among both depths for *M. annularis*, and -0.86 for *P. astreoides* (Table 1). An R^2 value of 0.01 for *M. annularis*, and 0.07 for *P. astreoides* (Fig. 4) suggests that there is no relationship between the presence of parrotfish and percentage of biting observed among all transects. This is in agreement with the highly mobile behavior of parrotfish because they were observed passing through and foraging on algae every few meters. These results also support the exclusion of the smallest size category of parrotfish, as they were usually found in schools and relatively immobile. Their addition to the regression analysis would have further decreased the relationship between biting and parrotfish abundance, but would have been unsupportive of idea that it was because of highly mobile activity.

Both the hypotheses: parrotfish will be more abundant at shallower depths, and that parrotfish will prefer feeding upon *M. annularis* more than other coral species, were not rejected. The last hypothesis regarding a positive relationship between parrotfish presence and biting amounts was rejected. This research has reinforced current studies on parrotfish behavior and personal observations made while collecting data. A highly mobile foraging behavior was represented in the data analysis as well as parrotfish abundances and coral biting preference. While more research must be conducted to examine why parrotfish intentionally graze on coral, this research focused on the extent of parrotfish corallivory on the reefs at the Yellow Sub dive site. In order to understand reasons for corallivory, the more practical question must be asked; how harmful is this behavior? In order to quantify the effects of corallivory on marine ecosystems, the scope of this initially destructive behavior must be measured. This research at the Yellow Sub dive site can serve as a springboard for understanding the severity of corallivory and its effects on the reef. Results presented here will allow

researchers to better allocate resources when collecting data on parrotfish populations or corallivorous behavior. Understanding this phenomenon may eventually lead to new methods of reef conservation and protection programs. It could also shed light on general characteristics of primary consumers and lead to a better knowledge base from which we understand marine trophic levels.

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Coastal marine water quality based on the presence of Polychaetes, coliforms, *Escherichia coli*, and enterococci in Kralendijk, Bonaire, Dutch Caribbean

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Abstract

A lack of sufficient wastewater treatment practices on the island of Bonaire, Dutch Caribbean suggests that contaminated groundwater seepage or runoff could be impacting the health of the coastal habitats and fringing reefs that surround the island. Bonaire does not monitor the health of its coastal waters, although effects of pollution have been observed. This study aims to learn more about the coastal water quality at three stations along the coast of the city of Kralendijk, Bonaire. Both indicator bacteria (coliform bacteria, *Escherichia coli*, and enterococci) and polychaete assemblages were monitored. IDEXX Colilert™ and Enterolert™ test kits were used to monitor bacteria levels in samples taken over a five-week study period. Polychaetes were identified to the family level from soft sediment samples. Polychaete family abundance, richness, and diversity were compared between stations. Grain size of the soft sediments at each station was measured. Polychaete assemblages varied between stations, but not enough data was gathered to eliminate the possibility of unexpectedly large grain size differences in explaining this variability. Typical water samples from each station showed narrow variability within U.S. Environmental Protection Agency standards for indicator bacteria levels. Two outliers occurred. The Marina station experienced a wider variance in all bacteriological indicator levels than the other two stations, and all surface stations experienced a significant spike in enterococci levels after a heavy rainstorm. If elevated enterococci levels consistently occur during heavy rainstorms, this could present a

Introduction

Human pressures on coastal environments have been well-documented around the world throughout the history of marine biology (Pearson and Rosenberg 1978; Del-Pilar-Ruso et al. 2010). Anthropogenic pollution such as runoff containing excess nutrients, oil, sediments, and discharge from seawater desalination plants can disturb reef ecosystems and marine flora and fauna (Pearson and Rosenberg 1978; Bak et al. 2005; Kline et al. 2006; Del-Pilar-Ruso et al. 2009). Monitoring the status of these pollution levels is an important step in preventing severe environmental degradation.

The island of Bonaire in the Dutch Caribbean does not currently monitor coastal marine pollution levels, despite having a

potential for creating anthropogenic pollutants (Peachey 2009). Bonaire has a population of ~16,000 people with a large tourism industry bringing visitors to the island's many resorts each year. Currently, Bonaire lacks a permanent wastewater treatment plant or a sufficient landfill (Peachey 2009). Current sewage management practices include the use of septic tanks and the dumping of untreated sewage into unlined ditches and cesspits. These practices have reduced the quality of the island's groundwater (van Sambeek et al. 2000). It has been speculated by researchers and environmental groups that contaminated groundwater from open waste pits could be seeping into the marine waters (Peachey 2009). However, no conclusive studies have been conducted to determine fecal

contamination in coastal waters, nor have other marine pollutants been monitored.

The indicator organisms coliform bacteria, *Escherichia coli* (*E. coli*), and enterococci are used worldwide to monitor both freshwater and saltwater for the presence of fecal contamination (Anderson et al. 2005). These bacteria live in the gastrointestinal tracts of humans and other warm-blooded animals, and indicate the presence of disease-causing bacteria. Both *E. coli* and enterococci are widely used by the U.S. Environmental Protection Agency (EPA) as indicator organisms for the presence of fecal contamination (U.S. Environmental Protection Agency 2000). Specifically, enterococci are recommended to be used as indicator bacteria to predict incidence of gastrointestinal illness in swimmers in marine water (Wade et al. 2003).

Coastal marine water quality can be more broadly monitored by observing benthic invertebrates, which respond rapidly to environmental stresses including anthropogenic pollution (Pearson and Rosenberg 1978; Elías et al. 2005; Del-Pilar-Ruso et al. 2009). Polychaete assemblages are particularly valuable for monitoring water quality worldwide (Pocklington and Wells 1992). Polychaetes live in soft marine sediments and assemblages are highly sensitive to changes in their environment. Different polychaete species are found in environments ranging from pristine to highly polluted. Therefore, the abundance or absence of certain polychaetes in marine sediments gives an indication of the quality of the marine environment.

This study provides information about the indicators of pollution in the coastal waters of Bonaire's capital city of Kralendijk (Fig. 1). Three sample stations were selected. Station 1 (Marina) is adjacent to the mouth of Kralendijk's marina, which houses boats, restaurants, and resorts. The high concentration of human activity at the marina suggests that the waters surrounding it might be subject to higher levels of anthropogenic pollution. Station 2 (Playa

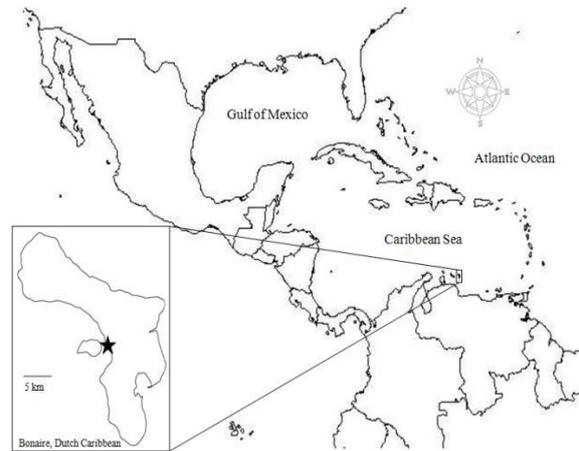


Fig. 1 The island of Bonaire, Dutch Caribbean. The site of data collection, near the capital of Kralendijk, is marked with a star. Geographic coordinates are 12°9'36.47" N, 68°16'55.16 (Alvez 2012)

Lechi) is 250 m south along the coast from the marina, across from Kaya Playa Lechi. There are few boats anchored, but little traffic. Station 3 (Yellow Sub) is 250 m south of the second site, and is adjacent to a residential area and the Yellow Submarine dive shop. Here there is noticeable dive and boat traffic, but less activity than Marina. These three sites were selected in order to explore the possibility of a pollution gradient between the Marina and the other stations. Two hypotheses were proposed.

- H₁: Bacteria levels are highest at the Marina, and spread from there to the less-trafficked locations.
- H₂: Polychaete indicator species reflect these differences in water quality between locations.

This study ultimately sought to gain more information about water pollution in this area of the Kralendijk coastal area through exploring the location, intensity, and temporal change in bioindicators: polychaete assemblages, coliform bacteria, *E. coli*, and enterococci.

Methods

Sampling

Samples were taken in three stations along the Western coastline of Kralendijk, Bonaire. The location of Kralendijk in relation to Bonaire is shown in Fig. 1. Three types of samples were collected in each of these stations at a depth of 5 m: soft sediment samples from the ocean floor, benthic water samples, and water samples from the ocean surface. First, a 100 ml sample of surface water was collected at the study site in a clean plastic bottle. Then, a 100 ml sample of benthic water from the same water column was collected next to the ocean floor using a clean plastic bottle. Finally, the soft sediment samples were collected using a 10cm-diameter PVC pipe and caps. The open-ended pipe was pushed 10 cm into the soft bottom sediments, then the top end was capped. The pipe was removed from the ground, creating suction. This prevented the collected sediments from falling out the bottom of the tube. The tube was put into a ziplock bag and uncapped, depositing the sediment in the bag. Water samples were taken weekly at each station for five weeks during September 2012 and October 2012. The sample number allowed small temporal trends in water quality to be analyzed, and prevented single-event rainfall or runoff from skewing the overall analysis of water quality. Since endobenthic populations are not highly variable over a five-week time period (Pocklington and Wells 1992), two sediment samples were taken from each station near the beginning of the study. One additional sediment sample was taken using the same method, and used for a grain size analysis.

Since different polychaetes have different preferred sediment sizes in which they live (Méndez and Ruiz 1998), average grain size data were used to determine whether or not grain size impacted the measured polychaete assemblages. Grain size served as another factor of comparison between stations.

Lab treatments

All samples were returned to the lab for analysis. The water samples were evaluated for *E. coli* and enterococci colony presence using IDEXX Colilert-18TM and EnterolertTM test kits. The Colilert-18TM test kit has been proven to be as effective as other methods for detecting and quantifying *E. coli* in marine water (Palmer et al. 1993). Colilert-18TM and EnterolertTM are approved by the U.S. Environmental Protection Agency for detection of *E. coli* and enterococci respectively in freshwater (U.S. Environmental Protection Agency 2007). The marine saltwater samples collected were diluted with sterile water to 100 mL of a 10% solution in order to be used with the freshwater sample kits. This 100 mL solution was used in the Colilert-18TM and EnterolertTM test kits to test for the presence of coliform bacteria, *E. coli*, and enterococci. Colony count results were multiplied by 10 to account for the dilution.

Soft sediment samples were analyzed in the lab for the presence of polychaetes. Sediment samples were sifted through a 0.5 mm mesh in order to separate the macrofauna from smaller grains and animals (Del-Pilar-Ruso et al. 2009). Samples were treated and preserved for 24 hours with a 10% formalin solution in seawater and containing Rose Bengal to dye organic matter. After 24 hours the samples were transferred to ethanol, when the organic matter was picked out by sight. The macrofauna were identified to the family taxon and counted.

One sediment sample from each station was used to analyze grain size. Sediments from each station were dried in a 41°C oven for two days and sifted through a U.S. Standard ASTM sieve with ASTM E-11 mesh numbers 10, 18, 35, 60, 120, and 230. This separated the sample into size terms according to Wentworth (1922): pebbles, very coarse sand, coarse sand, medium sand, fine sand, very fine sand, and silt. The sample portions of each size were massed

and average grain size at each station was calculated.

Data analysis

Polychaetes from each sample station were identified to the family level and counted. Presence of indicator polychaetes such as capitellids and others described by Pearson and Rosenberg (1978) suggest high levels of organic enrichment in the community (Dean 2008). The identification of the polychaetes allowed these indicators to be counted. The data were also analyzed for family abundance, richness, and diversity. Family diversity was also analyzed using the Shannon Index. The Shannon Index (H') takes into account the number of families and the number of individuals in each family in order to describe the diversity of the community. Comparing results from each of the described stations indicated whether a station had healthier polychaete assemblages than others (Pocklington and Wells 1992). Higher family richness, high family diversity, and a high abundance in less tolerant taxa are indicators of a healthier environment than lower family richness and family diversity (Del-Pilar-Ruso et al. 2010). Grain size data was compared with polychaete data to determine whether or not grain size differences accounted for differences in polychaete assemblages.

E. coli, coliform, and enterococci counts were also compared between stations by Mann-Whitney U-tests and to U.S. EPA standards for bacterial counts in recreational waters (U.S. Environmental Protection Agency 2000). For each water sample, the average and standard deviation for each bacteria count was calculated. The water sample data were also analyzed as a time series to explore temporal change in bacteria counts.

The results from the invertebrate samples and the benthic water samples were compared in a principle components analysis (PCA) at each station to analyze any correlation between invertebrate abundance, invertebrate richness, average grain size, and

water bacteria colony counts. Data were log transformed in PAST: Paleontological Statistics software (Hammer and Harper 2005) and then plotted with the Var-covar matrix to a PCA figure.

Results

Polychaetes

A total of 55 individual polychaetes were found and sorted into seven families. The most abundant family was Magelonidae (36.4%), followed by Capitellidae (9.1%), Sabellidae (7.3%), Errantia (3.6%), Nereidae (1.8%), Maldanidae (1.8%), and Terebellidae (1.8%) (Table 1). A total of

Table 1 Polychaete families and individual abundances found at each station

| Family | Marina | Playa Lechi | Yellow Sub | Total |
|--------------|--------|-------------|------------|-------|
| Magelonidae | 20 | 0 | 0 | 20 |
| Capitellidae | 4 | 1 | 0 | 5 |
| Maldanidae | 0 | 1 | 0 | 1 |
| Errantia | 0 | 2 | 0 | 2 |
| Sabellidae | 0 | 0 | 4 | 4 |
| Nereidae | 1 | 0 | 0 | 1 |
| Terebellidae | 1 | 0 | 0 | 1 |

38% of polychaetes found were unidentifiable due to missing body parts, and were not classified by family but still included in the total individual count for their respective sample stations.

Twice as many individuals were found at the Marina than at the other two stations (Fig. 2). The fewest individuals were found at Playa Lechi with 13% fewer than at Yellow Sub, and 52% fewer than at the Marina. The number of families found at each station followed a similar trend (Fig. 3).

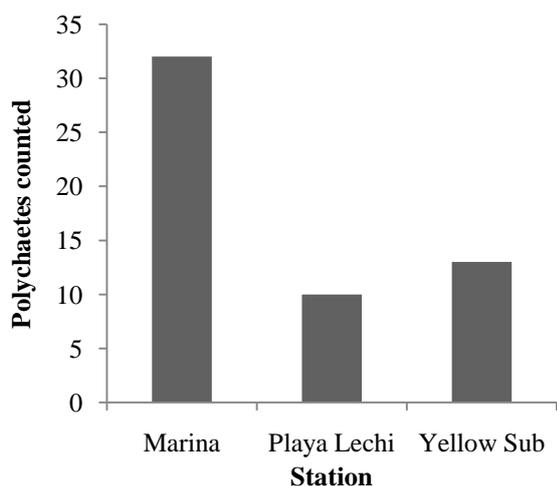


Fig. 2 Polychaete richness by sample station. Plotted are the numbers of polychaetes found, regardless of taxon, in sediments taken from the Marina, Playa Lechi, and Yellow Sub

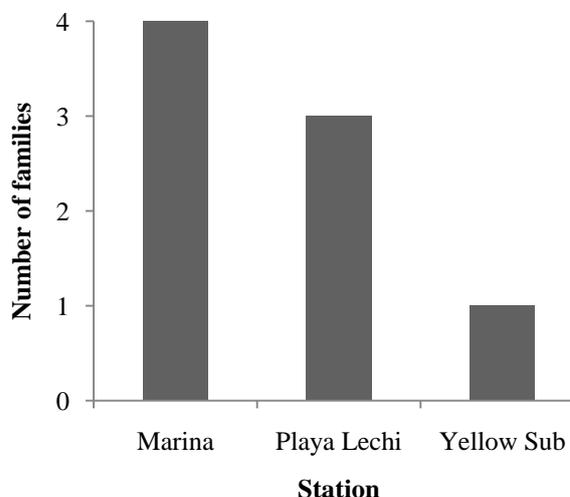


Fig. 3 Number of polychaete families identified from sediments taken at the Marina, Playa Lechi, and Yellow Sub stations

More polychaete families (four families) were identified at the Marina than at the other two stations (Table 1). Three families were identified at Playa Lechi, and one family, Sabellidae, was found at Yellow Sub. The only family found at multiple stations was Capitellidae, which was found at both the Marina and Playa Lechi.

The Shannon Diversity Index for families at each station was calculated to further describe these data. A higher H' indicates higher diversity. An H' of 0

indicates that all members of the community are equally distributed among the same families. The Playa Lechi station had the highest diversity index value ($H'=1.01$). Marina had a moderate diversity index value ($H'=0.74$), while Yellow sub had a diversity index value of $H'=0$. The zero diversity was due to all individuals being members of the family Sabellidae.

Indicator polychaete families were identified in order to learn more about the health of the benthic environment. Table 2 shows commonly-used indicator families and the abundances of individuals in those families found at each station. The only indicator families identified in this study were Capitellidae and Nereidae.

Table 2 Indicator polychaete families. Families are shown with what they typically indicate and the number of individuals found at each station. (Pockington and Wells 1992)

| Family | Typically Indicates | Number of Individuals Observed | | |
|---------------------|---------------------|--------------------------------|-------------|------------|
| | | Marina | Playa Lechi | Yellow Sub |
| <i>Capitellidae</i> | organic enrichment | 4 | 1 | 0 |
| <i>Spionidae</i> | organic enrichment | 0 | 0 | 0 |
| <i>Malacoceros</i> | organic enrichment | 0 | 0 | 0 |
| <i>Dorvillea</i> | pristine conditions | 0 | 0 | 0 |
| <i>Nereidae</i> | pristine conditions | 1 | 0 | 0 |
| <i>Cirriformia</i> | pristine conditions | 0 | 0 | 0 |

Grain Size

Polychaete assemblages are influenced by the texture of the sands in which they live. A grain size analysis was performed at each station in order to assess the role of different sediments in differences in polychaete assemblages (Fig. 4, Table 3).

Table 3 Average grain size from each location. Grain sizes are described according to Wentworth (1922)

| | Marina | Playa Lechi | Yellow Sub |
|--------------------------------|-------------|-------------|------------------|
| Average Grain Size (mm) | 0.179±0.027 | 0.326±0.029 | 0.536±0.077 |
| Grain Size Description | medium sand | coarse sand | very coarse sand |

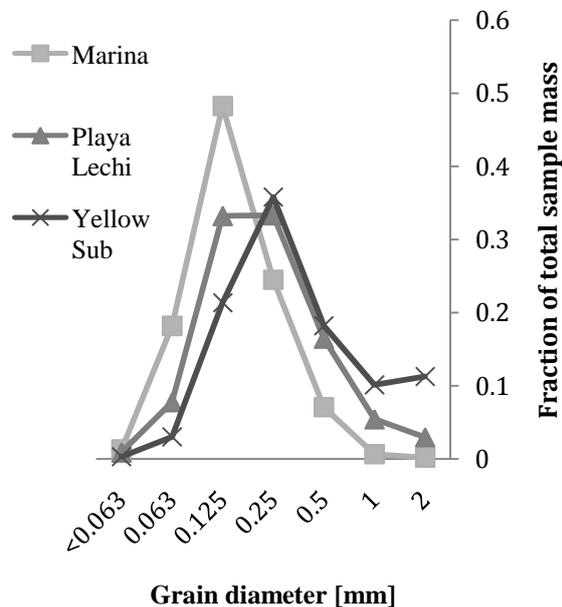


Fig. 4 Grain size distribution from each sample station. Light grey line with squares represents Marina. Darker grey line with triangles represents Playa Lechi. Darkest grey line with crosses represents Yellow Sub

It was observed that the sands at each sample site appeared very different. Sediments at the Marina were clearly finer and darker gray in color than sediments at the other two stations. Sediments at Playa Lechi and Yellow Sub were apparently

similar in color and texture, both having a tan color with a high number of small shells, but the sediment at Playa Lechi was also covered with coral rubble where there was minimal coral rubble at Yellow Sub.

Water samples

Bacteria levels in water samples were returned from the IDEXX kits as the most probable number (MPN) of bacteria colonies per 100 mL water. Each water sample was tested for coliform bacteria, *E. coli*, and enterococci. Mann-Whitney U-tests comparing the benthic and surface water levels taken at each collection showed that none of each station's surface bacteria levels were significantly different from the benthic bacteria levels ($n=5$, $p>0.05$). Thus, there was no differentiation between surface and benthic bacteria levels for future statistical tests.

Average coliform levels were calculated for each station (Fig. 5). Mann-Whitney U-tests were run on the numbers of coliforms per 100 mL at each station, showing that none of the three stations were significantly different from each other ($n=10$, $p>0.05$). However, values in coliform bacteria at the surface samples of the Marina station varied more than values at the other stations (Fig. 5). The standard deviation of the average of the Marina surface samples was 1233.5 MPN/100 mL, while all of the other standard deviations of the averages varied between 255.9 and 430.4 MPN/100 mL.

Similarly, the most probable number of *E. coli* colonies per 100 mL between stations was found using Mann-Whitney U-tests to be statistically insignificant ($n=10$, $p>0.05$). Fig. 6 shows the average MPNs per 100 mL and standard deviations. Standard deviations were found to be the highest at the Marina (52.8 MPN/100 mL on the surface and 40.6 MPN/100 mL at the bottom) than at the other stations (all between 16.1 and 22.8 MPN/100mL). All levels of *E. coli* were below the recommended EPA limit for *E. coli* colonies in recreational waters, 126 colonies/100mL.

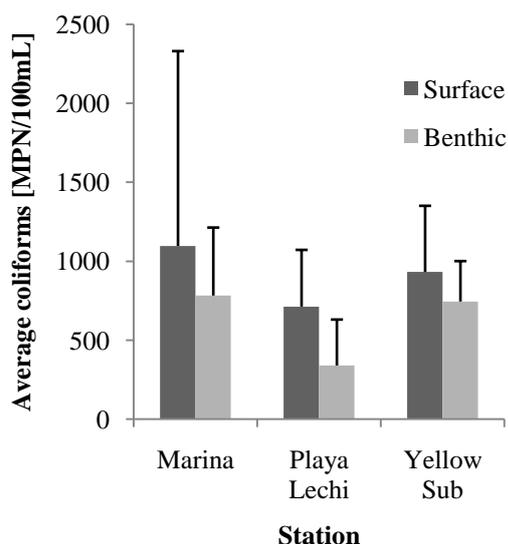


Fig. 5 Most probable number of coliform bacteria colonies for a 100 mL sample taken from the Marina, Playa Lechi, and Yellow Sub stations. Colony counts were averaged over six water samples. Error bars indicate standard deviation. Dark grey represents surface samples and light grey represents benthic samples

Enterococci levels were found to be below detection levels (<10 colonies/100 mL) in all samples except for samples taken on two different collection days (collections 3 and 4). No such trend was found in the *E. coli* or coliform levels from these collection days. Collection day 3 occurred during a large public festival which increased boat traffic. Significant enterococci levels were found in both the surface and benthic samples at Yellow Sub (31 and 30 colonies/100 mL, respectively), and in the benthic sample taken from the Marina (20 colonies/100 mL) (Fig. 7). The highest enterococci level was measured on the surface at Yellow Sub, and was lower than the recommended EPA limit for enterococci in bathing water, 33 colonies/100 mL by 6%.

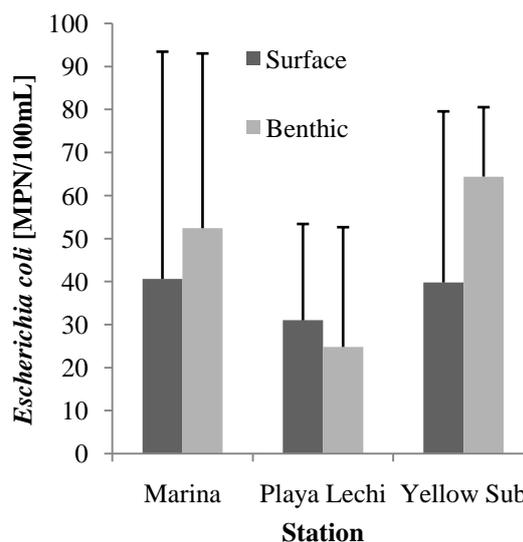


Fig. 6 Most probable number of *Escherichia coli* bacteria colonies for a 100 mL sample taken from Marina, Playa Lechi, and Yellow Sub stations. Colony counts were averaged over six water samples. Error bars indicate standard deviation. The U.S. EPA recommends that *E. coli* in bathing water not exceed 126 colonies per 100 mL. Dark grey represents surface samples and light grey represents benthic samples

On collection day 4, which took place directly after a large rainstorm, the enterococci levels measured at several stations were higher than its EPA limit (33 colonies/100 mL) (Fig. 7). The highest levels were measured at the surface Marina station (789 colonies/100 mL), followed by the benthic Marina station (317 colonies/100 mL), the surface at Playa Lechi (146 colonies/100 mL), and the surface at Yellow Sub (85 colonies/100mL).

Principal Component Analysis

A principle components analysis (PCA) was run using the data from the three bacteriological tests (Fig. 8). The first two components explained 83.9% of the variability.

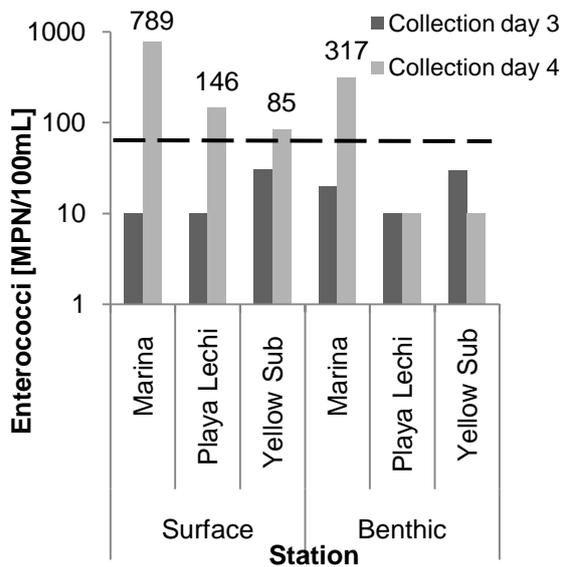


Fig. 7 Most probable number of enterococci colonies per 100mL measured from all stations on collection days 3 and 4. Enterococci levels on all other days were insignificant. The U.S. EPA recommends that enterococci levels in bathing water not exceed 33 colonies per 100 mL (dashed horizontal line). Dark grey represents collection day 3. Light grey represents collection day 4

The PCA echoes the previous figures in showing that although the three stations have relatively similar bacteria levels, the variability of the bacteria levels at Marina is much higher than the variability of the levels at Playa Lechi and Yellow Sub. The PCA was also run without including the anomalous collection day 4, with similar results (data not shown). Additional PCA analyses were performed including polychaete abundance and grain size, with results similar to the water-only PCA (data not shown).

Discussion

Polychaetes and grain size

Abundance of polychaete families ranged across stations. Theoretically, the station with the highest family abundance should be the most pristine station (Pocklington and Wells 1992). Assuming this, the Marina appears to have the healthiest assemblage, followed by Playa Lechi and Yellow Sub.

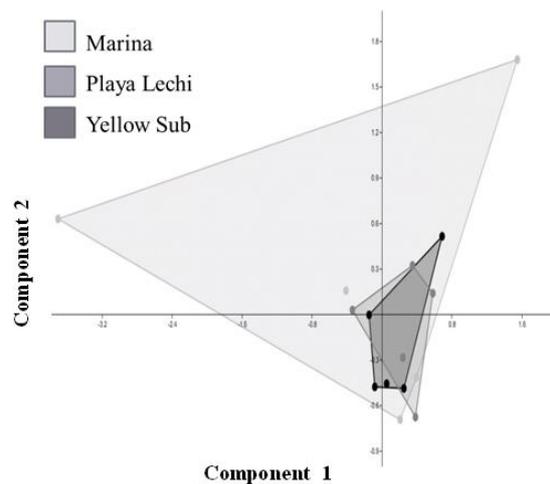


Fig. 8 Principle component analysis of bacteriological tests on water samples. The lightest grey represents all samples taken from the Marina. The middle grey represents the samples taken from Playa Lechi, and the darkest grey represents samples taken from Yellow Sub. Components 1 and 2 account for 83.9% of variability

Indicator species were narrowly found. The family Capitellidae, an indicator of organic pollutants, was found at both the Marina and Playa Lechi, suggesting that organic pollutants could cause large communities of these polychaetes. Only one individual of one indicator species of pristine conditions (Nereidae) was found at the Marina, which could indicate the presence of highly-polluted sediments at all stations. However, too few indicator species of disturbance were found to show significant differences between stations. Therefore, a lack of positive indicator species shouldn't be seen as a conclusive result.

The diversity between stations also differed, with Playa Lechi having the highest diversity of polychaetes, the Marina having an intermediate diversity, and Yellow Sub, where only one family was identified, having no diversity. This suggests that the Playa Lechi presents an environment most suitable for polychaetes, while Yellow Sub presents an environment suitable for a very limited assemblage of polychaetes.

These contradictory interpretations suggest that it is likely that the variance in polychaete assemblages between stations cannot be solely attributed to differences in pollution levels. Grain size differed greatly between stations, the average coarseness ranging from medium to very coarse. These differences are significant for polychaete assemblages (Nichols 1970) and may account for all of the variance between the stations. Further experiments would be needed to draw decisive conclusions about the variance of polychaete assemblages. These differences in average grain sizes could be caused by different sedimentation patterns and possibly linked to runoff patterns that also influence water quality.

Recommendations for further experimentation include increasing the quantity of endobenthic samplings and exploring explanations for differences in benthic sediment composition at these three spatially close sample stations.

Water samples

Findings from water sample data can be summarized into four points: (1) Bacteria levels in water samples between stations were not significantly different, (2) different stations, though they had similar levels of bacteria, had different variances in bacteria levels. Among these Yellow Sub and Playa Lechi stations varied much less than the Marina, (3) all bacteria levels were well below EPA standards except for anomalous events causing a spike in enterococci levels, and (4) a one-time spike in enterococci not seen in other bacteria caused extreme levels of enterococci at all surface stations and the Marina benthic station.

General water quality

Overall, this study showed that all stations generally have adequate water quality. Excluding enterococci samples on day 4, no station had average bacteria levels higher than U.S. EPA limits. Over the limited course of this study, normal variance

in water bacteria levels remained within safe limits. Future studies should include more samples to further determine “normal” levels and variance for these stations. No significant difference was found between bacteria levels at the different depths, nor was a significant difference found between bacteria levels at different stations. This rejects the hypothesis of pollution gradient between stations. This means that there is no evidence to suggest that pollution is moving from the Marina to the other, less-trafficked stations.

Variable bacteria levels

As illustrated by the PCA, although Yellow Sub and Playa Lechi had relatively similar normal ranges of bacteria levels, the Marina’s normal range of bacteria levels was much more variable. Although there is insignificant difference (seen in bacteria levels, grain size, and polychaetes) between stations, there is significant difference in variability of bacteriological levels, even excluding high enterococci levels on day 4. This suggests that the Marina environment is less stable bacteriologically, and more subject to swings in water quality due to one-time events. This may be due to the topography surrounding the Marina that is not present at the other locations. There is a large salina and waterway behind the Marina, fed by its own watershed. No such bodies of water are located on land near either of the other two stations. Runoff from rains at the Marina would include water from this entire watershed, whereas runoff from the other two locations would come from smaller sources. More research and experimentation could further develop this hypothesis.

High enterococci levels

One-time events can cause dangerous levels of enterococci in the water at several stations. Although only one such event occurred over the 5-week study period, it is unknown how often these “one-time” events

could be occurring over longer time periods. If several events causing high enterococci levels occurred over the course of a year, this would be cause for concern. It is imperative that further studies be done to determine how often these events occur to determine their impact on human and environmental health.

Presumably the elevated values in enterococci levels on day 4 at all surface stations could have been caused by runoff from the large rainstorm that occurred immediately prior to the sample collection. No other such rainstorms were observed within 12 hours of the other sample collections, but such rainstorms are common in the rainy season in Bonaire. If this is indeed the cause of the high bacteria levels, high enterococci levels off the coast may be very common. Further studies should be done comparing water quality in this area with frequency and intensity of rain and runoff.

Enterococci levels similar to those seen in the anomalous collection days 3 and 4 may represent a significant public health risk. Wade et al. (2003) found in their review of studies on bacteriological water quality indicators of gastrointestinal illness that eight out of 12 studies found that enterococci levels higher than the EPA limit were correlated with statistically significant relative risks of gastrointestinal illness, while only two of nine studies found similar risks below the EPA enterococci limit. Thus, they recommend that enterococci levels in marine water still be used as an indicator to predict gastrointestinal illness.

Recommendations

This study was severely limited in both time and sample size of all variables considered. Future studies with longer study times would expand the data collected here to draw firmer results. Studies exploring the relationship between rainstorms or the hydrology of the island and enterococci levels would be particularly interesting. However, it is strongly recommended that future publishers in *Physis* with

experimental designs of the same time limit focus on one of the relationships explored in this study: i.e. analyzing polychaete assemblages and grain size at one site.

Conclusions

In conclusion, this study's limited duration and sample size proved to be insufficient to draw the conclusions to the questions it originally asked. However, useful data was still gained from the inquiry. Polychaete assemblages varied between stations, but not enough data was gathered to eliminate the possibility of grain size differences in explaining this variability. All water stations had normal levels of bacteriological indicators of marine pollution, although the Marina station experienced more variance in bacteriological indicators. All surface stations and the Marina benthic station experienced dangerously high levels of enterococci following a heavy rainstorm. The Marina specifically may be more vulnerable to external effects on pollutant levels, specifically heavy rainstorms increasing enterococci levels. Researchers and the local government should be urged to continue looking into this variability in water quality in order to ensure public health and safety.

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The relationship between algae, depth, and abundance of terminal and initial phase parrotfish (*Sparisoma viride* and *Scarus taeniopterus*)

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Abstract

Coral reefs are undergoing phase shifts becoming dominated by algae rather than live coral. Algae on reefs is greatly impacted by herbivores such as parrotfish that use both the reef and shallow intertidal zone to feed. These two habitats are unique with differences in algal cover and algal community composition. The shallow intertidal may have greater risk of predation from ospreys and the habitat is more extreme in terms of turbulence and constantly changing microenvironments. It is unknown if feeding in the shallow intertidal zone provides a benefit that offsets the risk of feeding there. This study observed initial and terminal phases *Sparisoma viride* and *Scarus taeniopterus* to determine if there are differences in feeding by parrotfish between the shallow intertidal and the reef crest. The main goals of the study were 1) to compare algae from the shallow (higher predation risk) habitat to algae from the deeper (lower predation risk) habitats and 2) to compare abundance of terminal phase and initial phase. Transects at two different depths of 0.5 m and 10 m were placed at 10 different sites on the leeward side of Bonaire, Dutch Caribbean. Point intercept surveys and dry weight of algae samples were used to compare algal cover and fish surveys were conducted to measure parrotfish. Although the shallow intertidal zone had greater algal cover there was not a significant difference in abundance of parrotfish during either developmental stage studied, which may be explained by greater

Introduction

Coral reefs around the world are being damaged because of increased algae growth caused by excess nutrients from pollution in the water given off by agriculture or human excrement (Hughes et al. 2007). In addition to excess nutrients, there has also been a dramatic decrease in the populations of herbivores such as *Diadema antillarum* throughout Caribbean, which has led to an increase in algae growth. *D. antillarum* are important grazers of algae on coral reefs and the decrease in population has increased the importance of other grazers on the reef (Jackson 1997). Marine protected areas have been set up in the Caribbean to help conserve herbivore species that ameliorate the effects of increasing algae on reefs and avoid the shift from coral dominated to algae dominated reefs (Huntington et al. 2011). It is harder for corals to grow on algae dominated reefs because algae can

outcompete coral for space and interfere with larval settlement (Tanner 1995). Now more than ever it is important to study herbivores and how they keep algae under control on the reef.

Stoplight parrotfish, *Sparisoma viride*, and Princess parrotfish, *Scarus taeniopterus*, are important herbivores on Caribbean coral reefs (Choat et al. 2003). Parrotfish make up a large percentage of the herbivores on the reef and consume a large amount of algae found on dead coral. However parrotfish and other herbivores are being threatened by overfishing in the Caribbean (Bellwood et al. 2011). It is important to have healthy populations of parrotfish to consume algae but too many herbivores can also be harmful to coral health (Rotjan and Lewis 2006). For example, *S. viride* are an especially important species to study because repetitive biting by *S. viride* can damage coral tissue (Bruckner and Bruckner 1998). Studies of the depth distribution of parrotfish can help

understand their relationship to algal biomass.

It is essential to note what parts of the reef are important for herbivores such as parrotfish use since healthy populations of herbivores impact reef health. Substrate characteristics can influence the location of fish on a reef (Chabanet et al. 1997). Guidetti and Boreo (2002) conducted a study on the parrotfish, *Sparisoma cretense*, to determine the depth distribution of the population and found that *S. cretense* was most abundant at a depth of 12-15 m because of specific ecological requirements. These requirements could be physical such as water temperature and wave action or the requirements could be biological features such as benthic cover of algae (Guidetti and Boreo 2002).

More research could improve understanding of feeding behaviors of different phases of parrotfish and the distribution of the juveniles and adults of these important herbivores on the reef. Initial phase (IP) parrotfish can be either male or female whereas, terminal phase (TP) parrotfish are male and differ in color and are larger than IP (van Rooij et al. 1995). Larger TP parrotfish have higher grazing rates than IP parrotfish and metabolic requirements require the TP parrotfish to consume a lot of algae (Bruggeman et al. 1996).

Parrotfish are very abundant in Bonaire, and are found in the shallow region along the waterfront and other depths (Personal Observation). The presence of parrotfish in the shallow habitat is especially important to note because fish in shallow water could be more susceptible to fishing, dangerous tides and predators, such as ospreys that prey on parrotfish (R. Peachey, personal communication). TP parrotfish may be feeding in this shallow habitat because there may be greater biomass of algae present that supports the metabolic demands of TP fish more adequately than algal communities at deeper locations on the reef. This field study was conducted to study the relationship between algae and abundance of TP and IP

parrotfish in the shallow habitat compared to a deeper habitat, near the reef crest. The following hypotheses were tested:

- H₁: Terminal phase *S. viride* and *S. taeniopterus* are more abundant at 0.5 m than initial phase *S. viride* and *S. taeniopterus*.
- H₂: Initial phase *S. viride* and *S. taeniopterus* are more abundant at 10 m than terminal phase *S. viride* and *S. taeniopterus*.
- H₃: There is a greater algal cover and biomass at 0.5 m than 10 m that would provide more energy for parrotfish eating.

Understanding the relationship between developmental stages of parrotfish and use of more risky habitats could lead to better understanding of the requirements of these important reef herbivores.

Materials and Methods

Study site

The research took place in Bonaire, Dutch Caribbean, a desert island that is surrounded by a fringing reef with a gentle slope. The reef crest is located at a depth of around 8-10 m. The study was conducted along the reef near the center of the west coast, north of the capital Kralendijk (Fig.1). Species of parrotfish utilized in this study are very abundant at this location and the water is very clear making it an ideal place to study parrotfish feeding behavior.

Field and laboratory research

Ten sampling sites were randomly selected along 1 km long portion of the reef at sites that were at least 30 m apart. At each sampling site data on parrotfish abundance, algal cover and biomass was collected at two depths, 0.5 m and 10 m, which are depths that appear to be important for parrotfish grazing (Personal Observation).

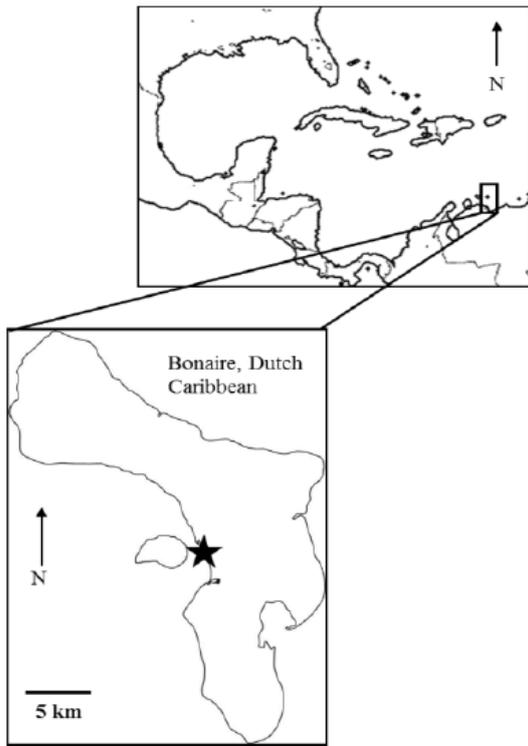


Fig. 1 Map of Bonaire, Dutch Caribbean with star indicating the research site ($12^{\circ}09'30.87''\text{N}$, $68^{\circ}16'52.77''\text{W}$) along the leeward coast where this study was conducted between September and November, 2012

Shallow sites were sampled by snorkeling and the deeper sites were sampled using SCUBA.

Sampling occurred between 15:00 and 17:00 h and all the study sites were sampled twice.

Starting points were predetermined using Google Earth and the actual starting points for the 10 m depth were located using fin kick cycles (approximating 1 m per kick cycle) to measure the distance from a pier or other object of known location, whereas 0.5 m starting points were located using a GPS. At both depths, a 30 m transect tape was laid to the north of each starting point. After an acclimatization period of 2 min, a fish survey of a 3 m wide belt was conducted along the transect yielding a 90 m^2 survey area.

Additionally, a point intercept survey was conducted along the transects to determine percent algal cover. The

substratum directly below the 1 m marks was categorized as turf algae, live coral, sand, newly dead coral, sponge, or rock/rubble. Samples of algae were taken at each of the sites by randomly selecting a number along the transect where a 4 cm quadrat was placed. Three random numbers were generated in case there was no algae under the transect line for the first number. Algae were scraped off the substratum inside the quadrat, collected in plastic bags and returned to the lab to determine the dry weight (mg algae cm^{-2}). In the lab the algae was placed in aluminum pans and dried in an oven for a minimum of 24 h at 100°C to determine dry wt.

Data analysis

A Mann-Whitney U statistical test was used to compare the mean density of IP and TP parrotfish (individuals m^{-2}) between the two depths, and also to compare the mean density of IP parrotfish to the mean density of TP parrotfish (individuals m^{-2}) at each depth with a 95 % confidence interval. The mean algae dry weight and percent cover was also compared between the two depths with a Mann-Whitney U statistical test using a 95% confidence interval.

Results

Ten paired surveys of 0.5 m and 10 m depths were conducted from September to November 2012. A total of 168 parrotfish were seen during this study. More parrotfish occurred at 10 m (total=119,98 IP, 21 TP) than at 0.5 m (total=49, 20 IP, 29 TP). The mean density of IP was significantly higher at 10 m ($0.12 \pm 0.05\text{ ind m}^{-2}$) than 0.5 m ($0.02 \pm 0.02\text{ ind m}^{-2}$) (Mann-Whitney U, $U=99.5$, $p=0.0002$) (Fig. 2). In contrast, the mean density of TP was higher at 0.5 m ($0.032 \pm 0.01\text{ ind m}^{-2}$) than 10 m ($0.026 \pm 0.01\text{ ind m}^{-2}$), however no statistically significant difference was found (Mann-Whitney U, $U=68$, $p=0.174$). At 10 m, there was a significantly higher mean number of IP ($0.12 \pm 0.05\text{ ind m}^{-2}$) than TP ($0.026 \pm$

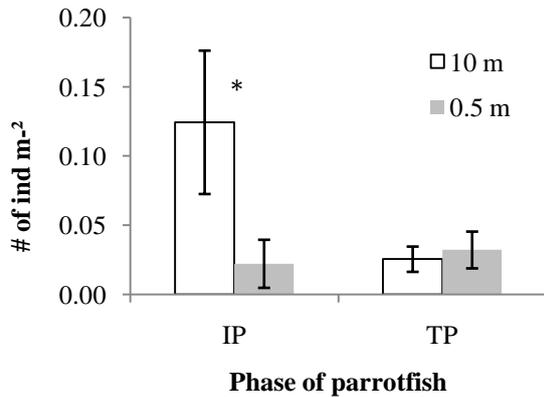


Fig. 2 Comparison of the mean number of parrotfish (\pm SD) of initial phase (IP) and terminal phase (TP) in the shallow intertidal zone (0.5 meters, grey) versus reef crest (10 meters, white) ($n=20$). Asterisk indicates data that shows significant difference (Mann-Whitney U, $U=100$, $p=0.0002$)

0.01 ind m⁻²) (Mann-Whitney U, $U=100$, $p=0.0002$).

However, at 0.5 m the mean number of TP (0.026 ± 0.01 ind m⁻²) was slightly higher than IP (0.02 ± 0.02 ind m⁻²), but the difference was not significant (Mann-Whitney U, $U= 68.5$, $p=0.162$)

The percent algal cover for the shallow intertidal zone (0.5 m) was much higher than the deeper sites and ranged from 60-73% whereas the mean percent algal cover for the reef crest (10 m) ranged from 20-47%. The mean percent algal cover was significantly higher at 0.5 m (66.7 ± 5.2) than 10 m (39.7

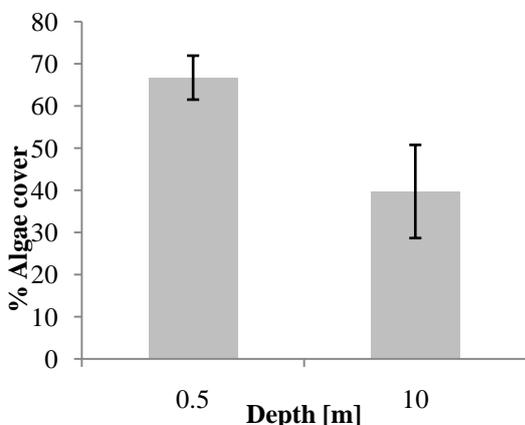


Fig. 3 Comparison of the mean percent algal cover (\pm SD) of the shallow intertidal zone (0.5 m) vs. the reef crest (10 m) ($n=20$) in Bonaire, Dutch Caribbean during a study from September to November, 2012

± 11.0) (Mann-Whitney U, $U=99$, $p=0.0002$) (Fig. 3).

The opposite relationship was found when comparing biomass of algae between the habitats. The algae dry weight at 0.5 m was much lower than the reef crest sites and ranged from 0.01 to 0.06 mg dry wt cm⁻², whereas at 10 m biomass ranged from 0.02 to 0.22 mg dry wt cm⁻². The mean dry weight was significantly higher at 10 m (0.13 ± 0.11 mg dry wt cm⁻²) than 0.5 m (0.05 ± 0.06 mg dry wt cm⁻²) (Mann-Whitney U, $U=77$, $p= 0.043$) (Fig. 4). General observations indicate that even though the total percent cover of algae was less at 10 m, it was thicker than algae at 0.5 m that was a thin layer that covered a greater area of the substrate.

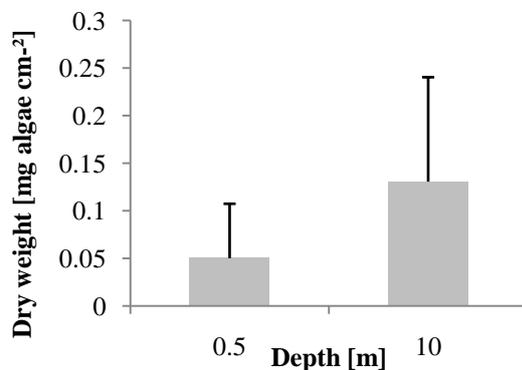


Fig. 4 Comparison of the mean dry weight of algae (\pm SD) in the shallow intertidal zone (0.5 m) versus the reef crest habitat (10 m) during the study in Bonaire, Dutch Caribbean from September to

Discussion

This study aimed to observe the different habitat preferences between IP parrotfish and TP parrotfish in relation to algal cover. There were more TP parrotfish at 0.5 m than IP parrotfish however, the difference was small and not statistically different, which does not support the hypothesis that there would be more TP than IP in the shallow intertidal (H_1). However, there was a significantly higher density of IP parrotfish than TP parrotfish at 10 m which supports the hypothesis that there would be more IP parrotfish at the

deeper site (H_2). There is a lower number of IP parrotfish in 0.5 m than 10 m this could be due to the difficulties IP parrotfish have accessing the shallow intertidal zone because of wave action. The study of the shallow intertidal had limitations due to different developments such as piers along the shoreline that may have affected species distribution. Observations of parrotfish behavior in the shallow water habitat were overtly different than behaviors displayed at the reef crest. Parrotfish were influenced by wave action and the fish needed to swim actively to avoid being pushed up onto the shore. This may explain why the number of IP parrotfish was much greater at 10 m than 0.5 m but the number of TP parrotfish stayed about the same between the two depths. Parrotfish would need to be strong and bigger, which is characteristic of TP parrotfish, to be able to feed in this region, which would be an interesting follow up study.

It is important to note that relatively the same density of TP individuals were found in the shallow region as the deep region. TP parrotfish are more brightly colored and are potentially more noticeable to predators such as ospreys that grab fish in shallow waters, which indicates there may be a trade-off between feeding in the shallow subtidal and some other ecological need such as nutrition (Robinson 1994). Parrotfish are a type of herbivore that are heavily overfished in some areas (Bellwood et al. 2012). Although, fishing for parrotfish is not common in Bonaire, fisherman would notice and have access to large parrotfish in the shallow region. One reason for parrotfish to choose the shallow habitat is that there may be less competitive pressure between individuals since very few parrotfish utilize this habitat compared to the deeper reef habitat and the algae covers a greater area and is more spread out.

The percent algal cover was higher in the shallow intertidal zone which supports the hypothesis that there would be greater percent algal cover and biomass in the shallow water habitat (H_3). Algal biomass

was greater and there was lower percent cover at the 10 m depth, which may sound contradictory, however, when scraping off the algae in the shallow region it was a thin layer that did not come off as easily from the rock. The algae in the deeper habitat was more fleshy and perhaps less palatable or less nutritious than algae in the intertidal zone. It was much easier to scrape algae off the substrate in the reef crest habitat, so another potential difference is that algae in deeper water are more readily grazed, which may explain why there was more IP parrotfish found in this depth. Perhaps they cannot graze the thin hard algae in the shallow intertidal zone. In conclusion, it is unclear if there is a nutritional benefit to feeding in the shallow intertidal zone (0.5 m) because even though there is greater algal cover, the algae growing there is not as thick and may be harder for parrotfish to access.

The difference in algal cover between the two depths may be influenced by the total number of parrotfish. The deeper depth had a lower algal cover but a higher total number of parrotfish of both phases. A study conducted by Hughes et al. (2007) that manipulated access of herbivores to different parts of the reef showed that areas that were not closed off had lower algal cover. Since the deeper depth in this study has low percent algal cover but higher number of total parrotfish this shows that grazer biomass relates negatively to algal cover (Williams and Poulunin 2001).

Since the algae was sampled at random points along the transects there was high variability. Line intercept surveys instead of point intercept surveys could be used to measure the total cover of algae at the different depths. More research is needed to compare the nutritional value of algae growing at different depths. This study only focused on two different depths to determine parrotfish abundance. Further studies of multiple depths may show more differences in habitat preference of different phases of parrotfish. It would also be beneficial to observe all the different species of parrotfish to see if there are differences in distribution

from the two species observed in this study. Studies such as this one are important for conservation practices including the establishment of Marine Protected Areas. Since herbivores are so important to reef health more research needs to be done on their habitat preferences so MPAs can be set up in the most efficient way to protect important species such as parrotfish.

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Physical and spatial differences between the three color morphologies of the Atlantic trumpetfish, *Aulostomus maculatus*

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Abstract

Mimetism allows organisms to blend into the environment and avoid detection by both predators and prey. The Atlantic trumpetfish, *Aulostomus maculatus*, uses three color morphologies blue, brown, and yellow to achieve this goal. The various colorations allow *A. maculatus* to stalk prey individuals and blend into tall gorgonians and sponges on the seafloor. Chromatophore and energy expenditure analysis showed these three morphs as steady and evenly dispersed. The present study focuses on behavioral and spatial differences between individuals of varying morphologies on a fringing reef in Bonaire, Dutch Caribbean. Length of trumpet fish, depth, and substratum association were noted on twelve 30-minute timed swims at varying depths and locations near Yellow Sub dive site. Results show no significant difference between the three morphology abundances, or between depth and color morphology. Only comparisons between brown and blue color morphologies were found to be significantly different with larger brown individuals and smaller blue individuals. Associations were found to be highest with sea-rods. Higher percentages of yellow color morphs associated with corals while more blue individuals tended to shadow fish. Brown morphs were found to have higher association rates overall which may account for their larger size. Trumpetfish that were not associated with one benthic substrate due to movement or hunting were considered to be active individuals. A difference in activity levels of the three color morphologies was not found to be statistically significant. Further research may include more investigation into the trumpetfish life history, and trumpetfish may serve as a model for color morphology studies in the marine environment.

Introduction

To any organism habitat provides a place for growth, reproduction and protection. An ability to match the background of a habitat as a form of defense against predators or mimicking prey can aid in protection and the ability to catch prey. This use of camouflage is found in marine and terrestrial ecosystems alike. Mimetism can take two forms, matching coloration in which an organism uses colors that represent a random sample of the background or disruptive coloration that works to break up the outline of the organism with the use of bold contrastive coloring (Cuthill et al. 2005; Houston et al. 2007).

Some organisms display multiple color morphologies that may aid in thermoregulation, camouflage or

intraspecific communication (Crook 1997; Venesky and Anthony 2007). In areas where predation provides the main selective pressure, color morphologies may serve as an adaptation for predator avoidance (Venesky and Anthony 2007). The Atlantic trumpetfish, *Aulostomus maculatus*, utilizes brown, blue, and yellow color morphologies to achieve this concealment. (Lochmann 1989). The color morphologies permit them to hide amongst gorgonians, sponges and sea rods in homogeneous environments (Aronson 1983; Houston et al. 2007). This also allows them to blend in with schooling Chromis or stalk benthic prey (Lochmann 1989). Several studies have shown that these color morphs are stable (Lochmann 1989; Cushman et al. 2004). Analysis of chromatophore composition revealed significant diversity in the abundances of

specific types of cells in different color morphs (Lochmann 1989). Furthermore examination of energy cost of changing from one morph to another was found to be higher than the camouflage gained (Cushman et al. 2004). If one color morph were to become more abundant, prey species may be able to recognize and avoid that particular color morph, leading to selection against the popular color (Aronson 1983; Cushman et al. 2004; Venesky and Anthony 2007). Even though color morphs may be stable and equally distributed throughout the population, whether they behave differently, are physically different, or are found along certain depths along the reef has yet to be thoroughly studied (Aronson 1983). According to Auster (2008) *A. maculatus* is a common piscivore in Bonaire, Dutch Caribbean, along the back reef habitats in coral environments. Therefore Bonaire is suited to study *A. maculatus* and was chosen for this study. The hypotheses developed for this study were:

- H₁: The three color morphs observed on the reef are equally distributed throughout the population.
- H₂: The yellow color morph are smallest in size, followed by blue, and the brown morph are the largest of the three.
- H₃: The yellow color morph is found shallowest of the three morphologies, followed by brown, and the blue individuals is found deepest along the reef.

To date, little research on the correlation between color morphologies, individual subject size and water depth has been conducted. Studies have shown that coral reef fish communities show habitat specificity, and have recognized the importance of understanding the assembly of fish communities at small special scales (Auster et al. 2005). Additionally, this study examines the interactions and associations between trumpetfish and sea rods. It was hypothesized that:

H₄: More trumpetfish are found in association with sea rods than any other substrate.

Materials and Methods

Study site

This study was conducted in Bonaire, Dutch Caribbean (Fig. 1). The island is surrounded by a fringing reef that ranges from 6 to 40 meters depth. The Yellow Sub dive site where this study was completed is on the leeward side of the island. Dives were conducted to the north and south of this site.

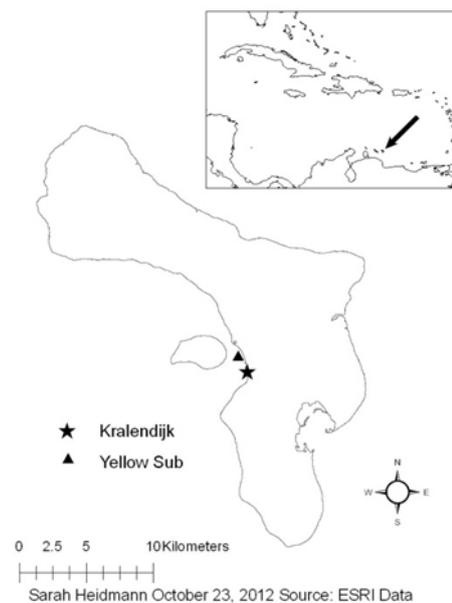


Fig. 1 Map of Bonaire, Dutch Caribbean in the context of the Caribbean Sea, insert above. Yellow Sub study site (marked by a triangle) is northwest of the capital, Kralendijk (marked by a star). Map created by Sarah Heidmann

Data collection

The methods used for this research paper are adapted from Chalfont (2012). Studies were run with twelve 30-minute timed swims using SCUBA. At each of the two locations (North and South), two swims were done at depths of 9, 12, and 15 m. Depths were selected after a preliminary dive, which found higher densities of

trumpetfish between 9 and 15 m. At each depth, each individual trumpetfish encountered within a 2 m belt was classified by color morph, measured lengthwise, and associations with substrate or environment were noted.

Data analysis

To determine the distribution of color morphs and the variety of depth per color morph, Kruskal-Wallis tests were performed using StatView. One-way ANOVA statistical analysis, in combination with Fisher's Protected Least Significant Difference (PLSD), were used from the same software to determine significance between the size of individuals and color morphologies. Associations were compared based on percentages with each substrate type.

Results

Distribution

A total of 102 trumpetfish were observed and measured throughout 12 timed swims. Out of those 58 were blue, 36 were brown and only eight were yellow. A Kruskal-Wallis test revealed that these differences in abundance between the three color morphologies was not significant ($df = 2$, $p > 0.05$).

Size

Observed sizes of trumpetfish ranged from 17 to 74 cm in length. Individuals recorded were separated by color morph into five categories; <26 cm, 26-40 cm, 41-55 cm, 56-70 cm, and >70cm (Fig. 2) Results showed that the blue and brown color morphs did not significantly differ in size to the yellow color morph (PLSD, $df = 2$, $p > 0.05$, for both tests). However, size of the brown color morph when compared to blue colored trumpetfish was highly significant, with the difference in brown size being larger than blue (ANOVA, $df = 2$, $p < 0.01$).

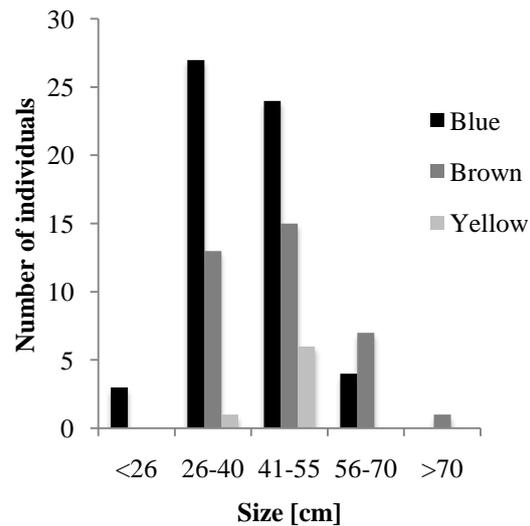


Fig. 2 A comparison of size distributions among the three color morphologies (blue, brown, and yellow) of *Aulostomus maculatus*. A statistical analysis with a parametric Fisher's Protected Least Significant Difference determined that the size of blue (black bars) and brown (dark grey bars) were not significantly different than the size of yellow (light grey bars) ($p > 0.05$). Blue individuals were significantly smaller than brown color morphs ($p < 0.01$)

Depth

Trumpetfish were divided by color morph for the depths of 9, 12 and 15 m and the total number of trumpetfish observed at each depth was determined (Fig. 3). No significant difference in color morph abundance between the depths was observed (Kruskal-Wallis, $df = 2$, $p > 0.05$). The total number trumpetfish seen at each depth showed no significant difference in trumpet fish abundance between the depths (Kruskal-Wallis, $df = 2$, $p > 0.05$).

Association

Of all blue color morph individuals, 24% were seen close to sea-rods, 10% were observed shadow-stalking fish, and only 5% were seen near corals. Brown individuals showed a similar trend with 28% close to sea-rods, but 14% seen with corals and only 6% seen with fish. More individuals of the

yellow color morph (25%) were seen close to corals instead of sea-rods. Another 13% were observed in sea-rods but none were observed (0%) shadowing fish (Fig. 4).

The rest of the individuals were classified into a category of ‘other’ various substrate types. Although yellow individuals appeared to be more active than the other color morphologies, this difference was not found to be statistically significant with a Kruskal-Wallis test ($p>0.05$) (Fig. 5).

Discussion

From the results, distribution of color morphologies although not equal were also not significantly different. This may be due to insufficient data set and therefore the original hypothesis is neither supported nor rejected. Chalfont (2012) in a similar study found the mean number of color morphologies of trumpetfish per transect to differ significantly, with yellow morphologies being the least abundant.

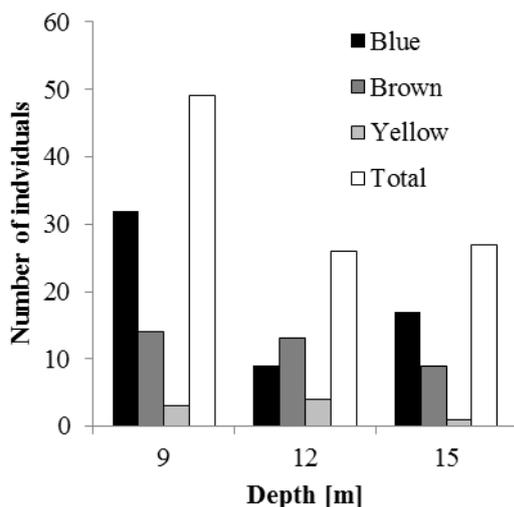


Fig. 3 A comparison of *Aulostomus maculatus* color morphologies, blue (black), brown (dark grey), yellow (light grey) and the total (white) and their distribution over three depths 9, 12, and 15 m. A Kruskal-Wallis test indicated that color morphs and their depth distribution were not statistically significant ($p>0.05$). The total trumpetfish seen at each depth were also analyzed using a Kruskal-Wallis test that demonstrated that there was no significant difference in total trumpetfish observed between the

different depths ($p>0.05$)

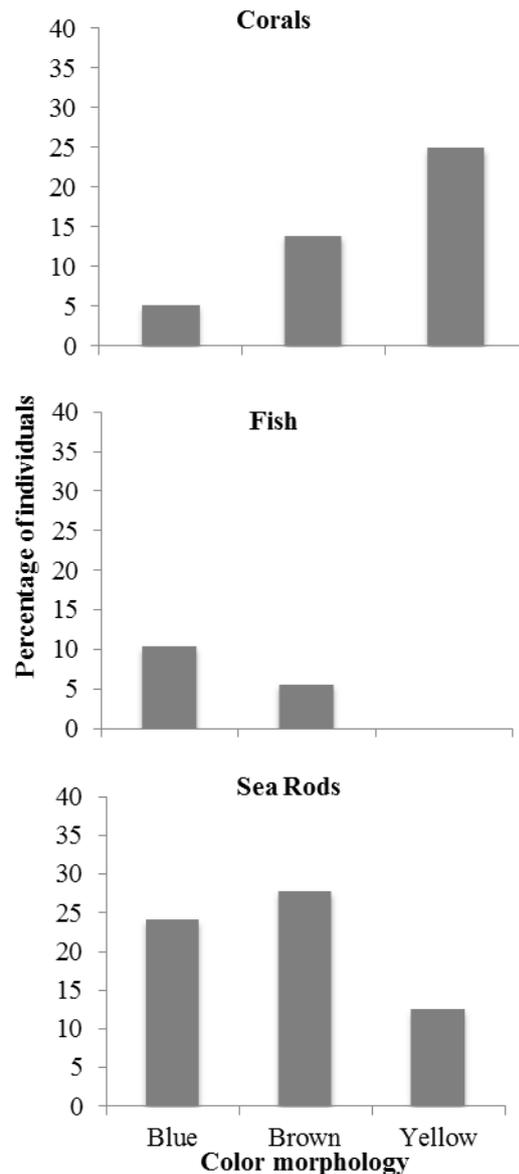


Fig. 4 Associations of *Aulostomus maculatus* with corals, fish, and sea rods calculated as percentages of each color morphology (blue, brown, yellow)

Chalfont (2012) attributed this to the possibility of a “double recessive allele” in the yellow variation that may make it naturally less abundant. These results suggest further research or long term studies are needed that may lead to more precise results.

Overall size comparisons of brown and blue color morphologies were found to be

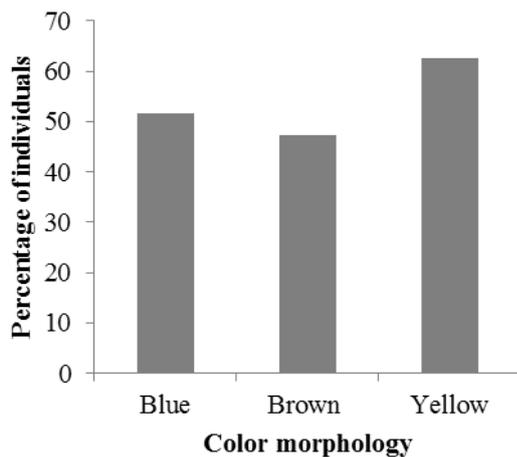


Fig. 5 Active individuals of *Aulostomus maculatus*, by color morphology (blue, brown, yellow), observed during 12 timed swims as percentage of total individuals seen

significantly different suggesting a trend in growth and coloration.

Brown color morphs were significantly larger than blue suggesting they may have higher camouflage or defensive abilities than blue and may have a higher survival rate. This may be attributed to the life history of trumpetfish and further research may gain an understanding of the roles coloration may play in sexual selection and other aspects of reproduction. It was predicted that yellow color morphologies would be the smallest due to a lack of camouflage and therefore higher predation rates, but this was not seen during association of substrate types mentioned later.

In contrast to results found by Chalfont (2012) indicating significance between depth and color morphology, this study suggests there is no significance between these two variables. This may be attributed to a lack in precision of depth measurements due to human error. Further studies may determine if differences in depth between these diverse morphologies exists.

Associations of the three color morphs with different benthic substrates show a blue color morph affinity for shadow stalking various species of fish. The opposite trend was present for yellow morphs in that none were observed shadowing fish but a higher

abundance were associated with corals. This association may account for the larger size variance of yellow individuals due to the increased protection of trumpetfish by intricate coral formations. Brown morphs were seen to associate with fish and corals leading to an increased camouflage ability and therefore possibly larger size ranges of individuals. Over 50% of all trumpetfish seen were found in association with sea rods indicating sea rods as a valuable habitat for these organisms. This supports the original hypothesis that trumpetfish would be seen to associate more with sea rods than any other substrate due to an increased camouflage ability. Activity levels suggest increased activity of yellow morphs, which may be due to increased hunting in the open due to a lack of camouflage amongst fish.

Further studies into associations with benthic substrata and hunting strategies as well as size, color variations and the correlations to the life stages and reproduction of this species are necessary. Long term research and application of tagging methodologies may assist in the understanding of this species and their ecological role. Trumpetfish are an important predator along Bonaire's reefs and could be used as a model in future marine color morphology studies.

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Behavior of the Princess parrotfish (*Scarus taeniopterus*): a comparison between daylight and sunset

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Abstract

Most animals are active either during the day or night, and at twilight, nocturnal and diurnal animals alike engage in behaviors to avoid predators, seek shelter, defend territory, or feed. Herbivorous fishes on coral reefs, such as parrotfishes, forage throughout daylight periods due to reliance on light and low nutrient content of algae. At sunset, parrotfishes seek cover to avoid predation during the night, resulting in less feeding and more aggressive behavior. This study compared how initial phase (IP) and terminal phase (TP) Princess parrotfish (*Scarus taeniopterus*) allocate time between daylight and sunset periods, specifically regarding feeding and aggressive behavior, on the fringing reefs of Bonaire, Dutch Caribbean. Using SCUBA, individual *S. taeniopterus* between 11 and 14 m depth were followed for 5 min after a 1 min acclimatization period, and time spent on each behavior was recorded. A two-way analysis of variance (ANOVA) was used to compare the amount of time *S. taeniopterus* spent feeding and in aggressive interactions between day and sunset periods, with phase and time of day as factors. Fish of both phases had a higher mean percent time feeding and a lower mean percent time being aggressive in the morning than at sunset. There was a significant difference in mean percent time feeding between phases, and TP fish had a significantly higher mean percent time being aggressive than IP fish. The changes in behavior found in this study increase the success of *S. taeniopterus* finding and keeping quality nighttime resting locations.

Introduction

Transition periods between day and night show a marked difference in the behavior of many animals, such as cattle, plankton, salmon, and tropical reef fishes (Hobson 1972; Enright 1977; Gonyou and Stricklin 1984; Fraser et al. 1993). Most animals are diurnal, nocturnal, or crepuscular: active during one part of the day, and inactive the rest of the time because adaptations for one light level tend to reduce ability in another (Fraser et al. 1993), resulting in a difference in abundance of visible species between day and night in a habitat. The period between daylight and darkness is transitional, when diurnal animals find cover for the night, and nocturnal ones emerge. During transitions, animals will be actively searching for prey, avoiding predators, seeking shelter, defending territory, feeding, or migrating.

An example of the unique behavior that may occur during transitions is the daily vertical migration of plankton that occurs across many species and locations (Enright 1977).

Herbivorous fishes on coral reefs are visual grazers and are most abundant and active during the daylight hours (Lewis 1986). One of the most abundant groups of herbivorous fishes is the parrotfishes, family Scaridae (Ogden and Buckman 1973). Parrotfish use fused, beak-like jaws to scrape algae turf from dead coral (Guidetti and Boero 2002), consequently ingesting calcium carbonate, which is used to grind plant matter in the pharyngeal mill (Randall 1967). The low nutrient content of algae means parrotfish must consume large quantities during the day to satisfy energy needs. Parrotfish must spend much of the day feeding; *Sparisoma viride* spends 84-

97% of diurnal time swimming, feeding, and hovering (Hanley 1984).

At night when low light levels make foraging difficult for parrotfish, resting conserves energy and reduces oxygen consumption (Curran 1992). At dusk, parrotfish settle on the soft bottom, usually under partial cover, and stay through the night, sometimes secreting mucus cocoons for protection from nocturnal predation (Hobson 1965). The function of the cocoons is not fully understood. Individuals may form cocoons only when injured or under stress (Hobson 1965). A cocoon may mask an individual's scent, reducing effective predation by morays (Winn and Bardach 1959), since moray eels are nocturnally active and use smell to locate prey (Bardach et al. 1959).

During the transition period between daylight and darkness, there is a marked change in behavior of many fish on the reef, including parrotfish. Transition behaviors are usually manifested as a decrease in feeding accompanied by an increase in migration and aggression (Hobson 1972), to search for and subsequently protect nighttime resting locations (Hobson 1965). Low-light conditions are favorable for large predators, so diurnal fishes, such as parrotfish, must roam cautiously while seeking cover (Hobson 1973). Terminal phase (TP) males use aggression to defend territories and protect initial phase (IP) female mates from other migrating males who may intrude (Streelman et al. 2002).

This study aims to determine how the Princess parrotfish (*Scarus taeniopterus*) allocates time for different behaviors during the day versus at sunset. The purpose is to determine whether time of day significantly affects time allocated to feeding or aggressive behaviors by testing the following hypotheses:

H₁: The mean percent time *S. taeniopterus* spends foraging is higher during daylight hours than at sunset for both developmental phases.

H₂: The mean percent time *S. taeniopterus* spends being aggressive is lower during daylight hours than at sunset for both developmental phases.

Studying behavior shows how a species interacts with its environment, including other species (Hobson 1972). A change in behavior reflects changing conditions, such as increased possibility of predation, which may not be directly apparent to an observer.

Materials and Methods

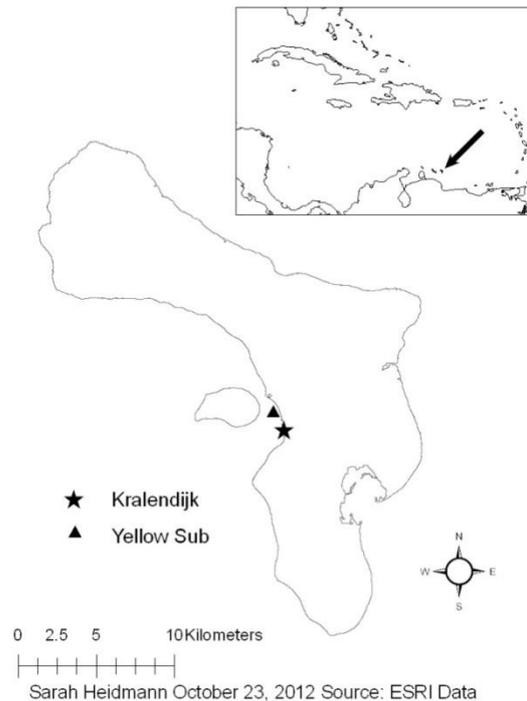


Fig. 1 Map of Bonaire, Dutch Caribbean in the context of the Caribbean Sea, insert above. Kralendijk, the capital, is marked with a star. The study site, Yellow Sub, is marked with a triangle

Study organism

S. taeniopterus show interesting behavior at sunset, such as tailstands, when an individual assumes a head-up position in the water column as daylight fades, a behavior which is not fully understood, but is thought to enlarge visual fields to detect predators and territory intruders (Dubin and Baker 1982). The range of *S. taeniopterus* is throughout the Caribbean, South Florida, Bahamas, and Bermuda. This study was

conducted from September through November, 2012, on the island of Bonaire, Dutch Caribbean (Fig. 1), at Yellow Sub dive site (12°09'36.47"N, 68°16'55.16"W), which is located on the west coast of the island.

Bonaire is a desert island with a tropical climate, resulting in good visibility on its fringing reef, which is close to shore, providing accessibility for behavioral studies of *S. taeniopterus*. Accessibility also means the site is heavily dived, so the parrotfish are familiar with the presence of divers and the use of SCUBA may not be as much of a disturbance to fish behavior as it would be at less dived locations.

Both IP and TP stages of *S. taeniopterus* were included in the study. Juveniles were excluded from the study since there may be a difference in behavior from adults. *S. taeniopterus* has a similar body shape, size, and markings to *Scarus iserti* (Striped parrotfish), and were differentiated by markings along the top and bottom of the tails of both phases of *S. taeniopterus*, which are absent in *S. iserti*.

Behavioral observations

All behavior was observed using SCUBA. The observation portion of each dive was approximately 40 min, at depths between 11 and 14 m, which has the highest parrotfish density at Yellow Sub according to Adler (2009). Daytime dives took place in the morning between 09:00 and 12:00 h. Hobson (1972) documented cover-seeking behavior that started 15 min before sunset and ended 45 min after sunset. However, during preliminary observations, it became apparent that behavioral observations without lights were not possible more than about 20 min after sunset, and fish are disturbed by artificial lights (Hobson 1965). Therefore, sunset behavioral observations began 20 min before sunset, and continued until 20 min after sunset. Sunset times were determined by a table produced online by the United States Naval Observatory (USNO) Astronomical Applications Department.

Individual *S. taeniopterus* were selected and followed for 1 min (to allow for acclimatization), then for an additional maximum of 5 min to record behavioral data. The observer remained at a distance of no closer than 2-3 m, above and behind the fish when possible, to avoid disturbing fish behavior, as suggested by Dubin and Baker (1982). Developmental phase was recorded (IP or TP), and equal numbers of each phase were followed at each time of day. Behavior was categorized as the following: feeding, defecating, swimming, stationary, aggression, and other. Aggression was subdivided into aggressor or offender, and the type of fish in the interaction was recorded: another *S. taeniopterus*, another parrotfish, or a non-parrotfish.

Swimming occurs between most other activities as a transition behavior that is non-specific, and the underlying purpose is not clear. Therefore, swimming was considered a separate behavior, recorded by subtracting cumulative time spent on other behavior from total observation time for each individual.

All other behaviors were timed using a watch recording to the nearest second. One continuous feeding period was defined as the amount of time an individual spent hovering at a single location. If the individual moved to another location, it was considered a new feeding period, and the interim was counted as swimming. Aggression was defined as apparent defense of territory, most often as direct chasing. Fin flaring was not counted as aggression even though it is an aggressive display, because an individual could also be doing another behavior, such as feeding, simultaneously.

Data analysis

For each fish followed, percent time spent on each behavior was calculated, and then averaged over all fish. Data were arcsine transformed to make the distribution more normal, and the analysis was performed using StatView 5.0. The effect of

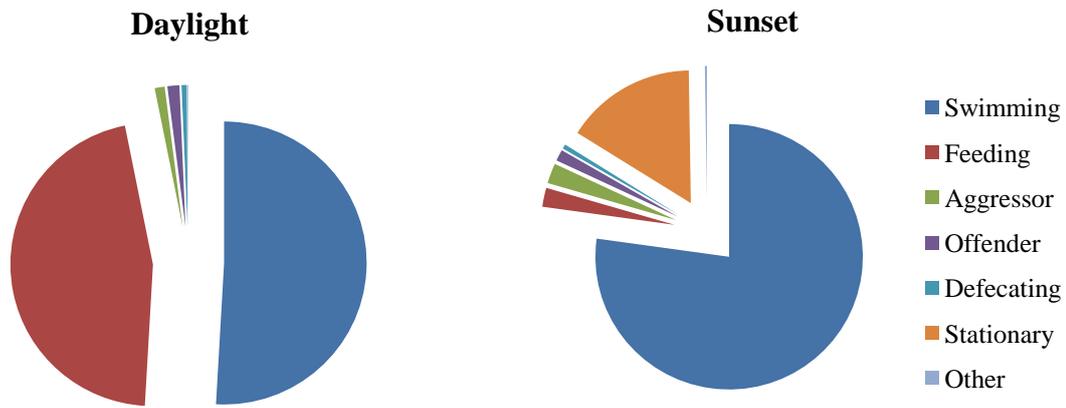


Fig. 2 Comparison of mean percent time spent in various behaviors of initial phase and terminal phase *Scarus taeniopterus* during daylight (0900-1200 h, n=62) and sunset (20 min before and after sunset, n=62) (total number of fish=124). Behaviors were categorized as swimming (dark blue), feeding (red), aggressor (green), offender (purple), defecating (light blue), stationary (orange), and other (periwinkle)

time of day (factor 1) and developmental phase of the parrotfish (factor 2) on the dependent variable, mean percent time spent feeding was analyzed using a two-way analysis of variance (ANOVA, $\alpha=0.05$). Additionally, the effects of time of day and developmental phase on mean time spent in aggressive behavior was analyzed using the same test and arcsine transformation.

Results

Data were collected during 10 daylight dives and 12 sunset dives. Each of the main effects, phase and time of day, had 2 levels and for each combination of factors (IP and day, IP and sunset, TP and day, TP and sunset), 31 fish were followed, bringing the total fish observed during the study to 124 fish (Fig. 2).

Parrotfish spent most of their time swimming during both times of day, increasing from 51% during the day to 77% at sunset. Mean percent time feeding decreased from 46% during the day to 2% at sunset, and there was no IP feeding observed at sunset. Mean percent time being aggressive increased from 1% during the day to 2.5% at sunset. There was no stationary behavior observed during the day, but at sunset the mean percent time spent stationary was 16%.

An ANOVA test on feeding (Table 1) found mean percent time feeding to be significantly higher during the day than at sunset for both phases ($p<0.0001$) (Fig. 3). IP fish spent a significantly higher mean percent time feeding than TP fish ($p=0.04$), even though there was no feeding observed by IP fish at sunset. There was a significant interaction between phase and time of day affecting feeding ($p=0.0002$).

An ANOVA test on aggression (Table 2) found that mean percent time being aggressive was significantly higher for TP fish than IP fish ($p<0.0001$) (Fig. 4). Mean percent time being aggressive was significantly higher at sunset than during the day ($p=0.005$). There was a significant interaction between phase and time of day affecting aggression ($p=0.01$).

Discussion

Both developmental phases of *S. taeniopterus* had a higher mean percent time feeding during daylight than at sunset, supporting the hypothesis that on average, *S. taeniopterus* feeds more during daylight compared to sunset periods. Time of day was a more significant factor affecting feeding than developmental phase, showing that variation in feeding is explained best by time of day. Previous studies have had

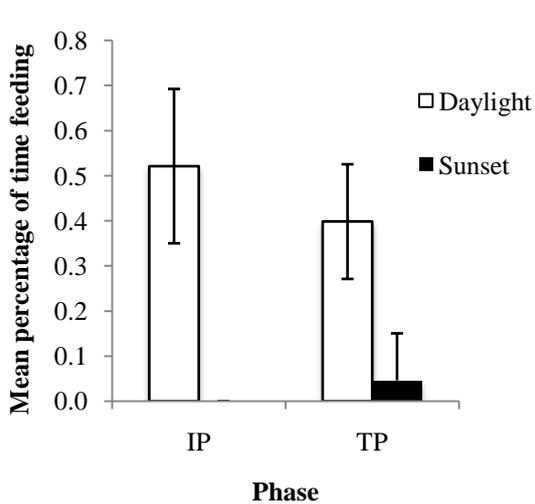


Fig. 3 Comparison of mean percent time feeding (\pm SD) between initial phase (IP) and terminal phase (TP) *Scarus taeniopterus* at daylight and sunset (n=31). White bars represent daylight periods, and black bars represent sunset periods

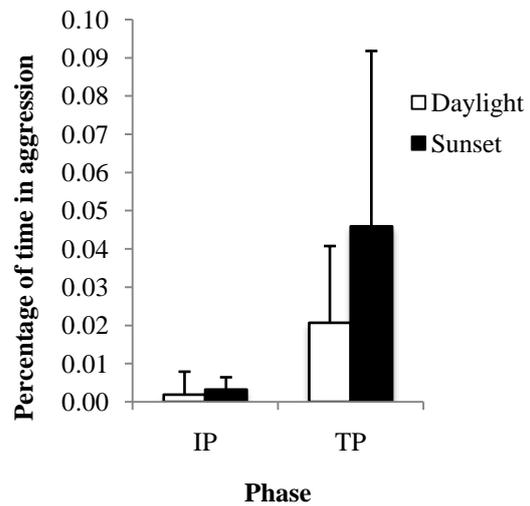


Fig. 4 Comparison of mean percent time spent being aggressive (\pm SD) between initial phase (IP) and terminal phase (TP) *Scarus taeniopterus* at daylight and sunset (n=31). White bars represent daylight periods, and black bars represent sunset periods

Table 1 Two-way analysis of variance (ANOVA) table percent time feeding (arcsine transformed) of *Scarus taeniopterus*. Factors tested each had two levels, developmental phase (IP or TP) and time of day (day or sunset)

| | DF | Sum of Squares | Mean Square | F-Value | P-Value |
|-------------------|-----|----------------|-------------|---------|---------|
| Phase | 1 | 0.082 | 0.082 | 4.161 | 0.0436 |
| Time of Day | 1 | 6.718 | 6.718 | 339.335 | <0.0001 |
| Phase*Time of Day | 1 | 0.301 | 0.301 | 15.227 | 0.0002 |
| Residual | 120 | 2.376 | 0.02 | | |

Table 2 Two-way analysis of variance (ANOVA) table for percent time spent being aggressive (arcsine transformed) of *Scarus taeniopterus*. Factors tested each had two levels, developmental phase (IP or TP) and time of day (day or sunset)

| | DF | Sum of Squares | Mean Square | F-Value | P-Value |
|-------------------|-----|----------------|-------------|---------|---------|
| Phase | 1 | 0.029 | 0.029 | 44.527 | <0.0001 |
| Time of Day | 1 | 0.006 | 0.006 | 8.362 | 0.0046 |
| Phase*Time of Day | 1 | 0.004 | 0.004 | 6.731 | 0.0107 |
| Residual | 120 | 0.079 | 0.001 | | |

similar findings during the sunset transition period, behavioral changes include decreased feeding for many reef fish (Hobson 1972). A small amount of feeding by some TP fish was observed at sunset, but no feeding by IP fish was observed. This

may be due to the timing of observations. TP fish are bigger, require more food to survive, and are less vulnerable to predation, so may continue to feed at sunset because risk of predation is lower and metabolic needs are higher than IP parrotfish.

When feeding, IP fish seemed to have longer continuous periods of taking bites, hovering near one head of coral, while TP fish would take a few bites before swimming to another location to feed or to chase away another *S. taeniopterus*. Discontinuous feeding of TP fish may account for the lower mean percent time feeding of TP fish than IP fish during the day. Some *S. taeniopterus* were observed to join feeding schools comprised mostly of Blue tang, but which also including Spanish hogfish, various parrotfish, Sergeant major, Atlantic trumpetfish, Schoolmaster, and trunkfish. Group foraging behavior has been previously documented, and, as some algal mats are well defended by damselfish, schooling may increase individual bite rates, because individual damselfish are overwhelmed by a school and can only defend against one fish at a time (Foster 1985). Future studies should examine biting rates of parrotfish in addition to time spent foraging, to compare amount of algae ingested and examine the hypothesis that feeding increases while schooling.

The mean percent time spent being aggressive was lower during daylight than at sunset for both developmental phases, as hypothesized. This study supports previous findings that during the sunset transition period there is increased aggression (Hobson 1972). Aggression was mostly directed at other *S. taeniopterus*. TP males were more aggressive than IP females at both times of day, as expected, male parrotfish must defend territories and mates from other male parrotfish (Streelman et al. 2002). Aggression is increased at sunset to address the increased migration of other fish into parrotfish territories (Hobson 1972), but phase was a more significant factor affecting aggression than time of day. Aggression seemed to become more pronounced as time went on during sunset periods, but this study did not examine changes in behavior over time within the sunset period. Fin flaring seemed to occur more often during sunset than daylight periods, but was not quantified in this study.

Time of day and developmental phase each separately affected the behavior of *S. taeniopterus* significantly. There was also a significant interaction between time of day and phase, meaning much of the variation in the results for feeding and aggression is due to the combined effect of both factors. Although differences in percent time swimming were not statistically tested, means were higher at sunset than daylight times for both phases. During sunset periods, some *S. taeniopterus* were observed performing tailstands, a head-up position in the water column thought to aid detection of predators and territory intruders (Dubin and Baker 1982). Some individuals also were observed swimming under coral heads and hovering stationary for short periods of time, as though trying out resting locations, but were not observed to return to any of them later, contrasting with previous studies showing strong affinity of some fishes to specific resting locations (Hobson 1972). Some *S. taeniopterus* were observed to swim under a coral head and stay there for the rest of the observation period, explaining the high mean percent time spent stationary during sunset periods.

Future work could include following one individual over several entire sunset periods to examine the level of affinity of individuals to certain locations. Through studying the behavior of *S. taeniopterus*, inferences can be drawn about the adaptations it has acquired to increase fitness and survival. Halting feeding in favor of increased aggression and migration during sunset periods allows individuals to protect suitable nighttime resting locations and avoid predation. Such adaptations are just one example of how coexistence is achieved among cohabiting organisms, the keystone of ecological success.

Acknowledgements

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Black spot disease of Ocean surgeonfish (*Acanthurus bahianus*) population in Bonaire, Dutch Caribbean

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Abstract

Diseases of oceanic species are difficult to research due to the ocean's vast size and the overall logistics involved in studying disease in organisms that live in aquatic environments. Disease in marine organisms may go undetected even when an outbreak occurs. A healthy organism's immune system can fight an infection, but if it is weakened due to stress the ability of the immune system diminishes. Increasing human impacts in the world's oceans stress organisms through exposure to pollution and global climate change, which can increase the number of diseases in marine organisms. In the last year an unidentified disease has been reported on the Ocean surgeonfish, *Acanthurus bahianus*. The disease causes black spots on the epidermis of the fish and the deterioration of the fins. This disease has only been observed in Bonaire, Curacao, and the Turks and Caicos, all in the Caribbean. The purpose of this study was to generate a disease scale to facilitate the quantification of the progression of the disease and to compare the results conducted during the warmer months to those of the cooler months. Additionally, timed swims were used to determine the frequency of disease at various depths on the reef. Ocean surgeonfish play an essential role as herbivores in the coral reef ecosystems and since this disease is affecting 82% of ocean surgeonfish in Bonaire; it is crucial to study the distribution of

Introduction

There are many causes of disease but the identification of the causative agents in marine organisms is lagging the identification of terrestrial diseases (Dobson and Hudson 1986). Marine diseases are especially difficult to research because of the special equipment needed, the variability of the ocean, and marine organisms often have a large distribution area (Vethaak and Rheinallt 1992). Excluding the effort needed to study marine diseases, it is also difficult to know how damaging marine diseases are because when a disease is identified there often is no baseline of information from beforehand to compare with new studies (Ward and Lafferty 2004). A marine disease can affect all aspects of a community. By reducing the numbers in one tropic level the other tropic levels are affected (Rodgers and Burke 1981; Raymundo et al. 2009).

Coral reefs are diverse ecosystems that

support many organisms and are subjected to natural and man-made disturbances (Ward and Lafferty 2004; Emslie et al. 2011). These disturbances lead to the loss of resilience and overall degradation of coral reefs (Ward and Lafferty 2004). Recently an outbreak of infectious diseases on coral reefs has become a significant cause of coral mortality and habitat loss in the Caribbean (Raymundo et al. 2009). The assorted effects caused by these disturbances include population decreases, diminished diversity, and abridged community structure (Raymundo et al. 2009). The population level effects then disrupt other higher-order community interactions that are directly connected to ecosystem stability that cushion the effects of mortality mediators such as diseases.

In Bonaire, Dutch Caribbean there is a new disease on *Acanthurus bahianus* (Ocean surgeonfish), that was first studied by Penn (2012) who found that 89% of *A. bahianus*

surrounding Bonaire have the disease. The disease causes black spots of various sizes on the epidermis is known as black spot disease. It is unknown what is causing this disease, however fish are subject to a number of parasitic, bacterial, viral and fungal diseases (Dobson and Hudson 1986). Additionally, immunity deficiencies due to changes in ocean salinity, global warming, or other environmental variables (Lahnsteiner et al. 2009) could allow for an otherwise innocuous causative agent to overcome the fishes defenses (Adedeji et al. 2012).

A. bahianus are found throughout the western Atlantic ranging from North America to Brazil and are very important to the coral reef community (Dunsmore 2008). Ocean surgeonfish provide important ecological services on coral reefs as herbivores that keep algal biomass from overtaking reefs (Lewis 1986; Reinthal and Lewis 1986), prey for carnivores (Lewis 1986; Reinthal and Lewis 1986), and have touristic value because divers seek to see them in their natural habitats (Dunsmore 2008).

The purpose of this research was to study black spot disease in *A. bahianus* population. The first objective was to identify the stages of the disease to facilitate the study of the disease in other locations. The second objective was to determine if there was a relationship between the time of year and percent of diseased fish by comparing results from the cooler part of the year (February – April) to the percent of diseased fish in the warmer months (September –November). Lastly, Penn (2012) proposed that the number of fish with disease decreased with depth and since disease spreads faster in warmer water (Boyett et al. 2007) that there would be more disease in fish in shallow water. There should be greater numbers of diseased fish in shallow water during the warmer months of the year if water temperature is a factor. Therefore, field research was conducted to test the following hypotheses:

H₁: The percentage of *A. bahianus* infected with the black spot disease is greater at the depths studied during the warmer months.

H₂: The mean number of individuals with spots decreases with increasing depth.

The results of this study will provide a comparison to Penn's (2012) research and provide an understanding of the stages, causative agent and role of the surgeonfish disease in Bonaire.

Materials and methods

Study site

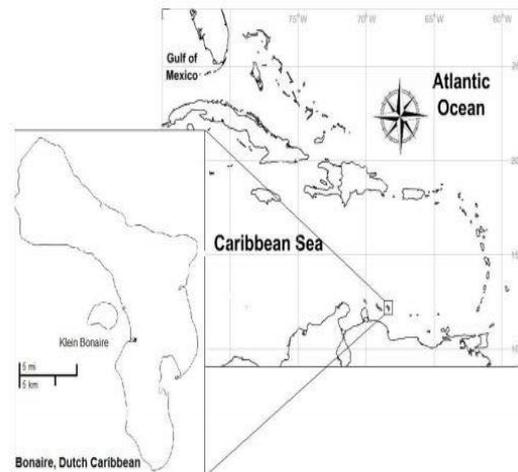


Fig. 1 Map of Bonaire, Dutch Caribbean, in the context of the Caribbean sea, insert above. The grey dot represents the study site, Yellow sub (N 12 09' 36.5'' W 068' 16'59.9'')

The study was conducted along the fringing reef of Yellow Submarine dive site in Bonaire, Dutch Caribbean (Fig. 1). Yellow Submarine dive site is just north of city of Kralendijk, where many people live and large cargo ships dock.

Bonaire also currently does not have a sufficient sewage treatment plan so much waste directly enters the ocean. The prevailing current usually carries any

pollution north by Yellow Submarine dive site. This is important because it could be a contributing factor in the spread of disease.

Methods

Photographs of infected and healthy *A. bahianus* were taken while SCUBA diving and snorkeling to provide a method for staging the disease in the field.

To determine if the percentage of *A. bahianus* infected with the black spot disease is greater during the warmer months, and if the mean number of individuals with spots decreases with increasing depth; 15 min timed swims were conducted while SCUBA diving. Divers entered the water haphazardly along the shore of the study site and always started against the current, to survey different areas each dive. First the surveyor descended to 18 m and swam slowing looking two meters in all directions and tallied the number of ocean surgeonfish on a slate. Observed fish were split into four categories: fish without spots, one to four spots, five to ten spots, and greater than ten spots. Only the first side seen of the fish was looked at when determining the number of spots on that fish. The diver continued with the two meter wide belt and ascending in a u pattern at 11 m, 5 m and 2 m (Fig. 2). The surveying process was repeated ten times over the course of five weeks (late September – early November).

Data analysis

The data collected was analyzed determining the mean number of individuals with spots at the four depths. An analysis of variance was also performed using a two way analysis of variance (ANOVA, $\alpha=0.05$). The percent of the diseased individuals at each depth was calculated and compared to the proportion of fish with disease during the cooler months of the year using a t-test.

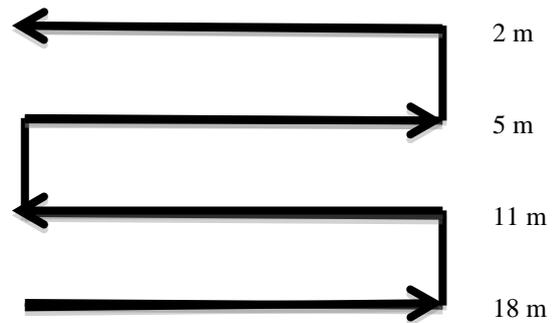


Fig. 2 Timed swims of 15 min were conducted at the depths of 2, 5, 11, and 18 m to survey the amount of diseased *Acanthurus bahianus* at Yellow Sub study site in Bonaire, Dutch Caribbean

Results

A total of 472 individuals were observed during 635 min of observation. Diseased fish were not observed at 18 m but were found at the other three shallower depths. The number of spots ranged on the diseased fish from one spot to over ten. Additional observations found a green plaster without spots on a few of the fish in the shallows.

The disease was categorized into three different stages. The healthy fish had zero spots. Stage one fish had one to four spots, Stage two fish had five to ten spots, and Stage three Fish had more than ten spots (Fig. 3). In stage three there was a variance between the degrees of infection, some fish had multiple spots on their fins while others were completely covered, had fraying fins, and were lethargic.

When testing the counts of various stages of the disease, the most significant result was the evenness among the disease stages (Fig. 4). The mean number of individuals that were healthy was $2.63 \text{ fish} \pm 1.27$, while overall there was a larger amount of mean diseased fish (5.32 ± 0.24). The largest difference of mean fish counts between stages was from healthy to stage one, while there were slight decreases found among the three stages.

Two-way ANOVA test of variance indicated that there were less diseased fish in that deeper areas and more diseased fish in the

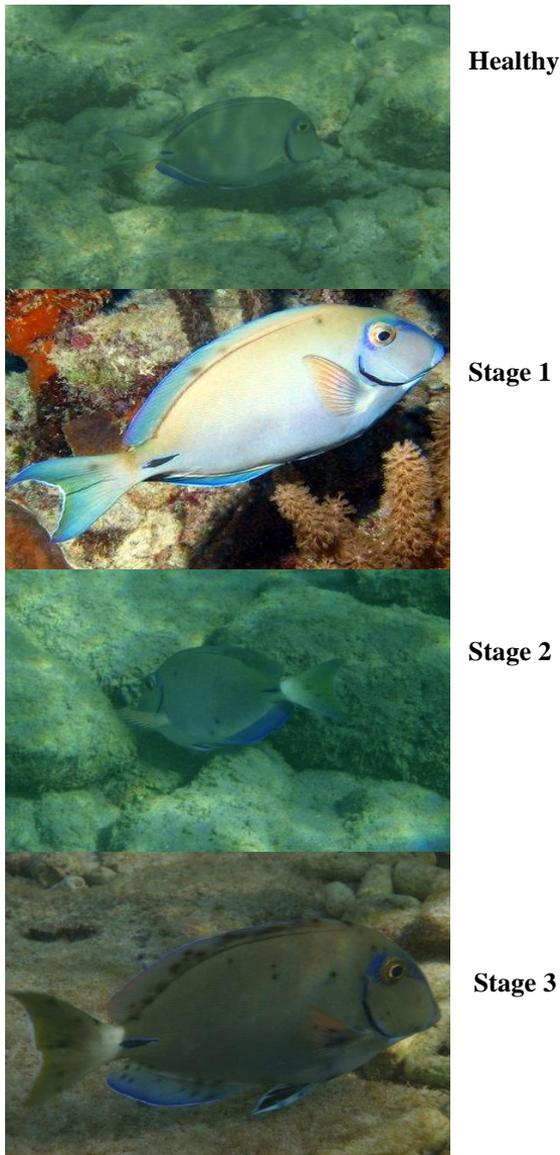


Fig. 3 The various stages of black spot disease in *Acanthurus bahianus* in Bonaire, Dutch Caribbean. A healthy fish has zero spots, stage 1 has one to four spots, stage 2 has five to ten spots, and stage 3 has more than ten spots. Photographs were taken in September 2012

shallow waters (Table 1). Further investigation using a Fisher's Protected Least Significant Difference (PLSD) test determined there was a statistical significance between the stages. The major differences were between healthy and stage one, stage one and stage two, and stage one and stage three (Table 2).

When estimating the overall percentages of infected individuals, 81.56% had black spot disease (n = 472).

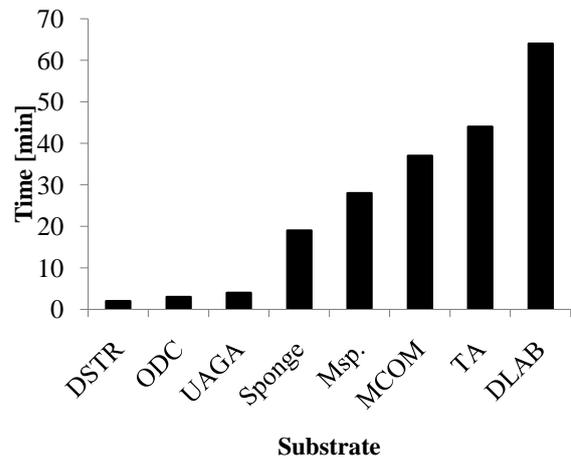


Fig. 4 At the depths of 18, 11, 5 and 2 m, 15 min timed swims were conducted at Yellow Submarine, Bonaire, Dutch Caribbean. The number of *Acanthurus bahianus* was surveyed with the number of black spots on each individual being recorded and placed in one of the four categories (Healthy, Stage 1, 2, and 3). The stages were separated by number of spots; Healthy had zero spots, Stage 1 had 1 to 4 spots, Stage 2 had 5 to 10 spots, and Stage 3 had greater than 10 spots

Table 1 Results of two-way ANOVA of level of disease among surgeonfish and depth of Yellow Sub dive site

| | P-value |
|------------|---------|
| 18 m, 11 m | 0.22 |
| 18 m, 5 m | < 0.01* |
| 18 m, 2 m | < 0.01* |
| 11 m, 5 m | < 0.01* |
| 11 m, 2m | < 0.01* |
| 5 m, 2 m | 0.13 |

When assessing the total percent of disease per depth, a relationship was found. As depth increased, the amount of disease decreased (Fig. 5). At 2 m there was an average of 93.78% diseased fish while there was 13.79% diseased at 18 m.

Table 2 Fisher's Protected Least Significant Difference test of surgeonfish density and level of disease identified

| | P -Value |
|------------------|----------|
| Healthy, Stage 1 | < 0.01* |
| Healthy, Stage 2 | 0.09 |
| Healthy, Stage 3 | 0.46 |
| Stage 1, Stage 2 | 0.02* |
| Stage 1, Stage 3 | < 0.01* |
| Stage 2, Stage 3 | 0.34 |

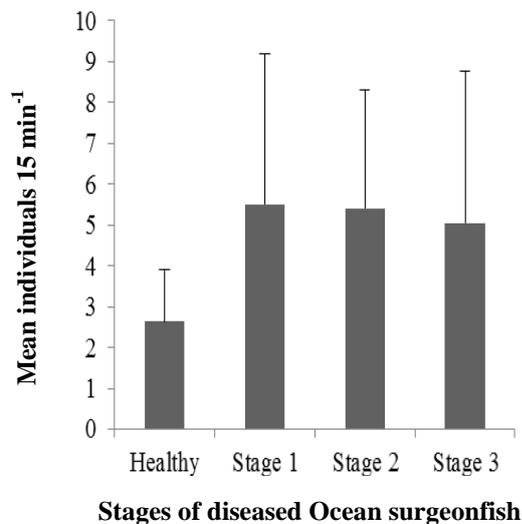


Fig. 5 The percentage of diseased *Acanthurus bahianus* across the depths of 18, 11, 5, and 2 m at Yellow Submarine study site on Bonaire, Dutch Caribbean. Data were collected during 15 min timed swims (n= 10 at each depth)

Using an ANOVA test of variance, this indicated a significant difference in density of fish at different depths ($p = <0.01$). Additionally, using the Fisher's PLSD test, significant differences were found between the abundance of surgeonfish populations and depth (Table 3). When comparing between the two shallow and the two deeper depths, the population densities were similar. However comparisons between a shallow

and a deeper depth showed population densities were significantly different.

Negative correlation between diseased fish and depth was also noted when looking at the mean of individuals per stage at each depth (Fig. 6). Healthy individuals were evenly spread throughout the depths while the other three stages varied in mean individuals per depth. Stage one had the highest mean number of individual fish at 5 m (8 ± 3) while the largest amount of disease was found in the 2 m depth. At 18 m there were no individuals found that were in stages two or three.

Table 3 Fisher's Protected Least Significant Difference test of surgeonfish density and depth

| | P- Value |
|---------------------------|----------|
| Depth (m) | < 0.01* |
| Disease Level | < 0.01* |
| Depth (m) * Disease Level | < 0.01* |

Discussion

While observing the diseased surgeonfish it was noticed that the disease seems to first appear along the dorsal fin with a few small circular spots. As the disease progresses it continues to affect the fins and other softer tissues until they are nearly covered. The spots do spread to other areas including the head and sides but the spots are more blotchy than circular. It also seems to be that the fish with the higher number of spots are more lethargic and can have deterioration of the fins; this could mean that the disease has more of an effect on the fish after it progresses. When comparing this study to Penn's (2012) study there is a decrease in black spot disease. Penn (2012) found that during 630 minutes of observation, 152 individuals were seen and estimated that black spot disease was affecting 89% of the Ocean surgeonfish. This decrease in black spot disease may be due to weather fluxes. When comparing data from the warmer months of September to

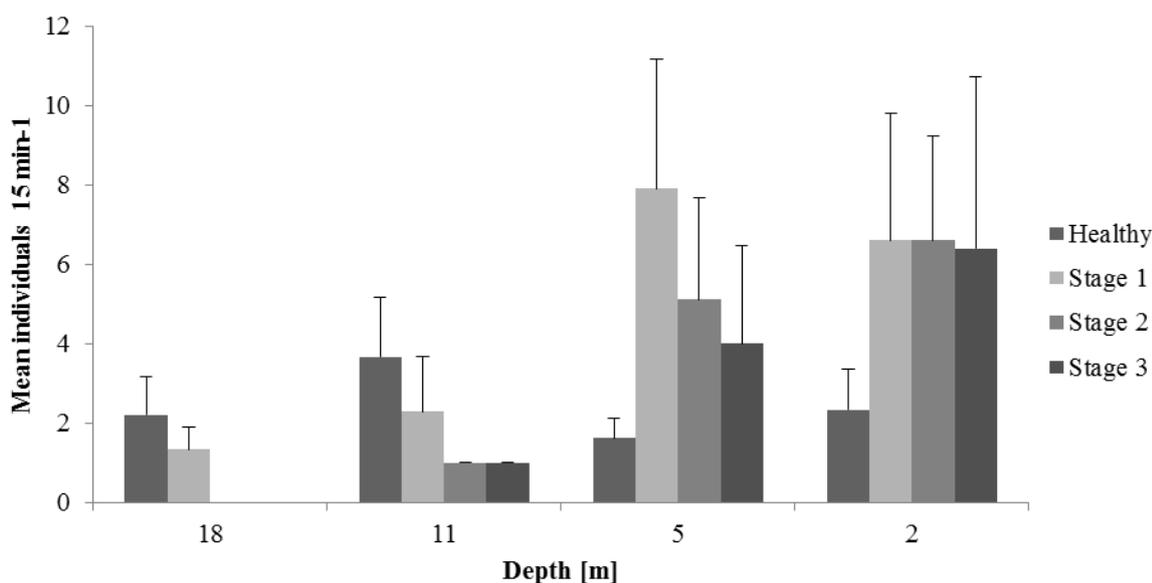


Fig. 6 At the depths of 18, 11, 5, and 2 m 15 min timed swims were conducted at Yellow Submarine study site Bonaire, Dutch Caribbean. The number of *Acanthurus bahianus* was surveyed with the number of black spots on each individual being recorded and placed in one of the four categories: Healthy fish had zero spots, Stage 1 had 1 to 4 spots, Stage 2 had 5 to 10 spots, and Stage 3 had more than 10 spots

October in this study to Penn's (2012) data of the cooler months of March and April, the percent of disease has decreased seven percent. However this decrease may also be because of larger sample size allowing for better accuracy. Penn (2012) surveyed only 152 fish while this study surveyed 472 fish thus this study is closer estimate of the actual percentage of disease.

More diseased fish were found at 18 and 11 m in Penn's (2012) study, while this study recorded more diseased fish in the shallower depths of 5 m and 2 m. Boyett et al. (2007) indicates that with an increase in temperature, there is an increase in disease.

However, this study found that during the warmer months, where the temperature of the water is warmer, there is a decrease in disease. Another indication of a temperature pattern could be the variation of distribution of surgeonfish across the four depths.

More surgeonfish were seen in the shallow waters (n=220), which is warmer, versus the deeper waters (n=87). In order to determine the flux between seasons and the distribution of surgeonfish in relation to

temperature more surveys are needed.

Results also indicated that the number of individuals with the disease does decrease with depth. A higher abundance of *A. bahianus* was found at 2 m than at 18 m. This distribution is at odds with Lawson et al. (1999) and Chapman and Kramer (2000) findings where most of the surgeonfish were found along the reef crest or deeper. With a shift from higher coral cover to higher algal cover estimated in Bonaire (Clements et al. 2009), Ocean surgeonfish individuals may be more abundant in areas with higher algal abundance such as the slope.

In addition to Ocean surgeonfish, black spot disease has been observed on Bar jacks, parrotfish, and Four-eyed butterflyfish during this study. With a decrease in total percent of disease found, currently 82% of Ocean surgeonfish are infected. This high incidence of disease has implications for marine disease management, applying to other species populations. Future research should first identify the pathogen causing this disease and determine the changes occurring on the surface of the fish as well

as inside of the fish. Second, water samples are needed to determine if black-spot disease is caused from natural or anthropogenic influences. Lastly, other places should be studied to create a baseline of this marine disease.

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Population demographic of *Pomacanthus paru* (French angelfish) in comparison to seafloor rugosity

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Abstract

Species conservation is becoming an important aspect in marine environments. Climate change and anthropogenic interactions have had a direct impact on the health of local species in all of the world's oceans, and coral reef ecosystems are no exception. Studies from around the globe have focused on angelfish demographics. In general, species within the family Pomacanthidae reside in places containing crevices and cliffs but are also seen in sandy areas with reef and seagrass patches. Some species are seen in groups of up to five fish, having one male in a harem of one to four females. Pomacanthidae are also known to predate on sponges and are generally found in areas which contain them. On the fringing reefs in the Dutch Caribbean, the species *Pomacanthus paru* (French angelfish) have not been extensively studied in terms of population in relation to maximum reef relief. To measure both aspects, 30 m transects were laid out at depths of 3, 6 and 15 m. The number of *P. paru* individuals as well as seafloor rugosity was documented and compared through statistical analysis during a five week period from September to October 2012. Results showed that most *P. paru* were found along the deeper transects of 6 and 15 m which had higher rugosity and juveniles were only seen in the less rugose, sandy flats at 3 m. Data collected will aid in providing a baseline for conservation efforts due to the declining state of natural environments, and in turn, the decline of individuals within populations.

Introduction

Ocean organisms all over the world have been exposed to deteriorating habitats. This is especially prominent on tropical coral reefs (Hughes et al. 2003). Overfishing, pollution and the constant need for reef resources are taking effect all over the world and are causing widespread habitat and species abundance loss (Hughes et al. 2003). It is therefore important to study the relationship between a species and its environment in order to protect habitats. This can be done by instituting conservation laws and by changing many harmful human practices.

The angelfish family Pomacanthidae has been found to occupy multiple habitat types in various tropical regions around the world, many of which have not been identified for every species within the family (Eagle et al. 2001). In the case of the *Pomacanthus paru*

(French angelfish), it is not clearly known where these fish generally reside.

Many different species in the Pomacanthidae family are known to predate on sponges (Pawlik et al. 1995). However, some angelfish species have been seen feeding on ascidians and tunicates (Bellwood et al. 2004). In the Sea of Cortes off the coast of Mexico, a study was conducted to investigate *Holacanthus passer* predation (Aburto-Oropeza et al. 2000). In order to determine feeding habits, *H. passer* were observed and their stomach contents were analyzed. The results showed that the species mainly fed on sponges but had three distinct feeding styles: 1) Predation on sessile invertebrates (e.g. sponges) 2) Planktivory and 3) Herbivory. A prime example of sessile invertebrate predation was observed in the Western Caribbean in 2009. Hawksbill turtles were feeding on sponges along the reef and it was common to

see angelfishes lurking nearby (Blumenthal et al. 2009). While the turtles ate, angelfishes would swim up and eat the pieces dropped or the parts of the sponge that were freshly uncovered. Gray, French, and Queen angelfish species were all observed in this commensal relationship with the turtles.

In regards to population and habitat type of angelfishes, a study was conducted in Japan on the species *Centropyge interruptus* including social groups of one to four females with the dominant male showing strong aggression towards other males (Moyer and Nakazono 1978). Hermaphroditism is occasionally found within the species. If the dominant male is absent from the harem (e.g. death, etc.) the leading female will go through a sex change to become the dominant male for the remaining group members. Although this relationship is not seen in all species, it does indicate that some angelfish species stay together in a group. The occupied areas mainly consisted of cliffs and tunnels, not always in places with coral cover (Moyer and Nakazono 1978). It was also noted that most groups populated ranges that were more fractured or had crevices (Moyer and Nakazono 1978).

Another study, performed on the Great Barrier Reef, investigated the populations of three smaller species of angelfishes (Eagle et al. 2001). The study showed a possible correlation between species abundance and topographic features within a habitat. All three preferred an environment consisting of mostly overgrown, dead branching coral but were also seen in areas of sand and at the reef crest. Although many individuals were found in the shallows, data showed that the fish were generally found in areas of complex corals (Eagle et al. 2001).

Angelfishes have been known to occupy several different microhabitats including turf algae, sand, rubble, and branching, massive, soft, and encrusting corals (Hobbs et al. 2010). In Abrolhos Archipelago in the Western South Atlantic, the cleaning practices performed by juvenile *P. paru*

were observed (Sazima et al. 1998). A total of 31 species of mostly rocky-bottom fishes were recorded swimming up to shallow cleaning stations that occurred in sandy areas with patches of reef and seagrass. The juvenile *P. paru* forms a mutualistic relationship with the larger fishes by eating debris and parasites off of the rocky bottom fishes.

This study aims to provide data on population demographics of *P. paru* in comparison to habitat type to gain information that may help in future conservation efforts for marine habitats in tropical regions worldwide. Little-to-no research has been published on the population demographic or the habitat type of *P. paru*. A baseline for both will be obtained for Bonaire so that future studies can show if the numbers of *P. paru* are rising or in decline. It will also be investigated to determine if there is a correlation between population demographics and reef rugosity. Based on results obtained by previous literature, hypotheses developed for this study are as follows:

- H₁: Juvenile and intermediate *P. paru* are more abundant in shallow areas (3 m) and terminal *P. paru* are more abundant in areas on the reef crest and reef slope (6-15 m).
- H₂: *P. paru* are more abundant in areas with higher rugosity and coral diversity.

Materials and Methods

Study site

The study used to determine habitat type and population size of *P. paru* was conducted at Yellow Sub dive site in Kralendijk, Bonaire, Dutch Caribbean (Fig. 1).



Fig. 1 Map of Bonaire Dutch Caribbean. The black star indicates Yellow Sub dive site in the capital of Kralendijk ($68^{\circ}16'55.16''\text{W}$; $12^{\circ}09'36.47''\text{N}$)

Field Research

To assess species richness, 30 m transects were laid out using SCUBA in shallow areas (3 m), the reef crest (6 m) and along the reef slope (15 m). Each transect was replicated five times at each depth for a total of 15 transects.

There was a 1-2 m space in between each transect so that the seafloor rugosity varied (Fig. 2). After each transect was laid, the observer waited 30 seconds before starting the survey in order to allow the fishes to come back. During the first pass along the line, all *P. paru* seen in the water column in a 5 m-wide belt were recorded as juvenile, intermediate or terminal phase (Fig.

3). The second pass along the transect line assessed seafloor rugosity. Reef relief was measured every meter using a 0.5 m stick. Data were collected in September and October 2012 over a span of five weeks. All transects were conducted between the hours of 10:00 hrs and 15:00 hrs.

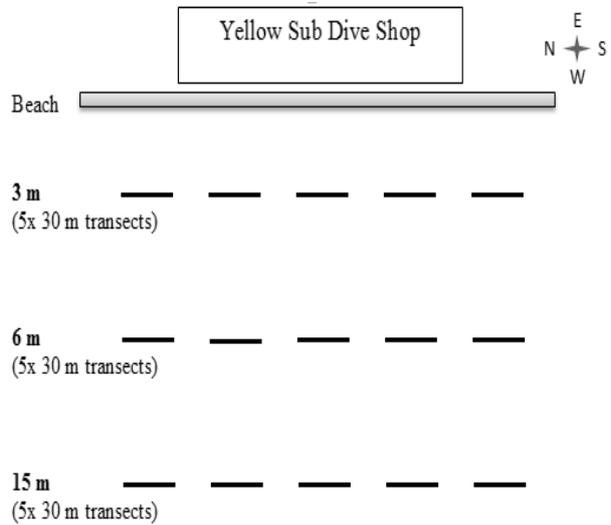


Fig. 2 Map of transect locations for population demographic study of *Pomacanthus paru* and reef relief. Five 30 m transects were laid out at each depth, 3, 6, and 15 m, for a total of 15 transects. There was 1-2 m in between each transect

Data Analysis

Sample size was averaged separately from reef relief at each depth. All results were compared and averaged into different categories of population size and rugosity in



A



B



C

Fig. 3 Different life stages of *Pomacanthus paru*. A) Juveniles have bright yellow vertical bands around the body with a yellow band around the tail B) Intermediates have light vertical bands on the body with varying degrees of yellow spots C) Terminals are darker, have no vertical lines on the body, and have many yellow spots

accordance to depth, and a correlation between average maximum reef relief of each transect and the number of *P. paru* in each transect was calculated. A graph comparing maximum reef relief and population size at each depth was generated and all data were evaluated through statistical analysis using the Kruskal Wallis test.

Results

Average seafloor rugosity had a tendency to increase with depth. At 3, 6 and 9 m the average maximum reef relief was 4 ± 9 cm, 12 ± 14 cm, and 77 ± 39 cm respectively (Fig. 4).

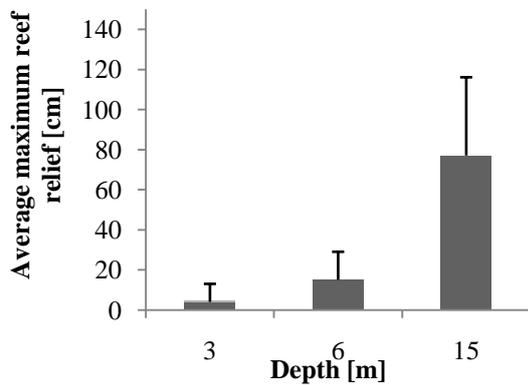


Fig. 4 Average maximum reef relief at 3, 6, and 15 m at Yellow Sub study site, Bonaire, Dutch Caribbean. Error bars indicate standard deviation. Data were collected using 15 transects of 30 m length and relief was measured using a 50 cm measuring stick

Although there was no statistical significance ($p > 0.05$) found using the Kruskal Wallis Test, the data showed that there were more terminal and intermediate *P. paru* at 6 m and 15 m than at 3 m. Juveniles were only found along the 3 m transects (Fig. 5). Although it seems that there was a weak positive trend in Fig. 6, the R^2 value of 0.0239 indicates otherwise, as there was no statistical significance.

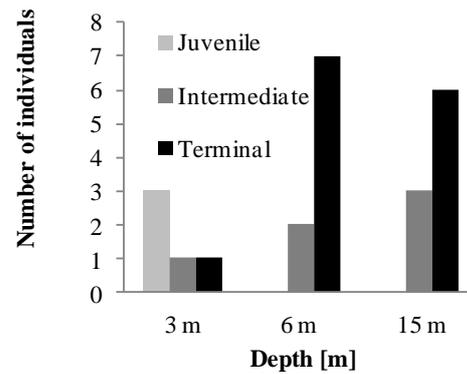


Fig. 5 Total number of individuals observed of *Pomacanthus paru*, and the life stage of each individual at depths of 3, 6, and 15 m at Yellow Sub study site, Bonaire, Dutch Caribbean. Life stage was categorized as juvenile (light grey), intermediate (dark grey), and terminal (black). Data were collected during September and October 2012 using 15 transects of 30 m length

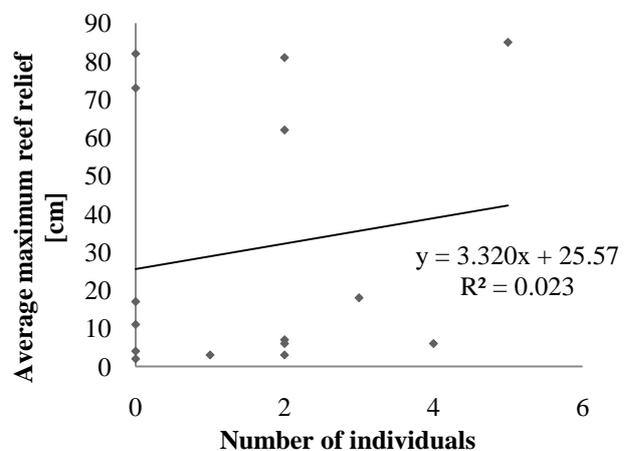


Fig. 6 Total number of *Pomacanthus paru* individuals observed compared to average maximum reef relief at Yellow Sub study site, Bonaire, Dutch Caribbean. Regression line is shown. The R^2 value indicates no statistically significant trend. Data were collected during September and October 2012 using 15 transects of 30 m length and a 50 cm measuring stick

Discussion

This study aimed to provide an understanding of the *P. paru* life stages in association with different habitat types. *P. paru* individuals were recorded more

frequently along the highly rugose 15 and 6 m transects and fewer individuals were recorded along the less rugose 3 m transects. These results supported a previous report which stated that some angelfish groups prefer fractured and more complex habitats (Moyer and Nakazono 1978). Interestingly, all life stages were found in the sandy flats which had less complex topography (3 m) but only intermediate and terminal *P. paru* were found in the deeper, more rugose areas (6 and 15 m). The data collected in the current study also showed a similar relationship to the study done by Eagle et al. (2001) where most individuals were recorded in areas with higher rugosity, but some were also recorded in the sandy flats. Topography is an important aspect for reef fish because highly rugose areas have an abundance of hiding places from predators and also contain more food sources than less rugose areas (Duckles and Stanesco 2010).

When different *P. paru* life stages were analyzed with rugosity, there was no statistical significance. The same was also true when analyzing the total number of individuals across the different depths. Only 15 transects were laid out thus a larger sample size would be needed for statistical analysis. It was therefore not possible to make any assumptions on habitat preferences for the different *P. paru* life stages. Further studies should be done to gain a better understanding of *P. paru* population demographic in comparison to habitat type.

Juveniles were only found along the 3 m transects and although there was no statistical significance this is important to note for future studies. The data indicates that both sandy areas near the shoreline as well as the reef are integral habitats for a fish during its lifetime. Without protection for these areas, *P. paru* and other species that act similarly could be in danger of population decline. This is especially important due to the declining state of our coral reefs worldwide. Marine Protected Areas, such as the one surrounding Bonaire, should not only focus on protecting the reef

but also the sandy areas due to the amount of juvenile fish that are found there.

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Reef fish assemblage and invertebrate cover in relationship to the degree of artificial reef isolation

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Abstract

Reef fishes and invertebrates are quickly losing their habitats due to widespread coral degradation. Artificial reefs are entering the spotlight as alternatives to this problem because they provide marine life with habitats. The role that artificial reefs will play in marine resource management is still unknown, partly because artificial reefs are often overlooked as alternatives due to a lack of knowledge about them. Without the right information on artificial reef placement, the reefs may be used inefficiently. This study focused on how reef fish assemblages and invertebrate coverage can be influenced by artificial reef isolation, distance from the natural reef. This study was conducted in Bonaire, Dutch Caribbean, at the Yellow Sub dive site. Six mooring blocks were visually censused for fish biodiversity and photographed to find percent invertebrate coverage. Three of the blocks were weakly isolated and the other three were strongly isolated from the natural reef. The study took place over a five-week span from September through October 2012. The only significant difference between the weakly and strongly isolated blocks was that there was higher fish abundance on the weakly isolated blocks. Fish biodiversity and percent invertebrate cover did not differ significantly between the two block isolations. A better understanding of what factors allow for more suitable habitats on artificial reefs will contribute to conservation efforts and could increase reef fish and invertebrate biodiversity and abundance.

Introduction

Biodiversity has long been considered an essential component to viable ecosystems. Due to negative anthropogenic effects on the earth, such as global warming, pollution, and over harvesting, the earth's biodiversity has decreased significantly (Naeem et al. 1999). With an extinction of a species all organisms in the ecosystem can be affected; coral reef ecosystems are no exception. Researchers are trying to find ways to preserve corals because reef fishes, as well as other marine organisms, species' biodiversity depends upon them (Richmond 1997). To remediate the rapid loss of coral reefs, the alternative solution of constructing artificial reefs is being explored. Artificial reefs have the potential to be very useful in fisheries management and reef restoration (Bortone 2011). The study of artificial reefs is a relatively young field (Bortone 2011) and

there is still a lack of knowledge that must be filled in order to create artificial reefs that increase species biodiversity.

Insufficient information is known in regards to constructing and employing artificial reefs effectively (Bortone 2011). There are many factors that must be taken into account when employing artificial reefs, including size, material, rugosity, design, and the placement distance from natural reefs. The abiotic factors that affect fish community assemblage on artificial reefs are still relatively unknown and researchers are still trying to determine optimum reef orientation and placement (Burt et al. 2009). There is also a need for quantitative data on artificial reefs, as most previous research done on artificial reefs has been qualitative (Bortone 2011).

This study aims to assess how artificial reef placement, particularly in regard to isolation from the natural reef, affects fish

biodiversity and invertebrate coverage. In a study done by Walsh (1985), it was found that isolation of an artificial reef can have a positive impact on fish diversity. Walsh (1985) credited this impact to decreased predation, reduced nest disturbance, and less competition for resources. Jordan et al. (2005) also found that fish abundance increased with increased reef isolation. Very little research distinguishes the biodiversity on isolated artificial reefs of juvenile fishes versus adult fishes. Similar to Walsh (1985) and Jordan et al. (2005), Carter et al. (1985) found high fish abundance on isolated artificial reefs but also observed that invertebrates with little or no motility were slow to colonize reefs that were more isolated.

It has been concluded that large concrete blocks, which were used in this study, are ideal for artificial reefs because they are structurally similar to natural reefs and can withstand harsh weathering by seawater longer than many other materials (Fitzhardinge and Bailey-Brock 1989). The present study was carried out on a flat area covered in sand at varying distances from natural reefs, which Turner et al. (1969) effectively showed would be an ideal environment for an artificial reef. The strongly isolated set of blocks was 35 m from the natural reef and the weakly isolated set of blocks was 15 m away from the natural reef.

Few studies have examined how the isolation from a natural reef affects fish communities. By looking for the degree of isolation that makes for the best habitat for fishes, data obtained in this study can advance the efforts to create the best habitats to conserve the earth's biodiversity. Three hypotheses are proposed for this study:

H₁: Strongly isolated artificial reefs have a greater fish biodiversity than weakly isolated artificial reefs.

H₂: There are more juveniles on strongly isolated artificial reefs than on weakly isolated artificial reefs.

H₃: There is more invertebrate cover on weakly isolated artificial reefs than on strongly isolated artificial reefs

Materials and Methods

Study site

The research for this study was conducted on the south side of the Yellow Sub dive site in Bonaire, Dutch Caribbean (Fig. 1). This site has a sandy flat area approximately 150 ft long between the shore and the reef. The sandy flat has many concrete mooring blocks located at different intervals between the reef and the shoreline (Fig. 2). The concrete blocks are ~1 m³ in size and were identified as artificial reefs. Two sets of three blocks were observed, one set that was strongly isolated, or 35 m, from the natural reef and the other that was weakly isolated, or 15 m, from the natural

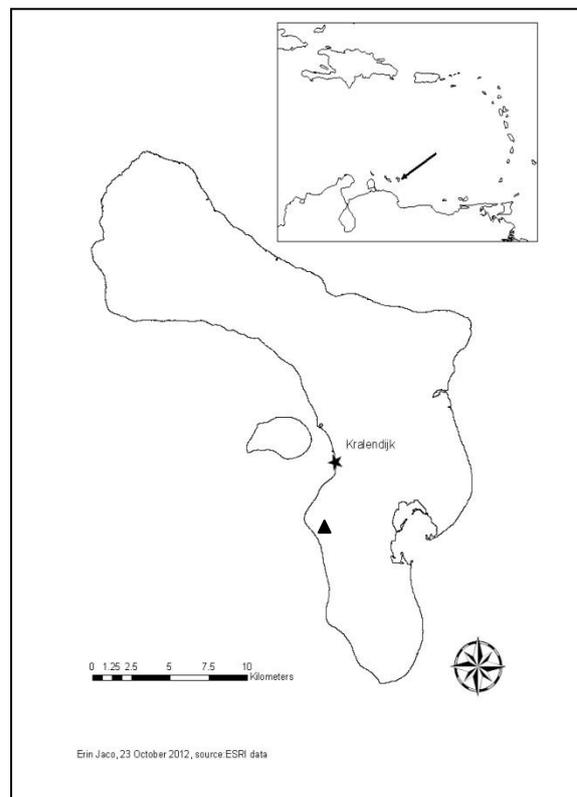


Fig. 1 Map of Bonaire, Dutch Caribbean. The black star marks Kralendijk and the black triangle marks the Yellow Sub study site (12°9'36.47"N and 65°16'55.16"W)

reef. The strongly isolated blocks were about 3 m underwater and the weakly isolated blocks were about 5 m underwater.

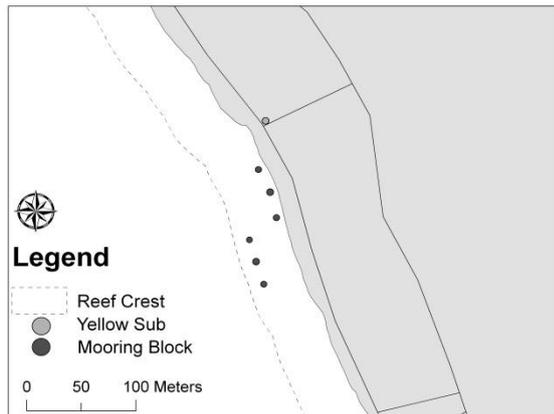


Fig. 2 Map of study site at Yellow Sub in Bonaire, Dutch Caribbean. The black circles mark the mooring blocks used in the study. The dashed line marks the reef crest. The grey circle marks the Yellow Sub dive shop (12°9'36.47"N and 6516'55.16"W)

Data collection

To assess the biodiversity of the fishes on the artificial reefs, a visual census was used. Following Walsh (1985), fishes were counted if they sheltered in the reef upon approach, stayed within a half meter of the reef during the survey, or if they returned directly to the reef after diver retreat. Fishes were initially surveyed while circling the mooring block in a 5 m radius to avoid scaring fishes away. The reef was then approached and examined closer. Holes and crevices of the block were examined to find smaller or shyer fishes. Care was taken to avoid recording fishes twice. Differentiation between adults and juveniles was made when possible. An underwater slate was used to record the fishes and an HD video camera helped with fish identification. Each block was censused three times over the duration of the experiment.

The invertebrate cover was analyzed using percent coverage. Estimations were made via photo analysis of the block sides. The sides were given a number between one and five with one being the side facing south and counting counterclockwise to four and side five being the top.

The blocks were observed between 9:00 hrs and 11:00 hrs for five weeks in the months of September and October 2012. The concrete mooring blocks south of the Yellow Sub dive site were mapped out to determine which blocks would work best for this study.

Data analysis

This study utilized descriptive and inferential statistics. The Shannon index was used to describe fish biodiversity. The Shannon index takes into account abundance and species evenness.

This index was used for, juveniles, adults, and all fishes. A Mann-Whitney U test was used to compare fish biodiversity and invertebrate coverage between the strongly isolated and weakly isolated artificial reefs. Mann-Whitney U tests are equivalent to t-tests but are used for non-parametric data.

Results

Fish assemblage

It was found that the two blocks with the highest fish biodiversity were weakly isolated blocks, with Shannon value (H') means (\pm SD) of 6.8 ± 0.88 and 7.12 ± 0.83 . However there was no significant difference using the Mann-Whitney U test ($p=0.275$) in biodiversity between the strongly isolated and weakly isolated blocks (Fig. 3).

A significant difference ($p=0.049$) was found between the average abundance of fishes on the strongly isolated and weakly isolated blocks (Fig. 4). All three isolated blocks had higher means of abundance (\pm SD), 108 ± 9.02 , 83 ± 14.57 , and 65 ± 12.06 , than the averages of weakly isolated blocks, 61 ± 19.5 , 52 ± 18.93 , and 63 ± 9.29 . There was not, however a significant difference ($p=0.827$) in the average species richness between the strongly isolated and weakly isolated blocks (Fig. 5).

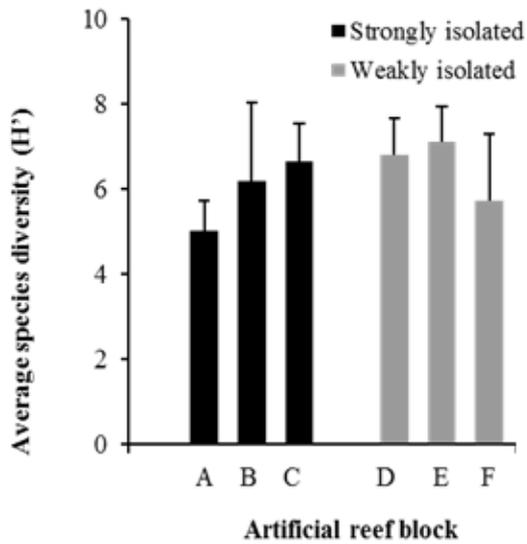


Fig. 3 Average species biodiversity for each artificial reef block, found using the Shannon diversity index. Error bars correspond to one standard deviation. There was no significant difference between the strongly isolated and weakly isolated blocks ($p = 0.275$)

There were only two fish species that had significant differences in abundances on the strongly isolated versus weakly isolated blocks. Both Slippery dick (*Halichoeres bivittatus*), with a mean (\pm SD) of 15.2 ± 8.07 on the strongly isolated blocks and a mean (\pm SD) of 24.4 ± 7.32 on the weakly isolated blocks ($p=0.01$), and Yellownose goby (*Elacatinus randalli*), with a mean (\pm SD) of 8 ± 2.74 on the strongly isolated blocks and a mean (\pm SD) of 12.8 ± 3.12 on the weakly isolated blocks ($p=0.027$), had a significantly higher average abundance on the weakly isolated blocks than the strongly isolated blocks (Fig. 6).

While the two blocks with the highest average juvenile abundance were weakly isolated blocks, with means (\pm SD) of 39 ± 7.77 and 44 ± 1.53 , there was not a significant difference ($p=0.275$) in the amount of juveniles on the isolated blocks versus the non-isolated blocks (Fig. 7).

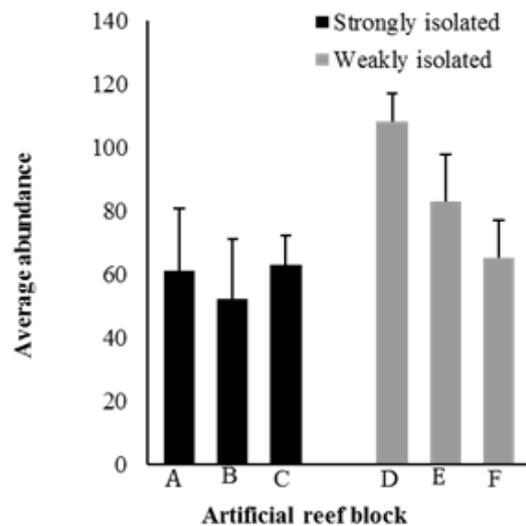


Fig. 4 Average abundance of fish for each artificial reef block, found using a visual census. Error bars correspond to the standard deviations. There was a significant difference between the strongly isolated blocks and the weakly isolated blocks (p -value = 0.049)

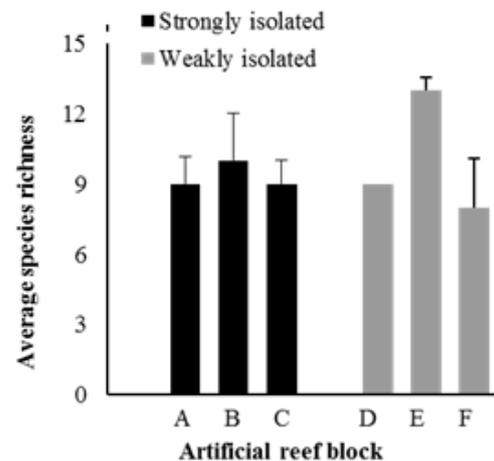


Fig. 5 Average species richness of fish, found for each artificial reef block using a visual census. Error bars correspond to one standard deviation. There was no significant difference between the strongly isolated and weakly isolated blocks (p -value = 0.827)

Taking into account the isolated blocks there were significantly more adults, with means (\pm SD) of 40 ± 18.72 , 50 ± 20.55 , and 42 ± 10.44 , found than juveniles, with means (\pm SD) of $21 \pm 13 \pm 2$, and 21 ± 10.02 , ($p = 0.049$), (Fig. 8). However, there was no significant difference ($p=0.383$) found between juveniles and adults on the non-isolated blocks.

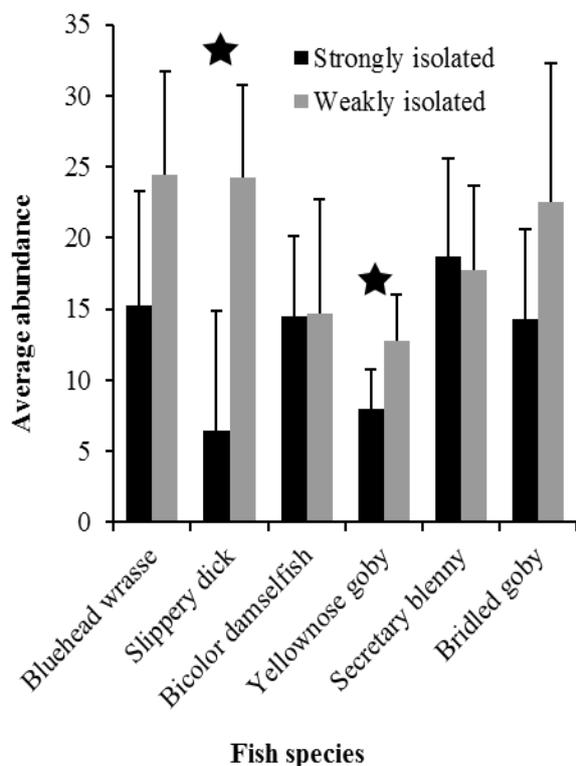


Fig. 6 Average abundance of the most common fish on the artificial reef blocks, found using a visual census. Error bars correspond to one standard deviation. The black stars indicate a significant difference. There was a significant difference found between strongly isolated and weakly isolated blocks for Slippery dick ($p = 0.01$) and Yellownose goby ($p = 0.027$), the rest of the fish showed no significant difference (p -value $> .05$)

Invertebrate cover

There was a wide range in percent invertebrate cover with the highest cover being Block F with 33.4% cover and the lowest being Block D with 14.7% cover (Fig. 9). Both the Block F and Block D are part of the weakly isolated group. There was no significant difference ($p=0.663$) of percent invertebrate cover between the strongly isolated blocks and the weakly isolated blocks (Fig. 9).

While no significant difference was found between the two sets of blocks, there was a significant difference found on the percent invertebrate cover between the

different sides of the blocks. Taking into account all six blocks together it was found that there was significantly more invertebrate cover (Table 1) on side 3 (the side facing north) with an average percent (\pm SD) of $34.3\% \pm 9.06$ (Fig. 10). It was also found that there was significantly less invertebrate cover (Table 1) on side 5 (the top face of the block) with an average percent (\pm SD) of $10.2\% \pm 4.9$ (Fig. 10). There were no significant differences for the rest of the sides of the blocks (Fig. 10).

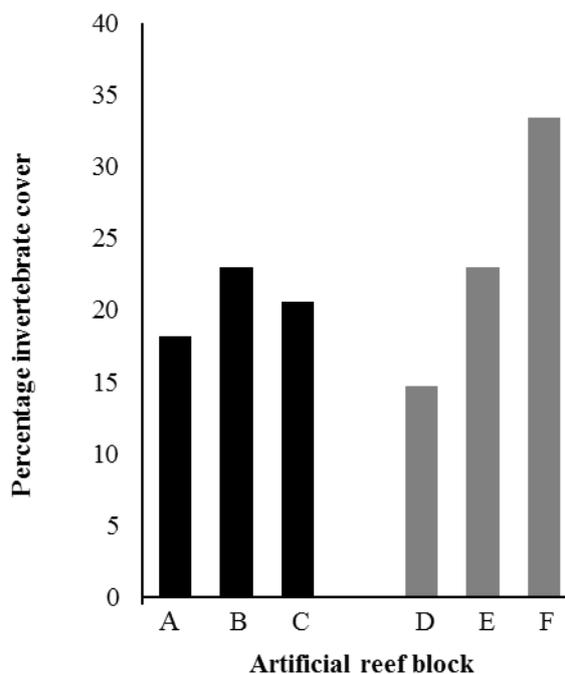


Fig. 9 Percent invertebrate cover for each artificial reef block, found using photos and a computer generated grid. There was no significant difference between the strongly isolated and weakly isolated blocks (p -value = 0.663)

Discussion

Fish assemblage

Using the Shannon index to calculate the fish biodiversity, no significant difference was found between the strongly isolated and the weakly isolated blocks, rejecting the first hypothesis. Fish species richness also presented no significant difference between the strongly and weakly isolated blocks.

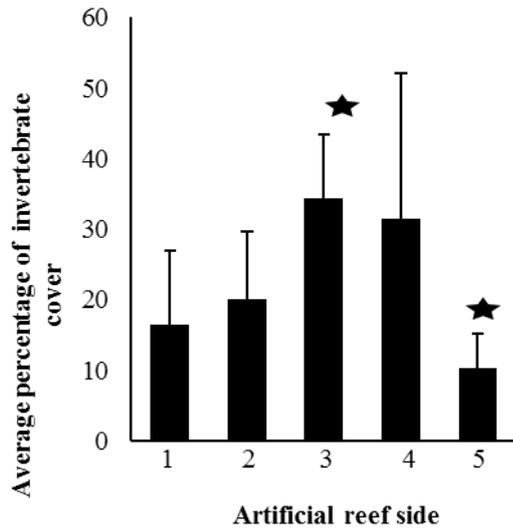


Fig. 10 Average percent invertebrate cover for each side of the six artificial reef blocks, found using photos and a computer generated grid. Error bars correspond to one standard deviation. Side three and five are marked with stars to indicate that they had significantly different percent cover than the other sides (Table 1)

Table 1 P-values of the comparison of the artificial reef block sides. The p-values were found using a Mann-Whitney U test. Asterisks indicate a significant difference

| Sides Compared | P-value |
|----------------|---------|
| 1v2 | 0.75 |
| 1v3* | 0.016 |
| 1v4 | 0.75 |
| 1v5* | 0.008 |
| 2v3* | 0.007 |
| 2v4 | 0.689 |
| 2v5* | 0.025 |
| 3v4 | 0.055 |
| 3v5* | 0.004 |
| 4v5 | 0.055 |

This indicates that fish biodiversity and species richness was similar between the blocks. Turner et al. (1969) found that while initially there was a difference in fish

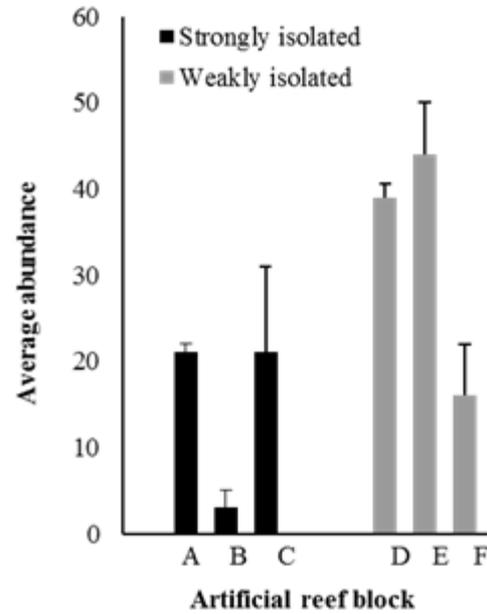


Fig. 7 Average juvenile abundance for each artificial reef block, found using a visual census. Error bars correspond to one standard deviation. There was a significant difference between the strongly isolated and weakly isolated blocks ($p = 0.275$)

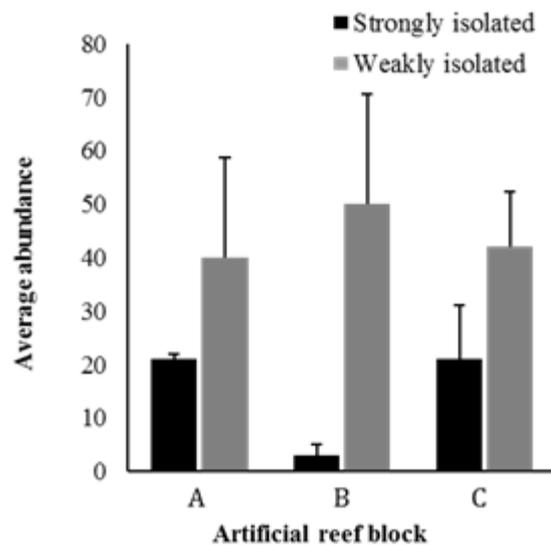


Fig. 8 Average juvenile and adult abundance for each isolated artificial reef block, found using a visual census. Error bars correspond to one standard deviation. There was a significant difference between the abundance of juveniles and abundance of adults ($p = 0.049$)

diversity between strongly and weakly isolated blocks; over time the fish populations reflected the source reef's equilibrium. Shulman (1985) reported that

the number of species present was relatively constant on reefs that were 20 and 40 meters away from their source reefs, which are roughly the distances used in this study. However Shulman (1985) did find that reefs that were 20+ m away from their source reefs had a significantly higher number of species than those less than 20 m away. Had this study also included a wider range of isolation distances, the results may have shown a significant difference between fish biodiversities and species richness. This would have corresponded better to the several studies, which found that isolation does affect fish assemblages (Walsh 1985; Jordan et al. 2005; Carter et al. 1985). There was a high connectivity between the artificial reefs and the natural reef in this study and may have contributed to the similarities in fish assemblages. Connectivity is a very important factor in fish settlement and may have affected the results of this study (Cowen et al. 2006).

While fish biodiversity and fish species richness were similar between the strongly and weakly isolated blocks, there was a significant difference in fish abundance. It was found that there were significantly more fishes on the weakly isolated blocks than the strongly isolated blocks. Five out of the six most common fishes found in the study had a greater abundance on the weakly isolated blocks, although only the Slippery dick and the Yellownose goby had a significantly higher abundance (Fig. 6.). Several studies found that fish abundance increased with a greater degree of artificial reef isolation from the natural reef (Walsh 1985; Jordan et al. 2005; Shulman 1985; Carter et al. 1985). This study is difficult to compare with past studies due to the relatively small-scale isolation and the large connectivity in the study. The strongly isolated blocks offered little of the benefits presented in studies by Walsh (1985) and Jordan et al. (2005) such as lower predation, less nest disturbance, and reduced competition for resources. The weakly isolated blocks may have been more ideal for fishes due to the close proximity of the resources that the natural reef provided.

Juvenile versus adults

The abundance of juveniles did not show a significant difference between the weakly and strongly isolated blocks, rejecting the second hypothesis. This indicates that juvenile abundance is similar between artificial reefs of varying degrees of isolation from the natural reef. In a study done by Walsh (1985) it was found that juvenile abundance was higher on strongly isolated blocks. One reason that the weakly isolated blocks had the same amount of juveniles may have been due to the fact that they were closer to the source of recruitment, i.e. the natural reef, which has been shown to effect juvenile abundance (Alevizon et al. 1985). Because there were significantly higher amounts of adults on isolated blocks than juveniles, results conclude that adults are more likely to colonize strongly isolated areas than juveniles.

Invertebrate cover

There was not a significant difference in the percent invertebrate cover between the strongly and weakly isolated blocks, rejecting the third hypothesis. This indicates that invertebrate cover is similar whether it is strongly or weakly isolated from the natural reef. This may be attributed to the high connectivity and the relatively small scale of this study as connectivity is considered a large factor in coral settlement (Cowen et al. 2006).

When determining the percent invertebrate cover, it was found that there was a significantly higher percent cover on the side facing north and a significantly lower cover on the top compared to the other sides when taking the average of each side of all of the blocks. Carter et al. (1985) found that the sand and detritus that settles on horizontal surfaces, i.e. the top of the blocks, affected benthic growth. This may be one explanation of why the top of the blocks had such little invertebrate cover. This may also explain why there was so much

invertebrate cover on the north face because the ocean current at the Yellow Sub dive site was observed to be a primarily southern current which may mean that there was more sediment and detritus, pushed by the current, on the southern faces and relatively little on the north faces.

The biggest limitation of this study was that only three replicates of each set of blocks were studied. In the future, more replicates of strongly and weakly isolated blocks should be observed. It is also recommended that future studies use artificial reefs that exhibit a greater difference in degrees of isolation, having only 20 meters between strongly and weakly isolated blocks was limiting.

Other than the finding that there was a higher abundance of fish on weakly isolated artificial reef blocks, this study rejects the hypothesis that there is a significant difference between weakly and strongly isolated artificial reef blocks from the natural reef in regards to reef fish assemblage and invertebrate cover. The finding that the amount invertebrate cover relates to the face of the block can help future studies understand what factors contribute to coral settlement.

While the scale of this study was small, it is still relevant to the deployment of artificial reefs as a conservation tool. The more information gained on artificial reefs, the more effective they will be. In order to conserve the biodiversity and abundance of and invertebrates alternatives must be explored to combat the degradation of coral reefs all over the world.

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***Vibrio* spp. communities associated with the azooxanthellate coral *Tubastraea coccinea* as compared to zooxanthellate shallow sea corals**

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Abstract

A coral is made up of key associations between endosymbiotic zooxanthellae, protists, bacteria, archaea, viruses, and fungi. These microbe-coral interactions can be very beneficial, some associations providing key functions in reproduction, nutrition, and antimicrobial protection. However, as a coral becomes thermally stressed, the ability to regulate microbe growth in its surface mucus layer becomes diminished and opportunistic pathogens are able to colonize. Corals may be able to adapt for the changing reef ecosystem by selecting for more beneficial associations: one of the facets of the coral probiotic hypothesis. The invasive azooxanthellate coral *Tubastraea coccinea* is able to colonize very shallow, hot and turbid areas that are not favorable for settlement by other species. However, not much is known about *T. coccinea* other than its invasive nature in the Caribbean. The purpose of this investigation was to determine if the surface microbial communities of *T. coccinea* are one of the factors aiding its survival. Culturing on Thiosulfate Citrate Bile Salt plates was used to visualize and compare the overall culturable *Vibrio* spp. communities present in *T. coccinea* and other widespread shallow corals. At each site, the numbers of *Vibrio* spp. were not significantly different between the three species, but numbers of a gram-positive bacteria, *Enterococcus* spp., were found to be significantly higher in *T. coccinea*.

Introduction

A coral does not operate as a single animal, but as a complex interaction of many different parts that include protists, algae, bacteria, archaea, viruses, fungi, and the coral host (Knowlton and Rohwer 2003). Together this system is referred to as the “coral holobiont”, which carries out its daily function using all these key players. Some host species are specific, and “winnow” during their planula stage to acquire a certain endosymbiotic zooxanthellae (Rodriguez-Lanetty et al. 2006). It has also been demonstrated that some species of scleractinian corals have specificity in their microbial communities (Rohwer et al. 2001). These associations are beneficial to the coral host in that the microbes are able to cycle nitrogen, to photosynthesize, and to provide antibiotic services (Barott et al. 2012). For example, species of *Porites astreoides* have been found to house cyanobacteria that are

hypothesized to aid in nitrogen cycling in the

coral (Wegley et al. 2007).

Many diseases affect coral reefs globally, and the frequency with which diseases are occurring and spreading through the Caribbean is rising (Carpenter et al. 2008). This increased instance of disease has caused this area to be considered a “hotspot” for microbial activity. This includes the island of Bonaire in the Dutch Caribbean, which is surrounded by a fringing reef and is affected by a variety of stressors including overfishing, sedimentation, diver damage, and nutrient effluent from the island’s outdated sewage system (Wieggers 2007), as well as global stressors like temperature rise and acidification. These factors all play a part in the positive feedback loop called the “DDAM hypothesis” (Rohwer and Youle 2010), which stands for Dissolved Organic Carbon, Disease, Algae, and Microbes. The increase in overfishing and removal of large

grazers causes an increase in algal cover. Algae are very good competitors with coral, and in most cases are able to overgrow the coral. This process occurs when the release of photosynthate from the algae causes an increase in dissolved organic matter. The microbes that live on the mucus layer, in the coral tissue, and in the coral skeleton feed on this dissolved organic carbon and create a hypoxic environment on the coral surface, which smothers the host coral (Barott et al. 2012).

Corals also have the ability to switch algal endosymbionts and microbial communities depending on environmental stressors (Ben-Haim et al. 2003). This discovery led researchers to develop the probiotic hypothesis (Reshef et al. 2006), which states that the dynamic changes in coral micro symbionts during periods of environmental flux will select for the most beneficial holobiont.

Temperature is one of the main abiotic stressors that cause a coral microbial community to shift from a beneficial community to a pathogenic one (Remily and Richardson 2006). Although the world of marine culturable microbes is very small, and researchers are increasingly turning to the genomic techniques to identify communities, the *Vibrio* genus is an ecologically and economically important bacteria, and has been researched mainly for certain species' ability to cause severe illnesses in humans. *Vibrio* spp. is a saltwater genus associated with many marine functions, from bioluminescence to coral bleaching. Many of these proteobacteria are also thermally tolerant, and prefer to reproduce in higher temperatures, allowing them to colonize a mucus layer after thermal stress. The seasonal bleaching of *Oculina patagonica* in the red sea occurs due to a species of *Vibrio shiloi* and only causes bleaching during the warmest months of the year (Kushmaro et al. 2001). The outbreak of *Vibrio* spp. induced diseases such as white band, yellow band, and white plague are also correlated with high temperatures (Bruno et al. 2007).

Tubastraea coccinea is an ahermatypic and azooxanthellate invasive coral species in the Caribbean. Its introduction in 1943 is hypothesized to have been from ship hulls or other ship transit, and the coral is now very widespread. In Bonaire, *T. coccinea* colonizes the shallows in a range of 0-3 meters (Creed 2006), but can also be found as far down as 30 meters. This shallow range is in an area of high sedimentation, nutrient flow, and higher temperature. *T. coccinea* is an azooxanthellate coral and therefore does not obtain the same byproducts of photosynthesis that zooxanthellate corals do. Many azooxanthellate corals employ other survival strategies to live especially in extreme environments. For example, the deep sea reef-building azooxanthellate scleractinian coral, *Lophelia petrusa*, houses a diverse microbial community that is hypothesized to provide beneficial services to the coral host (Galkiewicz et al. 2011). In shallow waters, *T. coccinea* also employs competitive colonization strategies. One of these strategies employs a "runner" that is a protrusion without polyps that detects colonizable area. It has also been observed that *T. coccinea* has faster colony growth compared to other corals (Vermeij 2005). *T. coccinea* is therefore able to out-compete many other hard corals that inhabit the shallow reef. In a study by Koh (1997), the antibiotic potential of coral surface microbes were tested using bioassays and culturing techniques. Several other corals in the genus *Tubastraea* proved to have the most bacterial resistance in these tests, but these studies were restricted to Pacific corals, and *T. coccinea* was not tested. Antimicrobial activity will be tested as a competitive strategy used in *T. coccinea* using culture techniques. The following hypothesis was formulated:

H₁: The surface mucus communities of *T.coccinea* harbors less bacterial members of the genus *Vibrio* than the surface mucus of shallow scleractinians *M. annularis* and *P. astreoides*.

Materials and Methods

Sampling sites

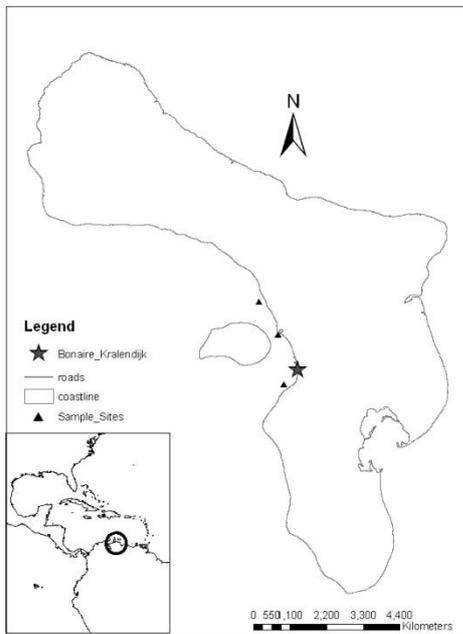


Fig. 1 Map of Bonaire, Dutch Caribbean, in the context of the Caribbean Sea, insert below. Sample sites are marked by triangles from North to South: Cliff, Something Special, and Windsock

Three dive sites along the western coast of Bonaire, Dutch Caribbean were sampled. The sites were chosen in terms of proximity to the main capital city, Kralendijk, as well as the presence of all key sample species (Fig. 1).

A site north of Kralendijk, Cliff ($12^{\circ}10'26.44''\text{N}$, $68^{\circ}17'26.62''\text{W}$) was sampled, along with Something Special ($12^{\circ}09'36.47''\text{N}$, $68^{\circ}16'55.16''\text{W}$), and a site south of Kralendijk, Windsock ($12^{\circ}7'58.50''\text{N}$, $68^{\circ}16'59.40''\text{W}$). All samples were collected using mask, fins and snorkel.

Culturing *Vibrio* spp.

Five mucus samples each of *Porites astreoides*, *Tubastraea coccinea*, and *Montastraea annularis* were taken at each

site with a 60 ml syringe. The coral was coaxed into producing mucus by aggravating the surface with seawater from the syringe. The sample therefore contained a mixture of mucus and seawater, and the mucus was targeted for culturing. Thiosulfate Citrate Bile Salt (TCBS) agar plates were used to culture, isolate, and enumerate colony-forming units of *Vibrio* spp. Each TCBS culture plate was inoculated with 0.1 ml of mucus and incubated at 28 degrees Celsius for 24 hours (Dinsdale et al. 2008).

CFU classification

Each colony-forming unit (CFU) was grouped into a category based on the Hardy Diagnostics description of culturable *Vibrio* spp. morphologies. Large yellow units were categorized as *Vibrio cholera/ Vibrio alginolyticus*. Large blue colonies with a blue, green center were categorized as *Vibrio parahaemolyticus*. Lastly, small clear to yellow colonies were categorized as proteus/enterococci.

Data analysis

Each plate was photographed and analyzed for colony growth. Analysis was done using Fiji, an image analysis software, and StatView for statistical analyses. The mean-ranks of CFU counts were then compared using Kruskal-Wallis one-way analysis of variance, and the Wilcoxon test to determine significant differences between cultured populations (Dinsdale et al. 2008).

Results

Site differences

The mean-ranks of total CFU numbers at each site were significantly different (Kruskal-Wallis $df=2$, $p<0.0001$); the average number of CFUs at Cliff was 499.5 ± 360 , 42.1 ± 57.3 at Something Special, and 47.6 ± 35.0 at Windsock.

CFU counts at Windsock were lowest for *M. annularis* (28.6 ± 23 , $p < 0.05$) and highest for *P. astreoides* (67.2 ± 44.3 , $p < 0.05$). There was no significant difference between CFUs in both zooxanthellate species (*M. annularis*, *P. astreoides*) and *T. coccinea* at this site. At Something Special, *T. coccinea* had the lowest CFU counts (13.3 ± 10.2 , $p < 0.05$), and *P. astreoides* had the highest count (95.3 ± 70.8 , $p < 0.05$). At Cliff, all three species had significant differences between one another with *P. astreoides* having the lowest density (228.1 ± 179.3 , $p < 0.05$), and *T. coccinea* having the highest density (803.1 ± 223 , $p < 0.05$).

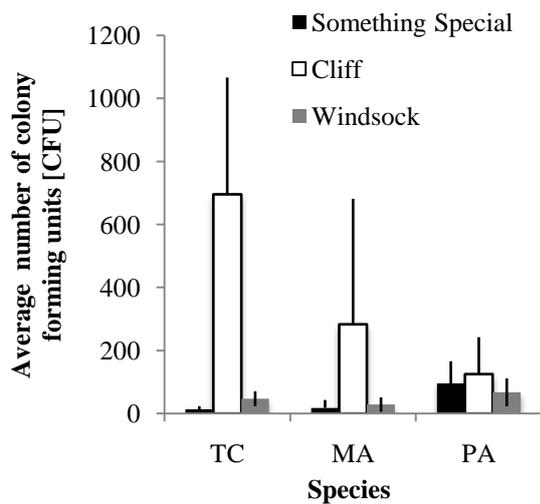
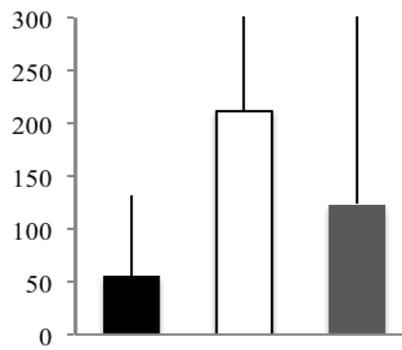


Fig. 2 Average number of colony forming units (CFU) counted on growth plates following inoculation for *Tubastraea coccinea* (TC), *Montastraea annularis* (MA), and *Porites astreoides* (PA) at sample sites Something Special (black), Cliff (white), and Windsock (grey). Total numbers of CFUs were counted on TCBS culture plates following a 24 hr incubation period at 28°C

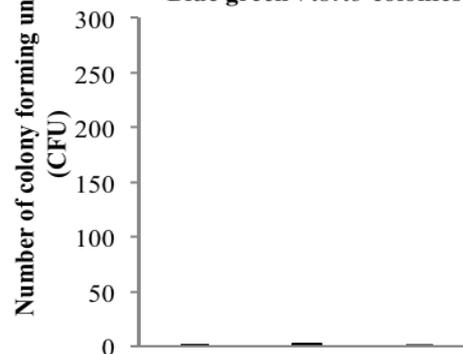
Species differences

Kruskal-Wallis test showed that mean-rank colony counts between species were significantly different ($df=2$, $p < 0.001$). The mean number of colony forming units counted for *T. coccinea* was 287.8 ± 389.8 , for *M. annularis* it was 171.2 ± 303.2 , and for *P. astreoides* it was 130.2 ± 132.2 (Fig. 2.). Bacterial population counts of *P.*

Small yellow *Enterococcus* colonies



Blue green *Vibrio* colonies



Large yellow *Vibrio* colonies

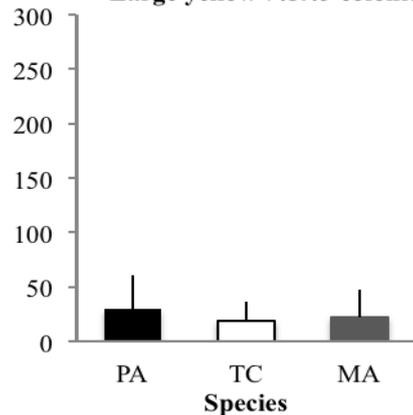


Fig. 4 Colony morphotypes (small yellow, blue green, large yellow) counted per species: *Porites astreoides* (PA, black), *Tubastraea coccinea* (TC, white), *Montastraea annularis* (MA, grey). Largest proportions of CFUs were identified as small yellow *Enterococcus* colonies and the smallest proportion was found in the blue-green colonies

astreoides were similar at Windsock and Something Special (Wilcoxon, $p > 0.05$), but were higher at Cliff ($n=228.1$, $p < 0.05$).

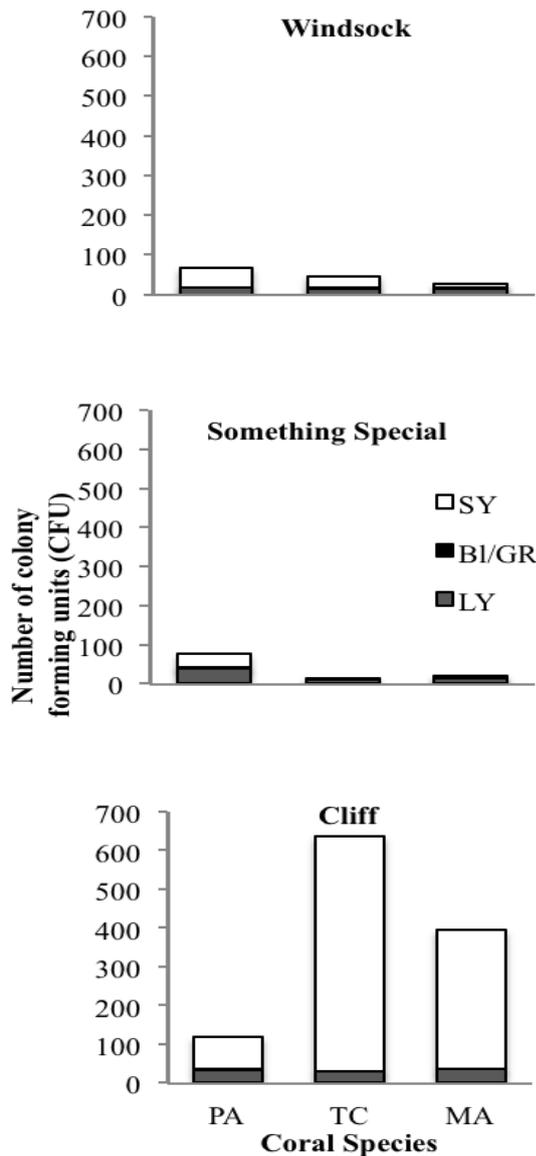


Fig. 3 Colony forming units (CFU) categorized counts at sample sites Windsock, Something Special, and Cliff. Large yellow colonies (LY, dark grey) are indicative of either *Vibrio cholera* or *Vibrio alginolyticus*. Blue green colonies (BL/GR, black) are indicative of *Vibrio parahaemolyticus*, and small yellow colonies (SY, white) were considered proteus/ enterococci

Populations of *M. annularis* were similar at both Windsock and Something special, but were also significantly higher at Cliff ($p < 0.001$).

T. coccinea had significantly low counts at Something Special (13.3 ± 10.2), then a count of 47.1 ± 23.5 at Windsock, and had significantly large densities at Cliff (803.1 ± 223).

CFU classification

The numbers of large yellow CFUs found in *P. astreoides* and *M. annularis* were not significantly different at any sites (Fig. 3). In *T. coccinea*, significantly higher amounts of large yellow CFUs were found at Cliff (29.4 ± 25.9) than at both Windsock (13.9 ± 7.3) and Something Special (11.4 ± 10 , $p < 0.05$). *T. coccinea* had highest amounts of blue green colonies at Windsock (3.6 ± 4.5), and lowest at Cliff (0.07 ± 0.03 , $p < 0.05$). Blue green colony counts for both *M. annularis* and *P. astreoides* did not differ between sites. Small yellow to clear colonies were significantly different in *T. coccinea* and *M. annularis* at all sites. Counts in *T. coccinea* and *M. annularis* were significantly highest at Cliff (605.7 ± 392.3 and 357.7 ± 373 respectively, $p < 0.05$).

Discussion

Based on observations and data collected over the course of this study there is not enough evidence to reject or accept my initial hypothesis. *T. coccinea* had the most total CFUs in each TCBS plate; however, a significantly large portion of CFUs counted were inferred to be *Enterococcus* spp., a gram-positive genera of bacteria. If these populations are taken out of the analysis (Fig. 4), the remaining *Vibrio* spp. populations at all three sites did not differ between coral species. For a more conclusive study, genetic techniques should be used to ensure bacteria species, as well as more frequent sampling of study species.

Zooxanthellate corals, *P. astreoides* and *M. annularis* had the most similarity between sites when it came to *Vibrio* spp. counts. Coral species exhibit specificity for endosymbionts and microbial communities, and will have similar baseline communities from site to site (Barott et al. 2012).

T. coccinea, although having the lowest amounts of *Vibrio* spp. did not have a significant difference between *Vibrio* counts of *M. annularis* or *P. astreoides*. *P. astreoides* demonstrated a high amount of

control for both its gram-positive (enterococci) and gram-negative colonies (e.g. *Vibrio* spp.). This suggests that *P. astreoides* may have high antimicrobial activity compared to other Caribbean corals.

A study by Lipp and Griffin (2004) demonstrated that surface mucus in shallow corals were very successful at bioindicating enterococci and fecal coliform contamination. Enterococci are an important water quality indicator, and *T. coccinea* mucus sampled in this study contained the highest amount of CFUs with an enterococci morphotype. The enterococci CFU population counts were significantly different between all three sites, with the lowest amount found at Something Special (0) and the highest found at Cliff. This suggests that *T. coccinea* mucus may be a beneficial biological indicator of water quality. The increased counts found were collected after a rainstorm, which may have led to effluent from the surface to runoff into the ocean. Large numbers of enterococci may have been caused by a change in incubation area, as our laboratory facilities became unusable. Although measures attempted to keep incubation temperature and sampling time the same, the change in incubation location may have had an impact on CFU numbers. Further analysis of water quality, especially at Cliff, would be necessary. In a previous *Physis* article, *T. coccinea* was found to facilitate growth of macroalgae that feeds epibionts and associated juvenile fishes (Pacheco 2008). Thus it would be beneficial to further investigate the internal metabolic processes of *T. coccinea*, and determine how it is able to survive macroalgal growth, high temperatures, surge, and sedimentation commonly found in the shallows.

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Status of reef health at Yellow Sub study site on Bonaire, Dutch Caribbean: progression of a coral - algal phase shift

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Abstract

Coral reefs around the globe are subject to environmental and anthropogenic stressors that are causing habitat degradation and a decline in reef resilience. Past studies of Caribbean reefs document a decrease in coral cover with a simultaneous increase in algal cover after significant stress, disturbance, or coral mortality. The long-term shift from coral dominated reefs to algae dominated reefs is known as a coral – algal phase shift. This study assessed the progression of a coral-algal phase shift at the Yellow Sub study site on Bonaire, Dutch Caribbean, by comparing current coral and algal benthic cover to historical data at a nearby study site. Research was conducted over a five-week period from September to October 2012. Twenty 10 m transects were filmed and analyzed through Coral Point Count software to determine percent live coral and algal cover. Mean coral cover at the study site was 14.25%, algae cover was 72.37% and the algae: coral ratio was 5.07. Diseases present were noted and included Yellow Band disease, White Plague, Dark Spot disease, and coral bleaching. In comparison to historical data at a nearby study site, a significant increase in the algae: coral ratio was observed, indicating the progression of a coral – algal phase shift at Yellow Sub study site. This study served to contribute to the scientific knowledge of Bonaire reef ecosystem resilience and the results obtained will help provide evidence and motivation to increase coral reef conservation efforts.

Introduction

Recent assessments of coral reef health place one third of the world's coral species at risk for extinction, indicating an immediate need for increased conservation efforts (Huang 2012). Some reefs are expected to be in irreversible decline within the next ten years (Titlyanov and Titlyanova 2008). Both global climate change and local anthropogenic stressors have been identified as primary causes for coral reef degradation and are targets of worldwide research to assess anthropogenic impacts (Aronson and Precht 2006). Coral reefs in the Caribbean are not exempt from this global trend. Case studies in the US Virgin Islands, Jamaica, Bonaire, Curacao, and other locations have documented declines in coral reef health by measuring the percentage of benthic substrate occupied by live coral over time (Goreau 1992; River and Edmunds 2001;

Bries et al. 2004; Bak et al. 2005; Rogers and Miller 2006).

The first scientific observation of a transition from coral to algal dominance was in a 25-year study in the Caribbean by Antonius and Ballesteros (1998). Coral-algal phase shifts occur as environmental conditions on reefs cross thresholds for optimal coral growth and make reversal to a coral dominated state unlikely (Fung et al. 2011). Algae recruit on dead substrate and compete with coral colonies for space (Aronson and Precht 2006). Furthermore, an over-abundance of algae inhibits coral recruitment (Titlyanov and Titlyanova 2008). Algal contact may also serve as a trigger for onset of coral disease (Nugues et al. 2004).

Notably, disturbances in Jamaica have led to the degradation of reefs that now show no evidence of recovery (River and Edmunds 2001). After strong hurricanes passed over the shores of Jamaica, the live

coral percent cover decreased considerably. Following this disturbance, an increase in the percentage of algal benthic cover was observed (Goreau 1992). In a 2001 study of Jamaican reefs, River and Edmunds reported the reefs had less than 5% live coral cover and more than 90% algal cover.

Bonaire and the surrounding Dutch Caribbean islands have been subject to environmental and anthropogenic threats in recent years. Stressors affecting these islands include overfishing that reduced herbivorous species (McManus et al. 2000), hurricanes and storms causing mass mortality (Bries et al. 2004), climate change affecting ocean temperature, reduction of the sea urchin *Diadema antillarum*, eutrophication, and physical damage by human traffic (Stokes et al. 2010).

On Bonaire, reefs serve as an important economic resource and are vital biological habitats. Increased stressors that affect the marine environment have resulted in a degradation of reefs around the island in the last 30 years (Stokes et al. 2010). A study completed by Stokes et al. (2010) gave a comprehensive view on the state of coral-algal phase shifts at multiple sites in 2008. Stokes et al. (2010) reported that at sites surveyed, live coral cover was half the recorded values in 1982 while algal cover increased between two and twenty fold, defining reef algal dominance as an algal-coral percent cover ratio of higher than one.

Disease presence is also considered a substantial threat to reef health in the Caribbean. Of the coral diseases described worldwide, 76% have been documented in the region (Steneck et al. 2011). In a study by Steneck et al. (2011) on Bonaire, 77% of species studied were identified as diseased. Yellow Band disease, Dark Spot disease, White Plague disease, Red Band disease, and White Band disease, and coral bleaching were considered most common (Steneck et al. 2011).

In order to advance scientific research on phase shifts and ecological implications, small-scale rate of change assessments need to be completed (McManus and Polsenburg

2004). This study contributes to the base of knowledge on phase shifts by conducting a small-scale survey on the island of Bonaire at the Yellow Sub reef dive site. The Yellow Sub reef is located within close proximity to the capital city of Kralendijk and is subject to island pollution and runoff, boat travel, and diver traffic. No data has been collected at this site in previous studies on the progression of a coral-algal phase shift. This study compared results to historical surveys at other locations on Bonaire in order to measure the health of Yellow Sub reef in contrast to other island sites. Results will contribute to the scientific knowledge of coral-algal phase shifts and serve as reference data for long-term reef health monitoring on Bonaire.

By studying coral and algal cover percentages, conclusions can be drawn on current reef health, degradation over time, and resilience (Stokes et al. 2010). This principle served as the base for this study. Verification of study hypotheses provides evidence of and reason to evaluate human impacts on near-shore reefs at Bonaire and to increase conservation efforts. The hypotheses developed for this study were as follows:

H₁: The algal-coral ratio on Yellow Sub reef is greater than one are higher than ratios observed in 2008.

H₂: Yellow Band disease, Dark Spot disease, White Plague, and coral bleaching are the most frequently observed diseases on Yellow Sub reef.

Materials and Methods

Study site

The study site was located in the southern Caribbean Sea on the island of Bonaire, Dutch Caribbean. Transect surveys were conducted at the Yellow Sub study site (12°09'36.47"N 68°16'55.16"W) at Kralendijk, on the leeward side of Bonaire (Fig. 1). At the Yellow Sub study site, five

areas were chosen for surveying (Fig. 2). The areas were centered at the site entrance marked by a small-scale artificial reef. The survey covered depths from six to fifteen meters.

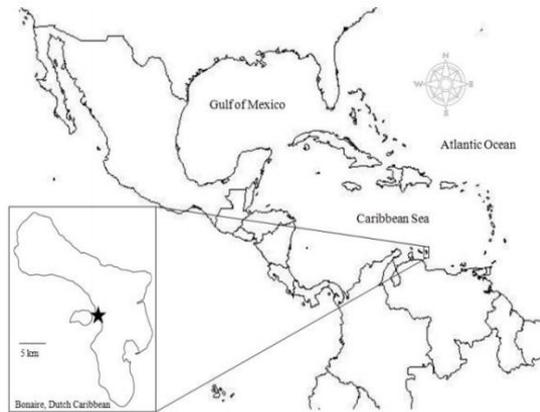


Fig. 1 Map of Bonaire, Dutch Caribbean, in the context of the Caribbean Sea. The study site, Yellow Sub (black star), is located in the capital of Kralendijk (12°09'36.47"N 68°16'55.16"W). Map modified from Alves, Physis Volume XI, 2012

Field research

In past studies of benthic cover, transect surveys were widely used and yield accurate data (Goreau 1992; Antonius and Ballesteros 1998; Bries et al. 2004; Stokes et al. 2010). Videography is accepted as a reliable method that allows for further analysis of benthic cover transects in a laboratory setting and storage of a permanent record of reef transects (Stokes et al. 2010). This study employed the use of video transects and laboratory video analysis to determine percent coral and algal cover as well as disease presence.

Within each of the five sample areas, 10 m transects were filmed at depths of 6 m, 9 m, 12 m, and 15 m (Fig. 2). Twenty total transects were surveyed. The 10 m tape was placed strategically to avoid damaging any sessile organisms. Using Ocean Images housing with aiming rod and Sony Handycam camcorder, transects were filmed at a height of 0.5 m on the east side of the

transect tape. Two to four transects were filmed per dive during daylight hours. Filming was completed at a slow and steady pace to optimize video quality. If a noticeable current was present, video was taken in the opposite direction.

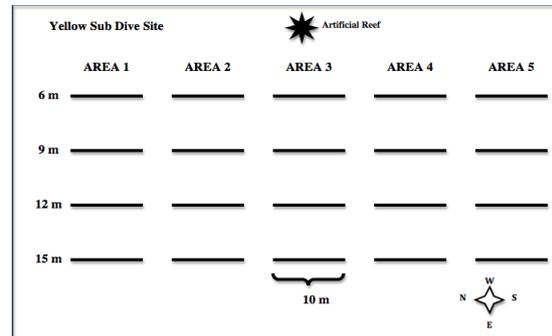


Fig. 2 Transect locations at Yellow Sub reef on Bonaire, Dutch Caribbean for coral and algal cover survey. Transects were 10 m in length at depths of 6, 9, 12, and 15 m. Distance between areas was 1-2 m

Laboratory research

Coral and algae cover

Video data from each transect was offloaded using the Picture Motion Browser (PMB) v5.2 PC software. Start and end times were recorded for each. Using the Microsoft Excel random number generator, 15 time points were selected as still frames. The PMB video-trimming function was used to save 15 still frames from video. Frames did not overlap.

Still frames were opened in Coral Point Count (CPCe) v4.0 Windows software. Fifteen randomized points were placed on each still frame. Each point was scored using pre-selected categories: coral, gorgonian, sponge, macroalgae, coralline algae, overgrowing organism, sand/rubble/pavement, other organism, and tape/wand/shadow. Corals were identified to species level. Macroalgae category includes turf algae along with other macroalgae identified to the genus level. A total of twenty transects were filmed, 300 still frames generated, and 4,500 points identified.

Upon completion of CPCe entry for each transect, data were exported and saved to a Microsoft Excel file containing the data summary. The CPCe program calculated total coral percent cover, coral species percent cover, and macroalgal percent cover. Mean coral and algal percent cover was determined for the study site, for each area, and for each depth. Algae: coral ratio was calculated by dividing the mean algae percent cover by the mean coral percent cover.

Coral disease

Original transects uploaded to PMB were analyzed for disease separately from percent cover. The entire length of the transects were surveyed to record presence of coral disease. Diseases were noted only on presence in transects, not severity. Diseases were recorded, noting disease name and coral species affected. The total number and types of diseases recorded and number of diseases and types observed affecting each species was calculated.

Data analysis

The Mann-Whitney U-Test was used to determine the significance of differences in coral and algae mean percent cover between each area and each depth in this study.

Comparisons were also made between coral disease presence between areas and between depths as an average and for each species in this study. Diseases recorded were totaled across depths, areas, and species affected.

The Mann-Whitney U Test was used to compare historical data sets to the current study results for both percent coral cover and algae: coral ratio. Coral cover and the algae: coral ratio in this study were compared with data collected in 1997 and 2008 as described by Stokes et al. (2010). The historical data referenced was collected at the Cliff study site (68°17'26.62"W 12°10'26.44"N) north of Yellow Sub study site on Bonaire, Dutch Caribbean (Fig. 3).

Cliff and Yellow Sub reef communities are similar in depth distribution and divers frequently visit both. The study sites were considered sufficiently similar in this study to allow for comparisons. Comparisons were made by choosing historical depth data points closest to those used in this study. Missing data points were estimated by trendline where possible. The 1997 data did not include cover at 15 m.

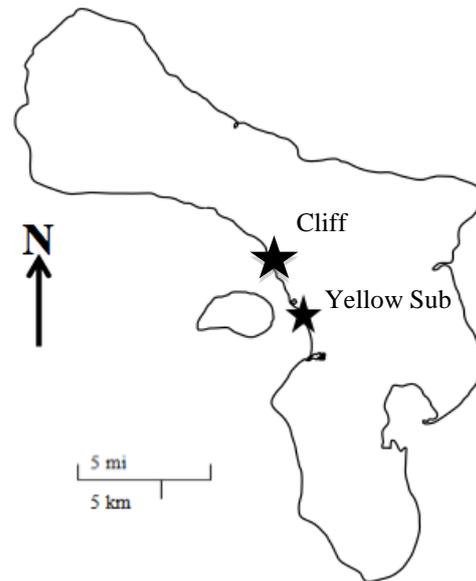


Fig. 3 Map of Bonaire, Dutch Caribbean. Study sites Cliff (68°17'26.62"W 12°10'26.44"N) and Yellow Sub (12°09'36.47"N 68°16'55.16"W) are marked with black stars. Map modified from Code, Physis volume X, 2011

Results

Mean percent coral cover

Mean percent coral cover at the Yellow Sub study site was $14.25 \pm 7.20\%$ (Table 1). The highest percent coral cover across depths was at 15 m with the least coral cover at 6 m (Fig. 4). Mean percent coral cover across depths was as follows: 6 m ($4.76 \pm 4.98\%$), 9 m ($12.65 \pm 7.11\%$), 12 m ($19.05 \pm 2.60\%$), 15 m ($20.56 \pm 7.93\%$). The highest percent coral cover across areas was in area 4 with the least in area 1.

Mean percent coral cover across areas was as follows: area 1 ($9.58 \pm 5.49\%$), area 2

(15.20 ± 10.88%), area 3 (14.35 ± 13.43%), area 4 (17.19 ± 5.04%), area 5 (14.95 ± 7.45%).

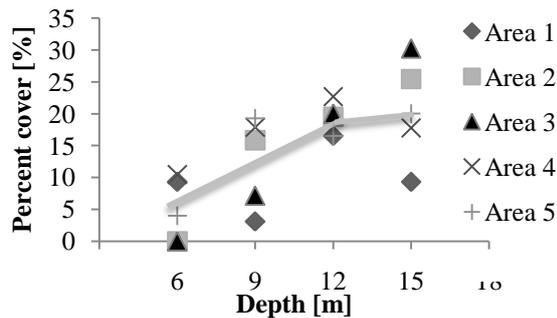


Fig. 4 Mean percent coral cover for areas 1–5 as a function of depth. Grey line depicts total mean percent coral cover. Data collected from Coral Point Count analysis of 20 video transects during September and October 2012 in Bonaire, Dutch Caribbean

The Mann-Whitney U-Test showed that percent coral cover was significantly higher at 12 m than at 6 m ($p < 0.01$) and at 15 m than at 6 m. There was a significantly lower percent coral cover ($p < 0.05$) in area 1 compared to area 4.

Coral cover by species

Species surveyed in this study include *Undaria (Agaricia) agaricites* (AAGA), *Montastraea annularis* (MANN), *Montastraea faveolata* (MFAV), *Meandrina meandrites* (MMEA), and *Porites astreoides* (PAST). These species yielded comparable data with percent cover greater than 0.20% (Table 2). Other species observed were *Diploria spp.*, *Siderastrea siderea*, and *Millepora spp.* that comprised less than 0.20% mean cover.

Highest percent cover by species was MANN closely followed by AAGA (Fig. 5). Lowest percent cover species was MMEA. Coral species cover is as follows: MANN (4.98 ± 3.94%), AAGA (4.49 ± 4.19%), MFAV (1.94 ± 2.84%), PAST (0.64 ± 1.10%), MMEA (0.45 ± 0.77%).

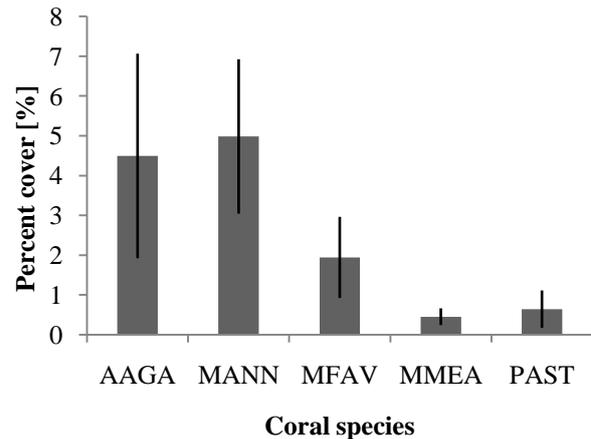


Fig. 5 Total mean percent cover by species (±SD). Coral species codes as follows: AAGA (*Undaria (Agaricia) agaricites*), MANN (*Montastraea annularis*), MFAV (*Montastraea faveolata*), MMEA (*Meandrina meandrites*), PAST (*Porites astreoides*). Data collected from Coral Point Count analysis of 20 video transects during September and October 2012 in Bonaire, Dutch Caribbean

MANN cover was highest at 12 m (8.66 ± 4.19%) and lowest at 6 m (2.14 ± 2.77%) (Fig. 6). AAGA cover was highest at 15 m (9.17 ± 4.40%) and lowest at 6 m (0.43 ± 0.43%). MFAV cover was highest at 15 m (3.47 ± 5.36%) and lowest at 6 m (1.02 ± 1.09%). PAST cover was highest at 12 m (1.21 ± 1.75%) and lowest at 6 m (0.00 ± 0.00%). MMEA cover was highest at 9 m (0.81 ± 1.13%) and lowest at 15 m (0.26 ± 0.40%).

The Mann-Whitney U-Test showed MANN had significantly higher mean coral cover than MMEA ($p < 0.01$), PAST ($p < 0.01$) and MFAV ($p < 0.05$). AAGA covered significantly more area than MMEA ($p < 0.01$), PAST ($p < 0.01$) and MFAV ($p < 0.05$). MFAV cover was significantly higher than MMEA cover ($p < 0.01$) and PAST ($p < 0.05$) mean cover of MANN and AAGA, MMEA and PAST ($p > 0.05$).

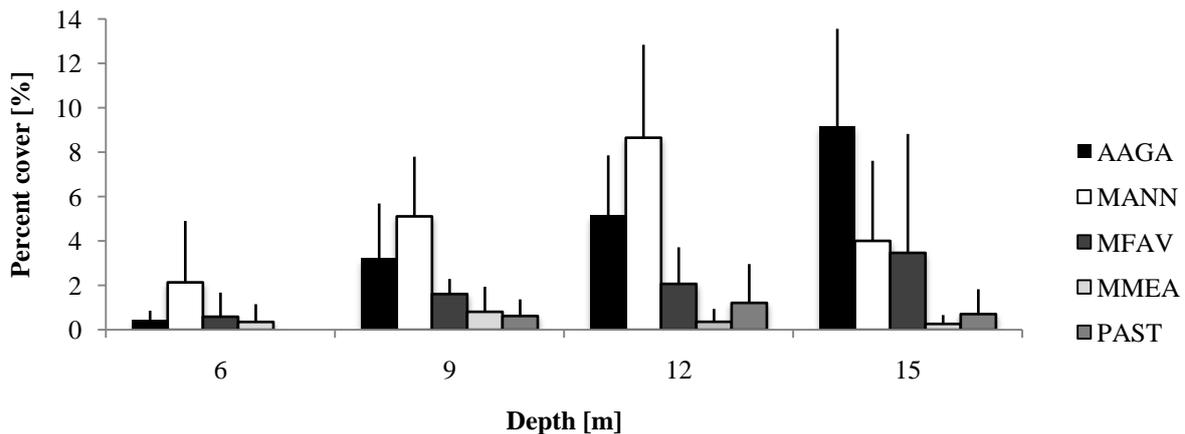


Fig. 6 Mean percent cover (\pm SD) by species as a function of depth. Coral species codes as follows: AAGA (*Agaricia (Undaria) agaricites*, black), MANN (*Montastraea annularis*, white), MFAV (*Montastraea faveolata*, darkest grey), MMEA (*Meandrina meandritis*, lightest grey), PAST (*Porites astreoides*, medium grey). Data collected from Coral Point Count analysis of 20 video transects during Sept ember and October 2012 in Bonaire, Dutch Caribbean

Mean percent algae cover

Mean algae percent cover was $72.37 \pm 11.63\%$ (Table 1). Algae cover in this study included macroalgae and turf algae. The highest algae cover across depths was at 6 m with the least at 12 m (Fig. 7). Mean percent algae cover across depths was as follows: 6 m ($87.91 \pm 12.40\%$), 9 m ($74.60 \pm 8.29\%$), 12 m ($62.73 \pm 11.00\%$), 15 m ($64.23 \pm 9.31\%$). The highest algae cover across areas was in area 3 with the least in area 2. Mean percent algae cover across areas was as follows: area 1 ($70.06 \pm 20.48\%$), area 2 ($69.63 \pm 20.77\%$), area 3 ($75.40 \pm 18.37\%$), area 4 ($72.13 \pm 2.06\%$), area 5 ($74.13 \pm 4.81\%$). The Mann-Whitney U-Test showed significant higher ($p < 0.01$) percent algal cover at 6 m compared to 12 m.

Coral disease

Yellow Band disease, Dark Spot disease, White Plague, and coral bleaching were observed on transects at the study site (Table 3). Yellow Band disease was seen affecting PAST, MANN, and MFAV. White Plague was found on MFAV and AAGA. Dark Spot disease was seen affecting

MFAV. Coral bleaching was observed in MMEA, MANN, AAGA, and PAST. No significant difference was found in disease occurrence across depths or across areas.

Yellow Sub site algae: coral ratio

The mean ratio of algae to coral cover was 5.07 ± 1.21 (Table 1). The algae: coral ratio across depths was highest at 6 m and lowest at 15 m (Fig. 8). Algal: coral percent cover ratio across depths was as follows: 6 m (18.70), 9 m (5.92), 12 m (3.30), 15 m (3.13).

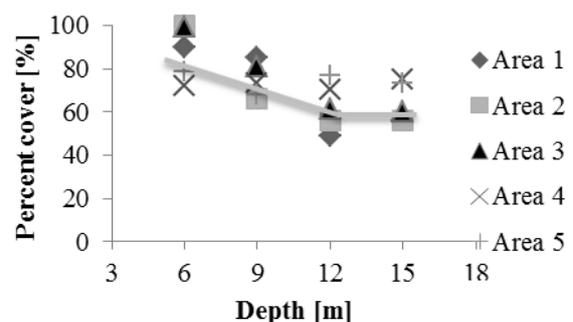


Fig. 7 Mean percent algal cover for areas 1–5 as a function of depth. Grey line depicts total mean percent algal cover. Data collected from Coral Point Count analysis of 20 video transects during Sept ember and October 2012 in Bonaire, Dutch Caribbean

Table 1 Summary of video transect percent coral and algae cover at Yellow Sub reef study site on Bonaire, Dutch Caribbean by area and depth over five-week study period from September to October 2012. Average cover and standard deviations calculated across areas and depth. Average algae:coral ratio was calculated using all transect data points. SD indicates standard deviation

| Depth | Area 1 | | Area 2 | | Area 3 | | Area 4 | | Area 5 | | Average | | | |
|---------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------|---------|-------|
| | % coral | % algae | % coral | SD | % algae | SD |
| 6 m | 9.33 | 89.78 | 0 | 100 | 0 | 99.11 | 10.46 | 71.97 | 4 | 78.67 | 4.76 | 4.98 | 87.91 | 12.40 |
| 9 m | 3.11 | 85.33 | 15.84 | 66.06 | 7.17 | 80.71 | 17.86 | 73.21 | 19.28 | 67.71 | 12.65 | 7.11 | 74.60 | 8.29 |
| 12 m | 16.53 | 49.11 | 19.52 | 56.19 | 20 | 61.33 | 22.67 | 70.22 | 16.52 | 76.79 | 19.05 | 2.60 | 62.73 | 11.00 |
| 15 m | 9.33 | 56 | 25.45 | 56.25 | 30.22 | 60.44 | 17.78 | 75.11 | 20 | 73.33 | 20.56 | 7.93 | 64.23 | 9.31 |
| Average | 9.58 | 70.06 | 15.20 | 69.63 | 14.35 | 75.40 | 17.19 | 72.63 | 14.95 | 74.13 | 14.25 | | 72.37 | |
| SD | 5.49 | 20.48 | 10.88 | 20.77 | 13.43 | 18.37 | 5.04 | 2.06 | 7.45 | 4.81 | 7.20 | | 11.63 | |
| Algae : coral | 7.32 | | 4.58 | | 5.26 | | 4.22 | | 4.96 | | 5.07 | | | |

The ratio across areas was highest in area 1 and lowest in area 4. Ratio across areas was as follows: area 1 (7.31), area 2 (4.54), area 3 (5.24), area 4 (4.19), area 5 (4.97).

Comparison to historical data

Coral cover and the algae: coral ratio was compared to available historical data as described in Stokes et al. (2010).

Coral cover at Cliff in 1997 was as follows: 6 m (32.00%), 9 m (30.30%), 12 m (29.30%) (Table 4). Coral cover at Cliff in 2008 was as follows: 6 m (5.86%), 9 m (26.6%), 12 m (28.00%), 15 m (30.10%). Overall average coral cover at Cliff in 1997 was $30.53 \pm 1.37\%$ and higher than 2008 Cliff cover of $22.64 \pm 11.28\%$ (Table 4). Both these means were higher than the 2012 Yellow Sub cover of $14.25 \pm 7.20\%$. No significance was found between this historical coral cover data and the data collected during this study.

However, the mean algae: coral ratio in 2008 at Cliff (2.38 ± 0.54) was significantly lower than at Yellow Sub in 2012 (5.07 ± 1.21) ($p < 0.05$). The ratio was lowest in 1997 and highest in 2012 (Fig. 9). The mean algae: coral ratios in 1997 at Cliff was as

follows: 6 m (3.40), 9 m (1.00), 12 m (0.70) with an overall mean of 1.7 ± 1.48 (Fig. 9). The mean ratios in 2008 at Cliff was as follows: 6 m (1.80), 9 m (3.10), 12 m (2.20), 15 m (2.40).

Discussion

Progression of coral – algal phase shift

The percent coral cover documented in this study is consistently below the ranges observed in the past. This indicates a possible decline in resilience and habitat degradation at Yellow Sub reef study site. The mean percent coral cover recorded in this study (~14%) is lower than historical mean coral cover on Bonaire, Dutch Caribbean. Coral cover on the island was consistently ~48% from 1999-2009 according to Steneck et al. (2011).

In 2008, the study by Stokes et al. (2010) shows an average percent coral cover of 23% - 38% on the leeward shore of the island. In 2011, estimates of island coral cover were ~38% (Steneck et al. 2011). The mean percent algae cover (~72%) at Yellow Sub study site was higher than values observed in previous studies on Bonaire. In 2008, the algae cover ranged from 10% - 65% (Stokes et al. 2010) and in 2011, the

Table 2 Summary of percent coral cover by species across area and depth. Coral species codes as follows: AAGA (Undaria (Agaricia) agaricites), MANN (Montastraea annularis), MFAV (Montastraea faveolata), MMEA (Meandrina meandritis), PAST (Porites astreoides). Average cover and standard deviations calculated for each species. Total average species cover calculated using all percent cover data points. SD indicates standard deviation

| Area | Depth | Species cover |
|---------|-------|---------------|---------------|---------------|---------------|---------------|
| 1 | | MANN | MFAV | AAGA | MMEA | PAST |
| | 6 m | 2.67 | 0.00 | 0.44 | 1.78 | 0.00 |
| | 9 m | 1.78 | 0.89 | 0.00 | 0.00 | 0.44 |
| | 12 m | 7.59 | 4.46 | 3.57 | 0.00 | 0.89 |
| | 15 m | 2.22 | 0.00 | 4.00 | 0.00 | 0.44 |
| | Avg | 3.57 | 1.34 | 2.00 | 0.45 | 0.44 |
| 2 | | MANN | MFAV | AAGA | MMEA | PAST |
| | 6 m | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 9 m | 6.33 | 2.26 | 4.07 | 2.71 | 0.00 |
| | 12 m | 8.57 | 1.43 | 4.76 | 0.48 | 4.29 |
| | 15 m | 8.93 | 0.89 | 14.73 | 0.00 | 0.00 |
| | Avg | 5.96 | 1.15 | 5.89 | 0.80 | 1.07 |
| 3 | | MANN | MFAV | AAGA | MMEA | PAST |
| | 6 m | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 9 m | 2.69 | 1.79 | 1.35 | 1.35 | 0.00 |
| | 12 m | 14.22 | 0.00 | 5.78 | 0.00 | 0.00 |
| | 15 m | 6.67 | 12.89 | 6.22 | 0.44 | 0.00 |
| | Avg | 5.90 | 3.67 | 3.34 | 0.45 | 0.00 |
| 4 | | MANN | MFAV | AAGA | MMEA | PAST |
| | 6 m | 6.69 | 2.51 | 0.84 | 0.00 | 0.00 |
| | 9 m | 7.14 | 2.23 | 4.91 | 0.00 | 0.89 |
| | 12 m | 10.22 | 1.78 | 2.22 | 1.33 | 0.44 |
| | 15 m | 0.44 | 0.89 | 12.44 | 0.00 | 0.44 |
| | Avg | 6.12 | 1.85 | 5.10 | 0.33 | 0.44 |
| 5 | | MANN | MFAV | AAGA | MMEA | PAST |
| | 6 m | 1.33 | 0.44 | 0.89 | 0.00 | 0.00 |
| | 9 m | 7.62 | 0.90 | 5.83 | 0.00 | 1.79 |
| | 12 m | 2.68 | 2.68 | 9.38 | 0.00 | 0.45 |
| | 15 m | 1.78 | 2.67 | 8.44 | 0.89 | 2.67 |
| | Avg | 3.35 | 1.67 | 6.14 | 0.22 | 1.23 |
| SD | | 3.94 | 2.84 | 4.19 | 0.77 | 1.10 |
| Total | | | | | | |
| Average | | 4.98 | 1.94 | 4.49 | 0.45 | 0.64 |

average algae cover was ~50% (Steneck et al. 2011). This inverse relationship supports documentation of a coral – algal phase shift and indicates that coral – algal competition exists for space (Aronson and Precht 2006).

In studies conducted in the last five years, coral cover is consistently low at shallow depths (Stokes et al. 2010; Steneck et al. 2011). Before these studies, shallow coral cover was higher, in the range of 26% - 32% and primarily comprised of *Acropora spp.* (Stokes et al. 2010). In this study,

MANN and AAGA were consistently observed as the most abundant coral species in this survey and occupied a high percentage of cover at the deeper depths. Coral damage and loss at these depths and shifts in species composition are attributed to the disturbance of Hurricane Omar in 2008 as well as system wide stresses and human impacts (Stokes et al. 2010).

Table 3 Summary of coral disease observed across area and depth. Coral species codes as follows: AAGA (*Undaria (Agaricia) agaricites*), MANN (*Montastraea annularis*), MFAV (*Montastraea faveolata*), MMEA (*Meandrina meandritis*), PAST (*Porites astreoides*). Disease codes as follows: Bleaching (Bleach), YBD (Yellow Band disease), DS (Dark Spot disease), WP (White Plaque). Areas and depths with no disease observed marked N/A. SD indicated standard deviation

| Depth | Area 1 | | Area 2 | | Area 3 | | Area 4 | | Area 5 | | # Diseases | |
|-------------------|---------------|--------------|---------------|----------------------|---------------|--------------|---------------|--------------|---|--------------------------------------|------------|---|
| | Disease | Species | Disease | Species | Disease | Species | Disease | Species | Disease | Species | | |
| 6 m | Bleach YBD | MMEA PAST | Bleach | AAGA | N/A | | | YBD | MANN | YBD | MANN | 2 |
| 9 m | YBD Bleach | MANN MANN | Bleach YBD | MANN AAGA MANN | YBD | MANN MFAV | YBD | MANN | YBD Bleach Bleach WP Bleach | MANN MFAV DLAB SSID AAGA | 3 | |
| 12 m | YBD | MANN | YBD | MANN | Bleach YBD | AAGA MANN | Bleach | SSID | YBD | MANN | 2 | |
| 15 m | DS | MFAV | YBD | MANN | Bleach YBD | AAGA MANN | Bleach YBD | PAST MANN | WP WP | MFAV AAGA | 4 | |
| # Diseases | 3 | | 2 | | 2 | | 2 | | 3 | | | |
| Diseases Observed | 4 | | | | | | | | | | | |
| SD | Areas: 0.55 | | Depths: 0.96 | | Species: 0.71 | | | | | | | |

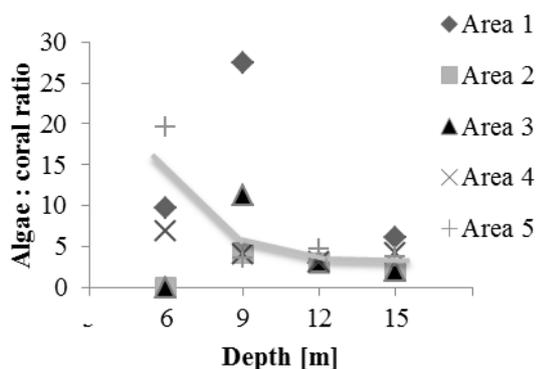


Fig. 8 Mean algae : coral ratio for areas 1–5 as a function of depth. Grey line depicts total mean algae : coral ratio. Data collected from Coral Point Count analysis of 20 video transects during Sept ember and October 2012 in Bonaire, Dutch Caribbean

The algae: coral ratio at Yellow Sub study site of 5.07 exceeds the algal dominance threshold of 1.00, as defined by Stokes et al. (2010). The increase in algae: coral ratio of the leeward study sites on Bonaire is

Table 4 Summary of mean percent coral cover by depth. 2012 data collected at Yellow Sub study site. 2008 and 1997 data from Cliff study site on Bonaire, Dutch Caribbean are from Stokes et al. (2010). SD indicates standard deviation

| Depth [m] | Mean percent coral cover | | |
|-----------|--------------------------|-------|-------|
| | 2012 | 2008 | 1997 |
| 6 | 4.70 | 5.86 | 32.00 |
| 9 | 12.60 | 26.60 | 30.30 |
| 12 | 19.00 | 28.00 | 29.30 |
| 15 | 20.50 | 30.10 | |
| SD | 7.20 | 11.28 | 1.37 |
| Average | 14.00 | 22.64 | 30.53 |

significant and indicates a possible progression of a coral – algal phase shift over a relatively short time period of four years.

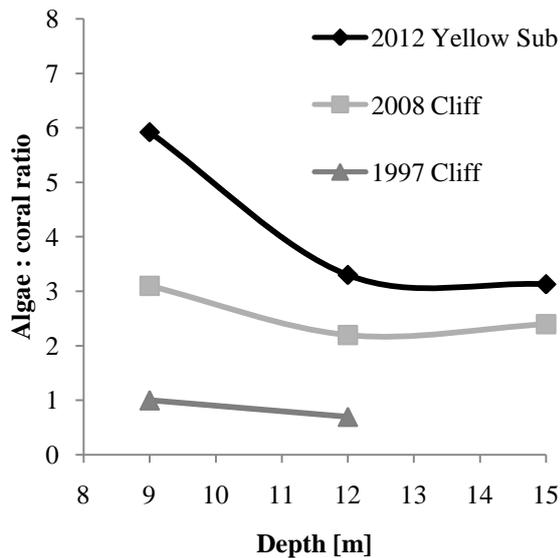


Fig. 9 Mean algae : coral ratio of 2012 Yellow Sub survey (black), 2008 Cliff survey (light grey), and 1997 Cliff survey (dark grey) on Bonaire, Dutch Caribbean as a function of depth. Error bars and 6 m data point removed for clarity. 2008 and 1997 Cliff data from Stokes et al. (2010)

Presence of disease

As hypothesized in this study, Yellow Band disease, Dark Spot disease, White Plague, and coral bleaching were observed. These diseases were also recorded in previous studies (Stokes et al. 2010; Steneck et al. 2011). Disease presence indicates deterioration of coral health and possibly has connections to the increase in algal cover as algal contact has been documented to trigger onset of disease (Nugues et al. 2004). Run-off from the urban center of Kralendijk may also decrease coral fitness and allow opportunistic pathogens to manifest (Steneck et al. 2011). Non-point source pollution such as rising sea temperatures may have the same effect (Aronson and Precht 2006).

Conclusions

This study indicates that Yellow Sub study site is experiencing a coral – algal phase shift and benthic cover is currently dominated by algae. The high algae: coral ratio and presence of disease indicates Yellow Sub reef is experiencing habitat

degradation due to external stresses. If coral – algal phase shifts continue on Bonaire reefs, thresholds for coral health will be exceeded and reversal to coral dominated systems will be unlikely (Fung et al. 2011). Deterioration of reef health at the study site could indicate that other sites on Bonaire are experiencing a similar shift to algal dominance. The long-term trend of deterioration in the Bonaire reef system is reflected by the short-term changes observed in the current and previous studies.

The shift to algal dominance in reefs has a direct effect on resilience and the ability of the ecosystem to revert to a coral dominated state (Fung et al. 2011). The degradation of reef habitat affects fish stocks, coastal resilience, and results in an immense decrease of biodiversity (Stokes et al. 2010). Bonaire reefs are a major component of the island economy and a decrease in biodiversity would affect the diving, fishing, and tourism industries. Therefore, conservation efforts and mitigation need to increase in order to reduce anthropogenic stresses on island reefs including pollution, sewage treatment, and overexploitation of reef fish (Stokes et al. 2010). Rising sea levels, increases of ocean temperature, and increased storm intensity due to climate change contributes to global reef degradation. It is vital to reduce anthropogenic impacts on coral reefs at local, regional, and global scales.

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Cleaner-client interactions and depth distributions among three cleaner species: *Bodianus rufus*, *Elacatinus evelynae*, and *Anclyomenes pedersoni*

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Abstract

Cleaning is a symbiotic behavior that has been observed in many animals, including both terrestrial and marine species. Cleaner species and their cleaning stations play a vital role in coral reef ecosystems by removing parasites, mucus, and diseased or dead tissues from their client fish. In this study, the depth distribution of cleaning stations and the interactions between cleaner and client species were observed with respect to three of the most prominent cleaner species in the Caribbean: juvenile *Bodianus rufus* (Spanish hogfish), *Elacatinus evelynae* (Sharknose goby), and *Anclyomenes pedersoni* (Pederson's cleaning shrimp). Research dives were conducted at Yellow Sub dive site on the leeward or west coast of Bonaire, D.C., a small island in the southern Caribbean off the coast of Venezuela. Two dives were conducted *ad libitum*, with observers recording all cleaning stations between 20 and 50 ft to determine the distribution of the three cleaning species. Observations on cleaner-client interactions were collected at several shallow stations on ten subsequent dives. Cleaner-client interactions at each station were categorized as inspected and cleaned, ignored, jolted, and scared away by another fish. Data analysis indicates that *B. rufus*, *E. evelynae*, and *A. pedersoni* do not have different depth distributions, but they all show the highest abundance of stations between 20 and 30 ft. The four cleaner-client interactions varied by both cleaner and client species. It is important to better understand the role of cleaning stations in coral reef ecosystems, as they are known to positively affect fish richness and diversity.

Introduction

Scientists have observed cleaning behavior in both terrestrial and marine animals; however, coral reef cleaning stations are the most common example of such behavior (Losey 1972). Marine cleaner organisms remove and consume ectoparasites, mucus, or diseased and injured tissues from client fish (Losey 1972). This behavior was reported as early as the mid-1920s (Côté and Soares 2011), but evidence that cleaner species actually ingest ectoparasites they glean from their clients was not presented until the late 20th century. Grutter (1999) conducted a study focusing on the cleaner fish, *Labroides dimidiatus*, and its effects on the abundance of gnathiid isopod parasites on client fish. The wrasses were discovered to ingest parasites, causing a temporary reduction in their abundance. In another study (Becker and Grutter 2004),

two species of cleaner shrimp: *Urocaridella* spp. and *Periclimenes holthuisi* were dissected in an attempt to provide evidence of their cleaning behavior. The gut contents of the shrimps showed ectoparasites, concluding that species of shrimp also present cleaning behavior.

Cleaning is a symbiotic behavior and, therefore, plays an important role in coral reef ecosystems. Cleaners ingest the parasites they collect from their client fish and rely on what they glean for food (Grutter 1999; Becker and Grutter 2004; Soares et al. 2007). Conversely, clients rely on the cleaner species for the removal of potentially harmful parasites. The abundance of parasites on client fish may be reduced by up to 75 percent after several cleaning events (Becker and Grutter 2004). With such a high percentage of parasite removal, cleaner species are of high value to coral reef ecosystems. Furthermore, over half of coral

reef fish species attend cleaning stations (Deloach and Humann 1999). Thus, the removal of a single cleaner species results in dramatic changes in the behavior of client fish and the remaining cleaner species (Losey 1972). And while the removal of cleaner species does not immediately affect fish abundance, after four to 20 months a notable decline in fish diversity becomes apparent (Bshary 2003). In conclusion, cleaning behavior does play a profound role in the health and biodiversity of coral reef ecosystems.

Cleaning stations are widely studied on coral reefs including those in the Caribbean. While over 40 species of shrimp alone have been recorded as cleaners in the Caribbean, the most prominent cleaning species include: *Bodianus rufus* (juvenile Spanish hogfish), *Elacatinus evelynae* (Sharknose goby, formerly Genus *Gobiosoma*), and *Ancylomenes pedersoni* (Pederson's cleaning shrimp, formerly Genus *Periclimenes*) (Wicksten 1995; Johnson and Ruben 1998; Wicksten 1998; Deloach and Humann 1999). These three species clean both benthic and pelagic species of fish (Deloach and Humann 1999).

Cleaning behavior of *B. rufus*, *E. evelynae*, and *A. pedersoni* has been observed at depths up to 30 m, although cleaning stations around 15 m are found to be most abundant and most productive (i.e. had the most clients and spent the most time cleaning during periods of observation) (Johnson and Ruben 1998). However, no studies have focused on the distribution of cleaner species along a depth gradient shallower than 15 m.

At these stations, cleaning is not the only behavior that occurs. When a fish approaches a cleaning station, several cleaner-client interactions may take place. The cleaner or cleaners may inspect and then clean, completely ignore, or cheat the client fish (i.e. bite its tissue, causing the client to jolt). Finally, the client fish may be scared away by a fish other than the cleaner, interrupting a cleaning event (Deloach and Humann 1999). Many of the studies centered

on cleaning stations mainly focus on the first type of cleaner-client interaction, observing which client fish species a cleaner was actually cleaning and as a result generating a list of client associations with certain cleaner species (e.g. Wicksten 1995; Grutter and Poulin 1998; Johnson and Ruben 1998). Not many studies, however, have been conducted in detail on the many cleaner-client interactions.

This study aimed to map the depth distribution of cleaning stations of three cleaner species: *B. rufus*, *E. evelynae*, and *A. pedersoni*, and to investigate the four main cleaner-client interactions as they relate to each of the three focus cleaner species. The following hypotheses were tested through analysis of field observations:

H₁: Each cleaner species has a different depth distribution on the forereef, and the abundance of each species' cleaning stations peaks in a certain depth range.

H₂: Cleaner-client interactions depend on the species of both the cleaner and client.

Methods

Study site

Cleaning stations were observed at Yellow Sub dive site on Bonaire, Dutch Caribbean. Bonaire is an island in the southern Caribbean off the coast of Venezuela. It is approximately 112 mi² with fringing reefs surrounding the island. Most diving is done on the west coast due to the large waves and rough nature of the sea on the east coast or windward side of the island. Yellow Sub dive site lies on the west coast near the capital of Bonaire, Kralendijk (Fig. 1) There is a significant amount of both boat and diver traffic at Yellow Sub. The reef begins at ~20 ft and ends along a sandy bottom at ~100 ft. Yellow Sub is a relatively healthy reef. Its coral cover and fish community is indicative of the general coral cover on the leeward coast of Bonaire.

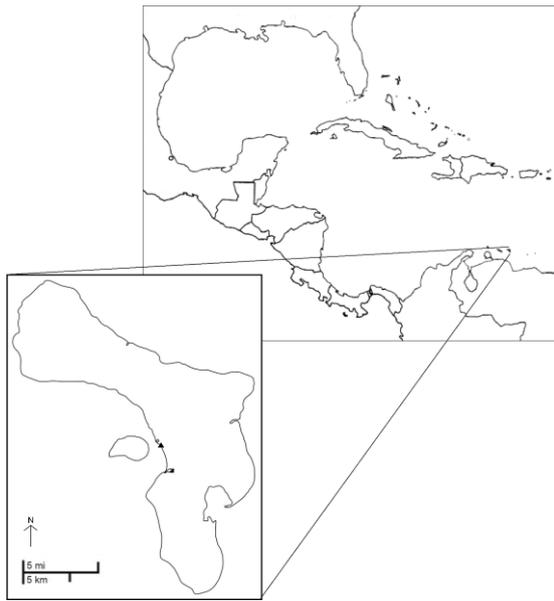


Fig. 1 Map of Bonaire in the Dutch Caribbean. The study site, Yellow Sub, is indicated by the black triangle. Yellow Sub lies on the leeward side or west coast of Bonaire. The site is located close to town and several piers. Caribbean map modified from <http://wps.ablongman.com/wps/media/objects/579/592970/BlankMaps/The%20Caribbean.gif>

Field observations

Based on Wicksten (1995, 1998), this study focuses on the three primary cleaners found in Bonaire: juvenile *B. rufus*, *E. evelynae*, and *A. pedersoni*. Research dives at Yellow Sub dive site were conducted between the hours of 10:30 and 12:30, as this was the most productive cleaning period (Wicksten 1995; Grutter and Poulin 1998; Johnson and Ruben 1998; Wicksten 1998; Soares et al. 2007; Velasquez 2008), during the months of September and October 2012.

Depth distribution of cleaning stations

Two dives were dedicated to collecting data on the location of cleaning stations along a depth gradient. On each dive, after a careful examination of the area by swimming freely, all cleaning stations with one or more of the three focus species between the reef crest at ~20 ft and 50 ft were noted. For each cleaning station found,

the depth and the total count and identity of cleaner species present were recorded.

Depth data were analyzed using descriptive analysis. The mean depth of cleaning stations for each species was calculated. The observed cleaning stations were also placed in three depth categories: 20 to 30 ft, 30 to 40 ft, and 40 to 50 ft. The percentages in each depth category were compared among cleaner species.

Cleaner and client behavioral observation

During 10 subsequent dives, cleaning activity at 14 cleaning stations was observed. The 14 stations were located within a depth range of 20 to 30 ft, and a reinforcing bar pole with orange tape marked each station so that observers could return to the same stations. On each dive, three to four cleaning stations were observed. Once a cleaning station had been identified, the observer noted the location of the station and the total counts and species of the cleaners present. The observer then moved approximately six feet away from the station and waited for two minutes for the surrounding reef fish to become accustomed to its presence. After the two-minute period, the cleaning station was observed for ten minutes. For every cleaning event, the species of the client fish, the client-cleaner interactions, and the duration of each interaction were noted. Behavioral interactions between cleaner and client were categorized as one of four interactions: inspected and cleaned, ignored, jolted, or scared away. In addition, any behavior of the client was recorded, including color changes and poses. A second observer noted the general composition of the surrounding species within a 3 m radius of the station during the 12 minutes of waiting and observation.

Data collected from the ten-minute observation periods were analyzed using descriptive analysis. A list of client species for all three cleaner species was generated, and the percentage of cleaning events for each client species was calculated. Percentages of each of the four cleaner-

client interactions were determined and compared among species.

Results

Depth distribution

Cleaning stations of *B. rufus*, *E. evelynae*, and *A. pedersoni* were observed on two dives between 20 and 50 ft. *B. rufus* cleaning stations were the least common, with only 12 stations found on the two dives. A total 20 *E. evelynae* cleaning stations and 14 *A. pedersoni* cleaning stations were also observed (Fig. 2). For all three species, cleaning stations were most abundant between 20 and 30 ft. (Fig. 2). *B. rufus* had the most even depth distribution of cleaning stations of the three species. Six stations were between 20 and 30 ft, three stations were between 31 and 40 ft, and three stations were between 41 and 50 ft. The depth distribution of *E. evelynae* was less even with 11, four, and five stations at 20 to 30 ft, 31 to 40 ft, and 41 to 50 ft respectively. *A. pedersoni* had the least even distribution with 11 of the 14 stations between 20 and 30

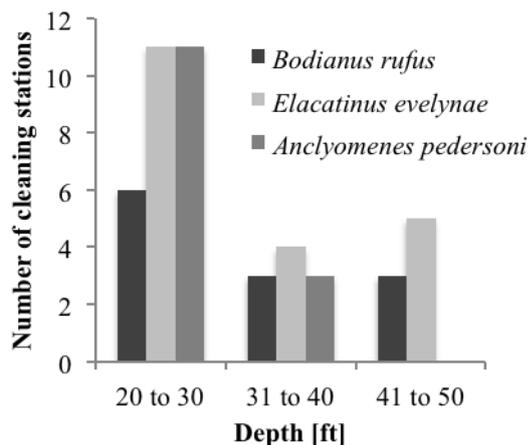


Fig. 2 Depth distribution of cleaner species *Bodianus rufus* (black), *Elacatinus evelynae* (light grey) and *Anclyomenes pedersoni* (dark grey) at Yellow Sub study site in Bonaire, Dutch Caribbean. Cleaning stations (n=46) were observed between depths of 20 and 50 ft. The depth and identity of cleaner species present were noted for each station observed

ft, three of the 14 between 31 and 40 ft, and no stations in the deeper category (Fig. 2).

Client preferences for cleaning stations

Thirty-four 10-min observation periods were made at cleaning stations of *B. rufus*, *E. evelynae*, and *A. pedersoni* between depths of 20 and 30 ft, and the general composition of fish species in the surrounding area along with the fish species visiting the cleaning stations were noted. The general composition of fishes surrounding the stations included 51 species, each of which was observed at more than one station. Of the surrounding species, 27.5% visited cleaning stations at least once. Fourteen client species were observed participating in cleaning events during this study, and the four most common clients for all three cleaner species were *Chromis multilineata* (Brown chromis), *Clepticus parrae* (Creole wrasse), *Cephalopholis cruentatus* (Graysby), and *Chromis cyanea* (Blue chromis) comprising 58.1%, 12.0%, 8.9% and 7.9% of all cleaning events respectively (Table 1). *C. multilineata* attended cleaning stations of all three species but the majority of their cleaning events occurred at *B. rufus* stations. *C. parrae* only attended *B. rufus* and *E. evelynae* stations. *C. cruentatus* and *C. cyanea* only attended *E. evelynae* and *A. pedersoni* stations (Table 1). *E. evelynae* and *A. pedersoni* shared many client species; of the six species cleaned by *A. pedersoni*, five were also cleaned by *E. evelynae* (Table 1).

Cleaner-client interactions

A total of 34 observation periods were conducted at cleaning stations of *B. rufus*, *E. evelynae*, and *A. pedersoni*. During every cleaning event at each station, the interactions between cleaner and client were placed in one of four categories: inspected and cleaned, ignored, jolted, or scared away by another fish.

Table 1 Summary of clients for each cleaner species. Numbers in the first three columns represent the number of visits each client species made to cleaning stations of a particular cleaner species. The fourth column represents the total number of visits to any cleaning station by each client fish species. The fifth column represents the percentage of visits made by each client fish species. n = 191 client fish

| Client | Cleaner | | | Total | Percent |
|---------------------------------|-----------------|--------------------|---------------------|-------|---------|
| | <i>B. rufus</i> | <i>E. evelynae</i> | <i>A. pedersoni</i> | | |
| <i>Chromis multilineata</i> | 78 | 31 | 2 | 111 | 58.1 |
| <i>Clepticus parrae</i> | 9 | 14 | | 23 | 12.0 |
| <i>Cephalopholis cruentatus</i> | | 9 | 8 | 17 | 8.9 |
| <i>Chromis cyanea</i> | | 14 | 1 | 15 | 7.9 |
| <i>Scarus taeniopterus</i> | | 1 | 4 | 5 | 2.6 |
| <i>Abudefduf saxatilis</i> | 5 | | | 5 | 2.6 |
| <i>Stegastes partitus</i> | | 2 | 2 | 4 | 2.1 |
| <i>Acanthurus bahianus</i> | 3 | | | 3 | 1.6 |
| <i>Stegastes planifrons</i> | | 2 | | 2 | 1.0 |
| <i>Haemulon chrysargyreum</i> | | 2 | | 2 | 1.0 |
| <i>Sparisoma viride</i> | | | 1 | 1 | 0.5 |
| <i>Paranthias furcifer</i> | 1 | | | 1 | 0.5 |
| <i>Ocyurus chrysurus</i> | 1 | | | 1 | 0.5 |
| <i>Haemulon sciurus</i> | 1 | | | 1 | 0.5 |

Inspected and cleaned

The most common behavior of cleaners toward client species in general was inspection and cleaning. Of the 241 observed behaviors, 107 corresponded to the cleaner inspecting and cleaning the client fish. The percentages of clients inspected and cleaned by *E. evelynae* (56.5%) and *A. pedersoni* (56.0%) was higher than that of *B. rufus* (29.6%) (Fig. 3).

Three of the four most common client species were inspected and cleaned during the majority of their visits to cleaning stations. *C. cruentatus*, *C. cyanea*, and *C. parrae* were inspected and cleaned during 65.4%, 63.6%, and 63.6% of their visits respectively (Fig. 4). The most common client species, *C. multilineata*, was inspected and cleaned during only 32.6% of its visits to cleaning stations of all three cleaner species (Fig. 4).

Ignored

Ignoring (i.e. when a cleaner does not clean the client and swims away) was the second most common behavior of cleaner species toward clients, accounting for 75 of

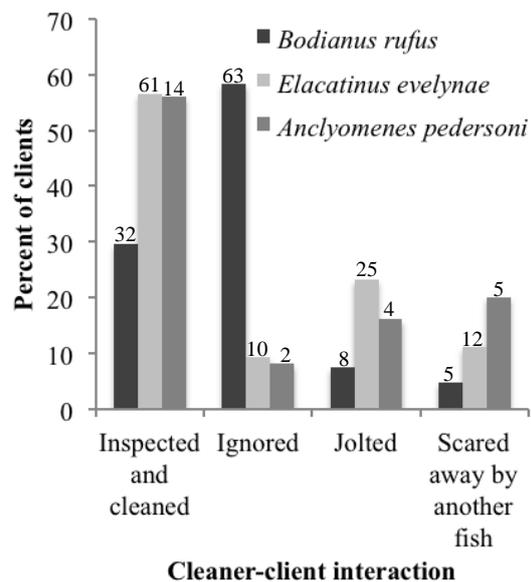


Fig. 3 Distribution of cleaner-client interactions for three cleaner species *Bodianus rufus* (n=108, black), *Elacatinus evelynae* (n=108, light grey), and *Ancliyomenes pedersoni* (n=25, dark grey) during 37 10-min observation periods. The behaviors of the cleaner species, toward a client were noted and categorized as one of four behaviors: inspected and cleaned, ignored, jolted, or scared away by another fish. Numbers above the bars indicate the total count of the specific behavior observed for that cleaner species

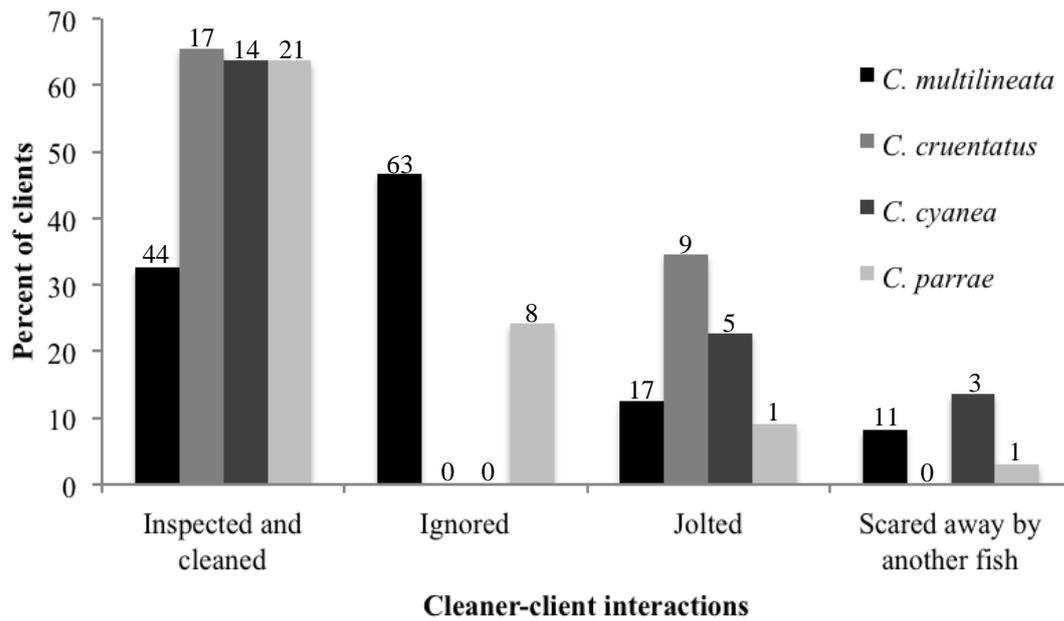


Fig. 4 Distribution of cleaner-client interactions for the four most common clients of *Bodianus rufus*, *Elacatinus evelynae*, and *Anclyonemes pedersoni*. During 37 10-min observation periods, cleaning behaviors of the cleaner species toward clients *Chromis multilineata* (n=135), *Cephalopholis cruentatus* (n=26), *Chromis cyanea* (n=22), and *Clepticus parrae* (n=31) were noted. Behaviors were categorized as inspected and cleaned, jolted, or scared away by another fish. Numbers above the bars indicate the total count of the specific observed behavior for that client species

the 241 behaviors observed (Fig. 3). *B. rufus* ignored the majority of the clients that visited its cleaning stations, as 58.3% of clients were ignored. *E. evelynae* and *A. pedersoni* ignored smaller percentages of their clientele at 9.3% and 8.0% respectively (Fig. 3).

Cleaner species were observed ignoring two of the four most common client species. Both *C. multilineata* and *C. parrae* were ignored by at least one cleaner species. *C. multilineata* was ignored during 46.7% of visits to cleaning stations. Cleaners also ignored *C. parrae* during 24.2% of its visits to the stations. *C. cruentatus* and *C. cyanea* were not ignored during any of the periods of observation in this study (Fig. 4).

As previously stated, *C. multilineata* was the client most often ignored by the three cleaner species. During the 10-minute observation periods, it was noted that *C. multilineata* visited cleaning stations alone or in groups of two or more. Fifty-two percent of *C. multilineata* visited cleaning stations in groups of two or more (Fig. 5).

When multiple *C. multilineata* visited a station, they were ignored during 88.9% of visits. Single *C. multilineata* were ignored during only 26.0% of visits (Fig. 5).

Jolted

A cleaner causes a client to jolt by cheating the fish and biting its flesh. Jolting was less common than inspecting and cleaning and ignoring, accounting for 37 of the 241 behaviors observed (Fig. 3). *B. rufus* jolted the fewest clients with jolting comprising only 7.4% of its cleaner-client interactions. *E. evelynae* jolted its clients the most at 23.1%, and 16.0% of cleaner-client interactions by *A. pedersoni* were jolting (Fig. 3).

C. cruentatus was the most commonly jolted client, as they were jolted during 34.6% of visits to cleaning stations. *C. cyanea* was jolted during 22.7% of its visits to cleaning stations. *C. multilineata* and *C. parrae* were jolted least, with 12.6% and 9.1% of visits resulting in a jolt (Fig. 4).

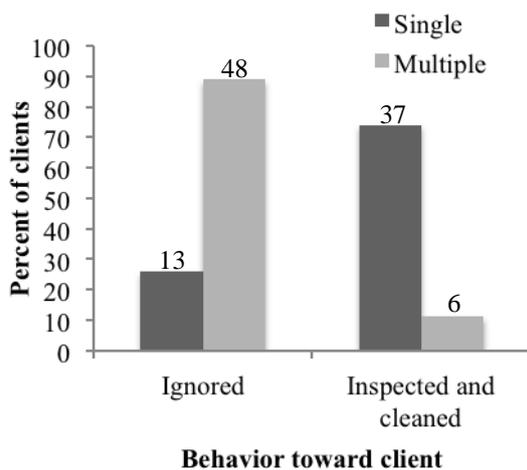


Fig. Behavior of cleaner species toward *Chromis multilineata* when single (n=50) or multiple (n=54) clients visit cleaning stations. *C. multilineata* visited cleaning stations either alone or in groups of two or more. During each visit to a station, the cleaner-client interactions were categorized as inspected and cleaned or ignored, and the number of clients at a given cleaning event was noted. Numbers above the bars indicate the total count of *C. multilineata* that experienced a given cleaner-client interaction

Scared away by another fish

The final cleaner-client interaction occurs when a cleaning event is interrupted as another fish scares away the client. This interruption was the least common behavior observed, involving only 25 of the 241 observed behaviors (Fig. 3). Of the three cleaner species, clients of *A. pedersoni* were scared away most often, as 20.0% of cleaner-client interactions were when the client was scared away. Clients of *E. evelynae* and *B. rufus* were scared away less often, with 11.1% and 4.6% of observed behaviors being scared away (Fig. 3).

Of the four most common client species, *C. cyanea* was scared away during 13.6% of visits. *C. multilineata* and *C. parrae* were scared away during 8.1% and 3.0% of visits, and *C. cruentatus* was not scared away during any periods of observation.

When cleaning events were interrupted by another fish scaring away the client, the

third party fish was often a Bicolor damselfish. Bicolor damselfish accounted for 91.3% of the interruptions. Threespot damselfish and Bar jack caused the other interruptions.

Discussion

Depth distribution

The abundance of cleaning stations of *B. rufus*, *E. evelynae*, and *A. pedersoni* varied with depth, indicating that the three cleaner species preferred certain depths. All three cleaner species had the greatest abundance of cleaning stations between depths of 25 and 30 ft, supporting the hypothesis that the abundance of cleaning stations would peak at a certain depth for each cleaner species. However, the distributions among the three species did not vary greatly. So, the hypothesis that each species would have a different distribution along a depth gradient was rejected. With a larger sample size, the trends in depth distribution may have been more conclusive.

A similar study by Johnson and Ruben (1998), which focused on the depth distribution of cleaning stations of *B. rufus*, *Thalassoma bifasciatum*, *E. evelynae*, and *A. pedersoni*, concluded that most cleaning stations were located around 50 ft. While this result appears to contradict the findings of this study, Johnson and Ruben (1998) only observed cleaning stations between 50 and 150 ft and included no shallower observations. In both studies, for *B. rufus*, *E. evelynae*, and *A. pedersoni*, the abundance of cleaning stations increased at shallower depths.

Client preferences for cleaning stations

Fourteen client species were observed visiting cleaning stations over the course of this study, with the four most common clients being *C. multilineata* (Brown chromis), *C. parrae* (Creole wrasse), *C. cruentatus* (Graysby), and *C. cyanea* (Blue chromis). Hence, the most common families

observed were Pomacentridae, Labridae, and Serranidae. A similar study, Wicksten (1998), that was conducted in Bonaire reported comparable results. Species of the family Serranidae, in particular *C. cruentatus*, were the most common clients followed by *C. multilineata*, species of the family Scaridae, species of the family Acanthuridae, and *C. parrae*. Overall, Pomacentridae species, especially *C. multilineata*, were reported to be the most common clients in many studies, which is congruent with the results of this study.

The frequency of visits by client species does not seem to be related to their abundance around the cleaning station (Wicksten 1998). As found in this study, approximately a quarter of the fish observed around the cleaning station were cleaned. *Stegastes partitus* (Bicolor damselfish), which are among the most abundant on Bonaire's coral reefs, was rarely cleaned, while *C. cruentatus* is much less abundant on the reef but is cleaned much more often. Commonness of client species may be correlated with the time of day. Through personal observations, predatory fish, such as *Aulostomus maculatus* (Atlantic trumpetfish), *Lutjanus apodus* (Schoolmaster snapper), and *Ocyurus chrysurus* (Yellowtail snapper), were observed visiting cleaning stations later in the day between 14:00 hrs and 17:00 hrs. Formal observation dives for this study were conducted between 10:30 and 12:30 hrs; however, personal observation indicates the fish composition surrounding a cleaning station and, therefore, client species, may change throughout the day.

Client species showed a preference for cleaners by visiting the cleaning stations of only certain species. *C. multilineata* visited stations of *B. rufus*, *E. evelynae*, and *A. pedersoni*, but the most visits were to *B. rufus* stations. This is consistent with Wicksten's studies (1995, 1998), which reported *C. multilineata* clients to visit both *E. evelynae* and *B. rufus* stations but with greater frequency to *B. rufus* stations. *C. parrae* visited only *B. rufus* and *E. evelynae*

stations, which is also consistent with the results reported by Wicksten (1998). Since both *C. multilineata* and *C. parrae* are pelagic species, perhaps they prefer being cleaned by *B. rufus* because it will travel farther from the cleaning station than *E. evelynae* and *A. pedersoni* to clean its clients (Wicksten 1998).

In general, *E. evelynae* and *A. pedersoni* cleaned many of the same clients. Two of the most common clients, *C. cruentatus* and *C. cyanea*, were both only cleaned by *E. evelynae* and *A. pedersoni*. This result is consistent with previous studies, Wicksten (1995, 1998), which stated that *E. evelynae* usually cleaned similar client species as *A. pedersoni* while *B. rufus* usually cleaned different species.

Cleaner-client interactions

The distribution of cleaner-client interactions varies among *B. rufus*, *E. evelynae*, and *A. pedersoni*. In addition, cleaner-client interactions vary among the four most common client species: *C. multilineata*, *C. parrae*, *C. cruentatus*, and *C. cyanea*. Thus, the results of this study support the hypothesis that cleaner-client interactions depend on the species of both the cleaner and client.

B. rufus was observed to ignore more clients than both *E. evelynae* and *A. pedersoni*. *B. rufus* and other fish species that tend to clean only as juveniles, such as (French angelfish) and *Thalassoma bifasciatum* (Bluehead wrasse), are facultative cleaners, meaning they do not rely on cleaning as their only food source (Grutter 1999). Because *B. rufus* has other food sources, it can afford to ignore a greater proportion of its clients. *E. evelynae* and *A. pedersoni* are obligate cleaners and rely solely on cleaning for food (Grutter 1999). These two cleaner species cannot ignore as many clients, as the gleaned ectoparasites, mucus, and tissue are their main source of food. As a result, both *E. evelynae* and *A. pedersoni* were observed to inspect and clean rather than ignore the majority of their

clients. No studies have been conducted on inspecting and cleaning versus ignoring behaviors of cleaners and the differences in the two behaviors among species.

C. multilineata and *C. parrae* were the two most commonly ignored clients. Although no studies have focused on the ignoring behavior of cleaner species towards clients, Wicksten (1998) observed that *C. multilineata* and *C. parrae* often visited cleaning stations in groups as well as individually. When clients arrive at a cleaning station in groups, they form a tight ball-like formation around the cleaners, a behavior called crowding (Wicksten 1995, 1998). In this study, *C. multilineata* was observed to crowd *B. rufus*, *E. evelynae*, and *A. pedersoni*, yet in past studies crowding by *C. multilineata* was only observed at *B. rufus* stations (Wicksten 1998). The majority of *C. multilineata* that crowded a cleaner during observation periods was ignored, while the majority of *C. multilineata* clients that visited cleaning stations individually was inspected and cleaned. In conclusion, clients in a group are more likely to be ignored by any of the three cleaner species than if they visit a cleaning station alone. In a study by Reinthal and Lewis (1986) that focused on the social behavior of species of the family *Acanthuridae*, it was reported that schools rarely visit cleaning stations. This may be because cleaners are less likely to clean client fish in groups.

Neither *C. cruentatus* nor *C. cyanea* was ignored during observation periods. Both species were cleaned by *E. evelynae* and *A. pedersoni*, which inspected and cleaned the majority of their clients for reasons previously stated. However, past studies have also concluded that cleaning gobies will inspect and clean a higher percentage of predatory clients, such as *C. cruentatus* (Soares et al. 2007; Côté and Soares 2011). It was suggested that the threat of predation led to a decreased proportion of clients ignored by a cleaner. Thus *C. cruentatus* was ignored less than other common client species.

Jolting is thought to occur when a cleaner cheats its clients by eating the fish's flesh instead of gleaning ectoparasites and damaged tissue. Grutter (1999) determined that fish flesh was more nutritious than ectoparasites and damaged tissue, giving a cleaner incentive to cheat its client and cause a jolt. Jolting was observed more in clients of *E. evelynae* and *A. pedersoni* than in clients of *B. rufus*. Both *E. evelynae* and *A. pedersoni* are obligate cleaners, so they may cheat their clients more frequently to obtain additional nutrition from their only food source.

The frequency at which clients are jolted depends on some characteristics of the species, such as their diet and home range. Past studies have reported that jolting occurs less in predatory species than in harmless species (Soares et al. 2007; Grutter 1999). This contradicts the results of this study, as the predatory species, *C. cruentatus*, was jolted the most frequently of the four most common client species. *C. multilineata* and *C. parrae* were jolted less frequently than *C. cruentatus* and *C. cyanea*. Both *C. multilineata* and *C. parrae* are transient fish species, which when jolted flee that station and switch to another cleaning station (Grutter 1999; Côté and Soares 2011). The jolting cleaner loses the "business" not only of that particular client fish, but also of other future clients (Grutter 1999). This "loss of business" may account for the lower rate of jolting in the two species.

The final and least common cleaner-client interaction occurs when a cleaning event is interrupted when a client is scared away by another fish. In most events, clients were scared away by Bicolor damselfish. The client species, *C. cruentatus*, was not scared away by Bicolor damselfish or by a fish of another species during the observation periods. A study focusing on the response of damselfishes to predators reported that damselfishes avoided Atlantic trumpetfish and would retreat into the benthic cover when approached (Helfman 1989). Another study, Coates (1980), described similar behavior in damelfishes

with other predators. Thus, *C. cruentatus* may not be scared away during cleaning events because it is a predatory fish and presents a threat to damselfishes.

Cleaning stations are vital to the health, richness, and biodiversity of coral reef fishes (Losey 1972; Bshary 2003; Becker and Grutter 2004). Thus, it is important to study cleaning behavior in depth. Several studies focus on only inspection and cleaning of clients (e.g. Wicksten 1995; Grutter and Poulin 1998; Johnson and Ruben 1998); however, cleaning events may involve several cleaner-client interactions. Many client fishes that visit a station are not cleaned, as they are ignored by the cleaners or scared away by other fish. Furthermore, this study provides a baseline for depth distributions of cleaning stations of *B. rufus*, *E. evelynae*, and *A. pedersoni* in Bonaire, D.C. Depth distribution of cleaning stations may change as reef degradation continues. Loss of habitats of cleaner species may have detrimental effects on coral reef environments.

The major limiting factor of this study was time. Compared to the study by Wicksten (1998) with over 700 hours of observations on cleaning stations, this study had fewer than six hours of observation on cleaning stations. With more time, perhaps trends in cleaner-client interactions with respect to cleaner or client species would be more apparent. As previously stated, the time of day might affect which species attend the cleaning stations. The study could potentially be improved or expanded by observing cleaning stations during different times of day. It could also be interesting to further observe the crowding behavior in *C. multilineata* and other client species. Another improvement that could be made on this study would be to standardize the methods used to determine the depth distribution of cleaning stations by using transects or timed swims at certain depths. Additionally, with a larger sample size of cleaning stations obtained along a depth gradient, it is possible that trends in depth

distribution for the three species would become apparent.

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Redlip blenny (*Ophioblennius macclurei*) territoriality and feeding behavior

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Abstract

Redlip blennies (*Ophioblennius macclurei*) are common reef fish in Caribbean coral reef environments. They are vitally important to reefs as primary consumers providing a link between algal production and secondary consumers. This research sought to discover the territorial interactions between *O. macclurei* and other reef species, as well as the amount of grazing pressure placed on reefs by Blennid presence. Videos were taken 2-3 times per week between 26 September and 4 November 2012, and were used to assess feeding behavior and territorial defense. The data collected has applications in monitoring reef energy transfer up the food web and the amount of grazing pressure placed on a reef. The average area grazed per day was found to be 1100.09 cm². Territoriality is useful to understand the complex relationships between population of reef fish and suitable territory area. It was found that blennies primarily hide from fish intruding into their territory, but will not allow others of the same species to overlap territories. The preferred substrates noted in the study were *Diploria labyrinthiformis* and turf algae. The findings have importance in blenny conservation by showing habitats preference. Findings also indicate to future researchers the importance of including small grazers in benthic studies.

Introduction

Grazing animals, also known as primary consumers, are vitally important everywhere from the plains of Africa, where buffalo feed on lush savanna grass, to the boreal forests of North America, where moose feed on pine needles to sustain their massive bulk. Primary consumers are the second branch in the food web transferring energy to higher consumers from the photosynthetic primary producers. In aquatic environments grazers are often small in relationship to their predators; the opposite of many terrestrial environments. In water the role of primary consumers is no less important.

Stevenson and Marshall (1974) concluded that “reference to suitable management regulations brings up the need for fundamental fishery biology information to accompany development efforts.” Primary consumers are vitally

important for the health of fisheries as prey fish for commercially important fish. Blennies of the family Blenniidae, often called combtooth blennies, are common in coral reef ecosystems all over the world. They receive their common name for their tooth morphology that closely resembles a comb (Lindquist and Dillman 1986). This adaptation has made them effective grazers. Multiple members of Blenniidae have been described as keystone grazers in Indo-Pacific reef systems (Choat, 1991).

In the Caribbean one of the most common blenny species is the Redlip blenny (*Ophioblennius macclurei*). They inhabit hard surfaces near algae. The substrate may be made of mixed live coral and eroded coral rock, or boulder debris from channel digging, shore collapse, broken concrete surfaces, etc. Feeding only occurs during daytime and individuals find holes to hide in at night (Nursall 1977). In Bonaire *O. macclurei*

are commonly found on mooring blocks at 3-5 m.

O. macclurei is a territorial fish (Nursall 1977). Smith and Tyler (1972) suggested that the defense of a home against intruders could be an important mechanism in community stability, by providing a stable base population against which visitors would have to compete. A similar species to *O. macclurei* from the Pacific Ocean, *Salarias fasciatus*, has been observed to chase away less than 10% of grazers, 90% of other blennies and 20% of Pomacentrids (Townsend and Tibbetts 2004). *S. fasciatus* and *O. macclurei* have very similar feeding behaviors, preferred benthos, and territorial protection (personal observation).

The four hypotheses proposed for this study are:

H₁: *O. macclurei* tolerate most other grazers and Pomacentrids, but not other blennies.

H₂: Larger individuals inhabit larger territory.

H₃: *O. macclurei* feed primarily on coralline algae.

H₄: Blenny feeding peaks at midday.

If H₁ and H₂ prove true it will be useful in studying blenny densities on coral reefs. For example, if the population of *O. macclurei* greatly increased, the decrease of competing blenny species could be predicted assuming that they share similar habitats. If this hypothesis proves true it will also aid in determining blenny populations for areas of reef.

It was assumed by Scott and Russ (1987) that blennies do not have a significant impact on the epilithic algal community (EAC) due to their small size. Townsend and Tibbetts (2004), however, showed that *S. fasciatus* consumed more organic carbon than the resident Pomacentrids. This shows blennies can have a greater contribution to grazing pressure than previously assumed.

H₃ was suggested by Labelle and Nursall (1977), but not proven in their experiment. They suggest that if H₃ is correct, *O. macclurei* provides an important link between coralline algae (and probably their bacterial associates) and a variety of piscivorous fishes.

H₄ is based on previous personal observations of the highest numbers of *O. macclurei* feeding behaviors during peak sunlight periods. This would be likely because most large predators on the reef are most active at periods of changing light conditions. Data on the feeding to light level relationship would provide a better understanding of blenny behavior and predator avoidance patterns in small reef fishes.

Materials and Methods

Study site

The study site was the dive site Yellow Sub off of Kralendijk, Bonaire located at 12°09'36.47"N, 68°16'55.16"W. The coral reef is on the leeward side of the island creating a very suitable habitat for *O. macclurei*. Preliminary research was done on two sets of mooring blocks that are one m³ in volume where the target species was present. *O. macclurei* is very territorial so it should be found in the same area on repetitive dives. Trial dives were conducted before data was collected by diving and searching for appropriate habitat and fish in 10-20 feet of water.

Data collection

Data was collected between 29 September and 4 November 2012 between 6:00 and 17:00 hrs. Videos of individual blennies were recorded while scuba diving using a Sony camcorder with Ocean Images housing. Individual blennies were filmed for no more than 10 minutes per dive to attempt to include a broader range of sized fish. Both sets of mooring blocks

were visited every dive to maximize blennies filmed each dive.

Videos were analyzed using QuickTime Movie Viewer. The time, size of the fish, number of bites taken, substrate on which the fish spent the majority of time, furthest distance traveled from start point, fish interacted with and behaviors were recorded every minute.

Data analysis

Bites per minute and size were used to calculate averages and standard deviations over the course of a day. Substrate preference was determined by finding the percent of minutes with each substrate being dominant. Percentages of time spent on three categories of activities were compared using a pie chart. Linear regression was used to find if there was a correlation between time of day and bites per minute. Linear regression was also used to determine the amount of correlation between the distance traveled per minute and time of day.

Data from Christianson et al. (2012) was used to determine size of bite per cm of *Salarias fasciatus*, a similar Blennid species to *O. macclurei* (2010). The bite size (cm) found previously was multiplied by the average size from the recorded data to find average bite size. The average bite size was multiplied by the average amount of bites per minute to find the average grazing area per minute. Average grazing area per minute was multiplied by 600 to represent a 10 hr feeding period per day.

Results

Data recorded

Videos were recorded 2-3 times per week between 26 September and 4 November 2012. During observation time, 220 mins of usable video were taken. Videos were analyzed for bites min^{-1} , fish size, preferred substrate, behavior, and fish species interacted with. Data were collected between 6:00 and 17:00 hrs. Blennies could not be found between

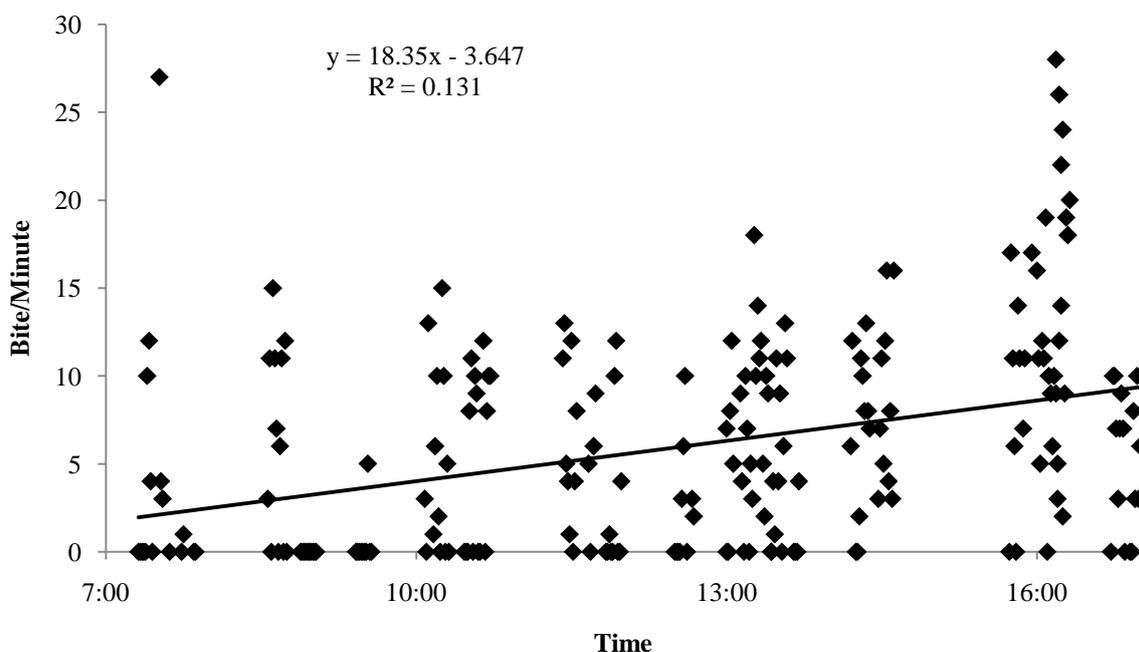


Fig. 1 Shows a comparison of bites per minute and time of day. Regression line is shown. There is a weak positive correlation $R^2=0.1311$ between bites per minute of *Ophioblennius macclurei* and time

06:00 and 07:00 hrs. The light was too dim to record usable video after 17:00 hrs. A minimum of 10 mins of data were collected in single hr categories (12:00-13:00 hrs and 15:00-16:00 hrs). A maximum of 38 usable mins of video were collected between 13:00 and 14:00 hrs.

Feeding

The average of bites per min was 5.90 ± 6.07 . Assuming a 600 min day, blennies take 3537 bites per day. According to Christiansen et al. (2010), the average bite size of an 8.7 cm *Salarias fasciatus* was 42.01 mm^2 . The average size of blennies from the data collected was 6.44 cm. Therefore, the average bite size of blennies observed was 31.1 mm^2 . The total area grazed per day by an average blenny was therefore 1100.09 cm^2 .

The number of bites per min and the time shown in Fig. 1 have a weak positive correlation ($R^2=0.1311$).

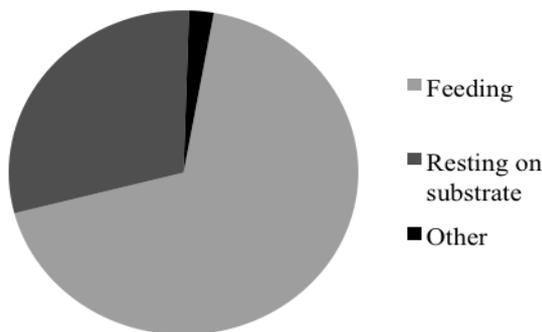


Fig. 2 Percent daily activity blennies spend feeding, resting on the substrate and other activities. Light gray indicates time spent feeding, medium gray indicates time spent resting on the substrate and the Black indicates other activities

Behavior

Blennid behavior was divided into three categories: feeding, resting on substrate and other. The primary activity included in other was hiding in holes from

larger fish in their feeding territory. The subjects spent 68.2% of time feeding, 29.5% of time resting on substrate and 2.3% on other activities (Fig. 2).

The interactions of the subjects with non-subject fishes occurred 45 times. The majority of interactions were with *Abudefduf saxatilis* (Sergeant major) (73.3%). The other 26.7% of interactions were with the species *Chromis multilineata* (Brown chromis), *Haemulon sciurus* (Blue striped grunt), *Stegastes partitus* (Bicolor damselfish), *Stegastes diencaeus* (Longfin damselfish), *Bodianus rufus* (Spanish hogfish), and *Acanthurus bahianus* (Ocean surgeonfish) (Table 1). The most preferred substrate from the data collected was *Diploria labyrinthiformis*, which was preferred 29.6% of times. *O. macclurei* were also found on *Diploria strigosa* (DSTR), old dead coral (ODC), *Undaria agaricites* (UAGA), Sponge, *Montastrea sp.* (Msp.), *Millepora complanata* (MCOM), and turf algae (TA) (Fig. 3). The distance moved and time shown in Fig. 4 had no correlation ($R^2=0.0204$).

Table 1 Number of fish interactions viewed during the observation periods sorted by species. This shows the range of fish species that interact with *Ophioblennius macclurei* including interactions within the species. The highest amount of interactions is with *Abudefduf saxatilis*. The other species shown are: *Chromis multilineata*, *Haemulon sciurus*, *Stegastes partitus*, *Stegastes diencaeus*, *Bodianis rufus*, and *Acanthurus bahianus*

| Fish Species | # of Interactions |
|------------------------|-------------------|
| <i>A. saxatilis</i> | 33 |
| <i>C. multilineata</i> | 4 |
| <i>H. sciurus</i> | 2 |
| <i>O. macclurei</i> | 2 |
| <i>S. partitus</i> | 1 |
| <i>S. diencaeus</i> | 1 |
| <i>B. rufus</i> | 1 |
| <i>A. bahianus</i> | 1 |

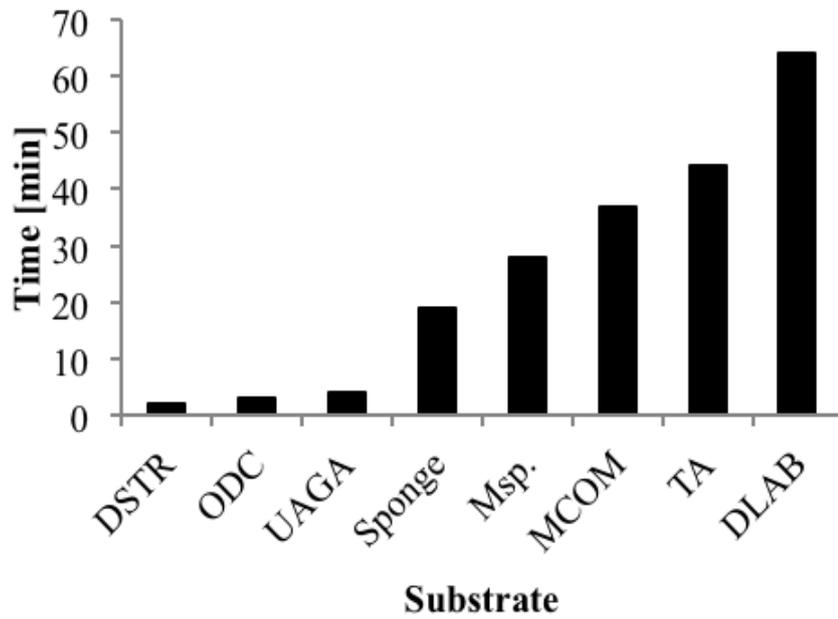


Fig. 3 Substrate preference shows number time blennies spent on each substrate during the observation periods. The substrata are: *Diploria strigosa* (DSTR), old dead coral (ODC), *Undaria agaricites* (UAGA), Sponge, *Montastrea sp.* (Msp.), *Millepora complanata* (MCOM), turf algae (TA), and *Diploria labyrinthiformis* (DLAB)

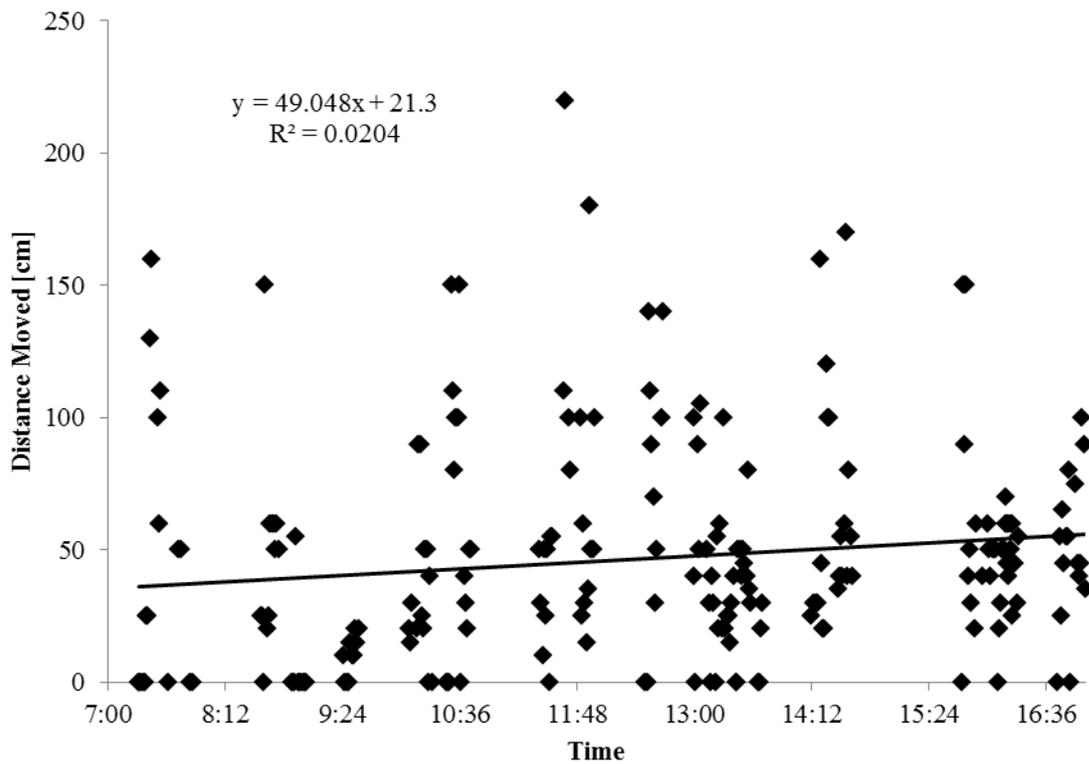


Fig. 4 The maximum distance the individual blenny moved from the point it was at when the minute started and the time of day during the observation period. Regression line is shown. The R^2 value indicates there is no correlation between movement and time of day

Discussion

The first hypothesis for this project was that *O. macclurei* tolerate most other grazers and Pomacentrids in its territory, but not other blennies. Observations showed that *O. macclurei* does allow other grazers into their territory, primarily because they do not appear to use fangs, which leave it defenseless against larger fish. *A. saxatilis*, a Pomacentrid, frequently attacked subjects as they fed near nests. *A. saxatilis* never did any apparent damage to subjects, but subjects did sometimes move to new territories to avoid interactions. Subjects preferred to hide underneath coral than to battle for territory.

The second part of this hypothesis proved harder to study than originally planned. However, the lack of Blennid interactions suggests that the fish do not share territory. Never was a subject recorded feeding on the same side of a block as another blenny. In the two inter-blenny interactions recorded the larger subject chased the smaller away from feeding territory that the smaller had moved to in order to avoid further interactions with *A. saxatilis*. Based on these observations, H_1 is not rejected.

This means that densities of other fish species are likely to have an effect on abundance of blennies in a reef ecosystem, because large fish stress individual blennies and feeding and territory size decrease. On Guam fringing reefs, as parrotfish density decreases, Blennid density increases to fill in the niche for grazers (personal observation). As reefs in the Caribbean become increasingly degraded and fishing down the food chain becomes more common, blennies may have an increasing importance in reef ecosystems. Therefore, knowing their diet and behavior is important to understanding grazing patterns on degraded coral reefs.

The second hypothesis was that larger individuals inhabit larger territory. Blennies found defending larger territory were shown by Nursall (1977). It was

assumed when designing the experiment that the territory would be smaller than one m^2 . It was found that the range can extend around and between the one m^3 blocks. Territory size was not able to be determined in this study. To determine territory size, a longer viewing time per fish would be needed. It would be beneficial for future studies if more cameras were placed from different angles around the blocks.

Information on the relationship between territory size and subject size would be useful to know, in order to conduct then studies relating area grazed and the territorial area to see if there is a preferred amount of algal cover for *O. macclurei*.

The third hypothesis stated that coralline algae are the primary food source of *O. macclurei*. Labelle and Nursall (1992) suggested this hypothesis while describing blennies as an important link between primary production in coralline algae and secondary consumers in the coral reef food web. This hypothesis was rejected in this study. Subjects bit exclusively turf algae growing on the mooring blocks. This matches what Christiansen et al. (2010) hypothesized: that members of *Blenniidae* are only functional algavores and target detritus that has settled in turf algae.

The diet of *O. macclurei* is important because as reefs change due to overfishing and human impacts smaller primary consumers become more important to reduce algae growth on hard surfaces. Concurrent research shows the percent coral cover of Yellow Sub reef (Meltvedt (Snow) 2012) and the mooring blocks (Jaco 2012) near the study site. Jaco (2012) shows that the mooring blocks that often have multiple blennies feeding on them have on average about five percent more coral cover than Yellow Sub reef.

The fourth hypothesis was that blenny feeding peaks at midday. This hypothesis was rejected. A small trend was shown that feeding increases as the day

progresses. The R^2 value was not statistically significant and additional research is needed to show a definitive trend. The data suggested that feeding and movement remain stable between 07:00 and 17:00 hrs.

More research needs to be done, but the data presented in this study could aid in future research of *O. macclurei* behavior. If feeding behavior is consistent during daytime, future researchers may not need to vary time of day during research. Also, the impact of blennies on reefs is consistent over each day making average feeding per day more accurate.

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