

PHYSIS

Journal of Marine Science



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Physis

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Editor-in-Chief: Cathryn Morrison

Editors: Bianca Zarrella, Kayley You Mak, Christina Mielke, Benjamin Foxman

Opening Pages: Marissa Neitzke

Photography Editor: Marissa Neitzke, William Duritsch

Layout and Formatting: Helen Jarnagin

Figures and Tables: Samantha Chase

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Front Cover: William Duritsch

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Forward (in order of appearance): William Duritsch, Christina Mielke

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Physis: φύσις

The ocean is undeniably powerful. Tides, waves, currents, and storms are some displays of the vast physical power of water. There is power in what the ocean provides for man; over one third of the planet depends on fish as a major source of protein or income. The sheer size and depth of the ocean, covering over 70% of Earth, contributes to its extensive power. Perhaps the most powerful quality of all is *Physis*, Greek for natural self-healing, which is the innate attempt by oceans to retain equilibrium after disturbances. *Physis* exists for all components of nature and combats disturbances caused by humanity. Our ancestors' perception of the ocean as an infinitely powerful and limitless resource has shaped the attitudes and actions of many people today. We have reached a point of such rapid destruction within the ocean that *Physis* is no longer enough to keep the long-term damage at bay. This rate of destruction has led to dire consequences; a study published by Dr. Boris Worm (2006) suggests that if our current global fishing habits continue, all fished taxa will collapse by 2048.

In an effort to shift current mindsets, ecologist and economist Garrett Hardin coined the term "ecolacy." As "literacy" is the ability to understand the true meaning of words, ecolacy is the ability to understand complex ecosystem interactions, such as those between humankind and the ocean. A shifting perspective that encompasses long-term interactions between people and the ocean is necessary to allow *Physis* to work to its fullest potential, thus hopefully avoiding future consequences such as those suggested by Worm.

"We do not inherit the earth from our ancestors, we borrow it from our children"
- Chief Seattle

Once humankind collectively begins working towards attaining ecolacy, we can begin to understand how our actions disrupt the powerful process of *Physis* and the oceans can begin to make progress on repairing the damage humanity has caused. The ocean has the power to heal itself—all we need to do is shape and build our restoration and conservation efforts in a way that supports this process.

During our semester on Bonaire, we have not only expanded our knowledge of the processes and organisms of the ocean that surround the island, but we have expanded our appreciation for them. Through our studies, we have come to understand not only the current efforts of humanity to heal the ocean, but also the efforts of the ocean to heal itself. What we have learned here and would like to share with you is that the power humanity has over the ocean is combatted by *Physis*, a force with the potential to be even more powerful than our own, *if* our future actions allow it to be.

We present *Physis: Journal of Marine Science*—our continuation of the discoveries made about the power of the Earth's oceans.

Jillian R. Neault
CIEE Research Station Bonaire, Spring 2015

Foreword

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

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

The Tropical Marine Ecology and Conservation program in Bonaire offers a one-of-a-kind opportunity designed for upper-level undergraduates majoring in Biology and other related fields. This program aims to provide an integrated and superlative experience in Tropical Marine Ecology and Conservation. The emphasis on field-based science is designed to prepare students for graduate programs in Marine Science or for jobs in Marine Ecology, Natural Resource Management, and Conservation. Student participants enroll in six courses: Coral Reef Ecology, Marine Ecology Field Research Methods, Advanced Scuba, Tropical Marine Conservation Biology, Independent Research in Marine Ecology/Biology, and Cultural & Environmental History of Bonaire. In addition to a heavy and comprehensive course load, this program provides dive training that culminates in certification with the American Academy of Underwater Sciences, a leader in the scientific dive industry.

The student research reported herein was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature, Bonaire, Dutch Caribbean. Projects this semester were conducted on the leeward side of Bonaire where most of the island’s population is concentrated. Students presented their findings in a public forum on 29 April, 2015 at CIEE Research Station Bonaire.

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



Faculty



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



Dr. Enrique Arboleda is the Coral Reef Ecology Faculty for CIEE and co-teaches Independent Research and Marine Ecology Field Methods. He is a marine biologist from the Jorge Tadeo Lozano University (Colombia), holds a specialization on Biodiversity and Evolutionary Biology from the University of Valencia (Spain) and obtained his 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. Patrick Lyons is the Tropical Marine Conservation Biology faculty for CIEE and co-teaches Independent Research and Marine Ecology Field Methods. Patrick received his B.Sc. in Marine Biology from the University of Rhode Island and his Ph.D. in Ecology and Evolution from Stony Brook University. His research has three different themes that all broadly touch on the behavior of organisms in the marine realm. The first theme is on the fascinating mutualism between alpheid shrimp and gobiid fishes, the different sets of behaviors that these organisms use, and how these behaviors may have evolved. His second theme is on the hunting strategies of piscivores, specifically of lionfish that use a novel "water jetting" technique while approaching prey. The last theme is on how the behavior of recreational SCUBA divers can alter the community composition of benthic reef organisms and the structural complexity of reefs.

Interns



Jack Adams is one of the Cultural and Environmental History of Bonaire Instructors. Jack studied Environmental Science at the University of Leeds in the United Kingdom. For his final project Jack travelled to Indonesia and studied habitat complexity of coral reefs and its effects on fish communities. After graduating from university Jack completed his Divemaster certification in Honduras.



Serena Hackerott is one of the teaching assistants for the Marine Ecology Field Research Methods and Independent Research courses. Serena received both her B.S. in Biology and M.S. in Marine Sciences at the University of North Carolina at Chapel Hill. Her undergraduate research focused on the possibility of biotic resistance against the lionfish invasion in Belize, Mexico, the Bahamas, and Cuba. Her graduate research quantified the effects of invasive lionfish on native reef fish community structure and composition along the Mesoamerican Barrier Reef in Belize.



Patrick Nichols is a teaching assistant for the Marine Ecology Field Research Methods class and for students' Independent Research. Hailing from the snowy north, Patrick spent his undergraduate career at the University of Miami where he worked extensively with the lionfish invasion and the molecular biology of coral communities in response to climate change. During his senior year Patrick participated in the CIEE Monteverde study abroad program in Costa Rica where he studied mosses and liverworts of tropical cloud forests. After graduating with a degree in Marine Science and Biology, Patrick decided to pursue other opportunities with CIEE, here in the heart of the Caribbean.



Martin Romain is the co-instructor for Cultural and Environmental History of Bonaire and intern for Tropical Marine Conservation Biology. Originally from Belgium, he graduated the Erasmus Mundus Master of Marine Biodiversity and Conservation (EMBC) in 2012. His thesis focused on the behavior of juvenile blacktip reef sharks (*Carcharhinus melanopterus*) of French Polynesia. He then joined the team of the Marine Megafauna Foundation where he studied the whale shark (*Rhincodon typus*) population of Mozambique (Tofo).

Staff



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



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



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



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



Amy Wilde is the Program Coordinator. She holds a B.S. degree in Business Administration, as well as, a Masters of Science in Management Administrative Sciences in Organizational Behavior, from the University of Texas at Dallas. Amy currently provides accounting and administrative support for staff and students at CIEE and she is the student resident hall manager.

Students



Samantha Chase
Colorado State
University
Biology
Denver, CO



William Duritsch
University of Dayton
Biology
Troy, OH



Benjamin Foxman
Colorado University
Environmental
Studies and Biology
Bethesda, MD



Helen Jarnagin
Occidental College
Biology
San Mateo, CA



Christina Mielke
Oregon State
University
Environmental
Sciences
Portland, OR



Cathryn Morrison
Villanova University
Environmental
Sciences
Atlanta, GA



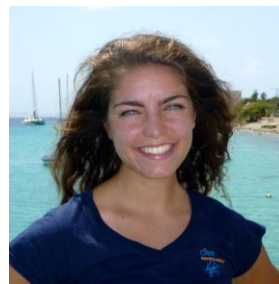
Jillian Neault
University of
Washington
General Biology
Port Ludlow, WA



Marissa Neitzke
Northland College
Biology and
Sustainable
Community
Development
New Richmond, WI



Kayley You Mak
Barnard College
Biology
San Francisco, CA



Bianca Zarrella
Vassar College
Biology
Leominster, MA

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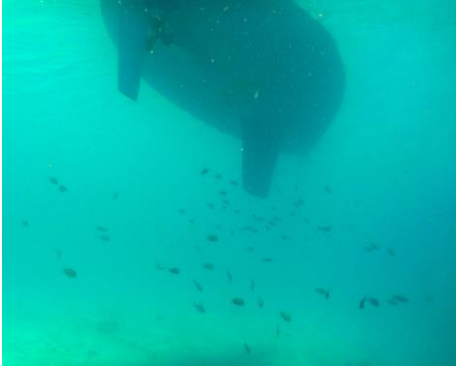
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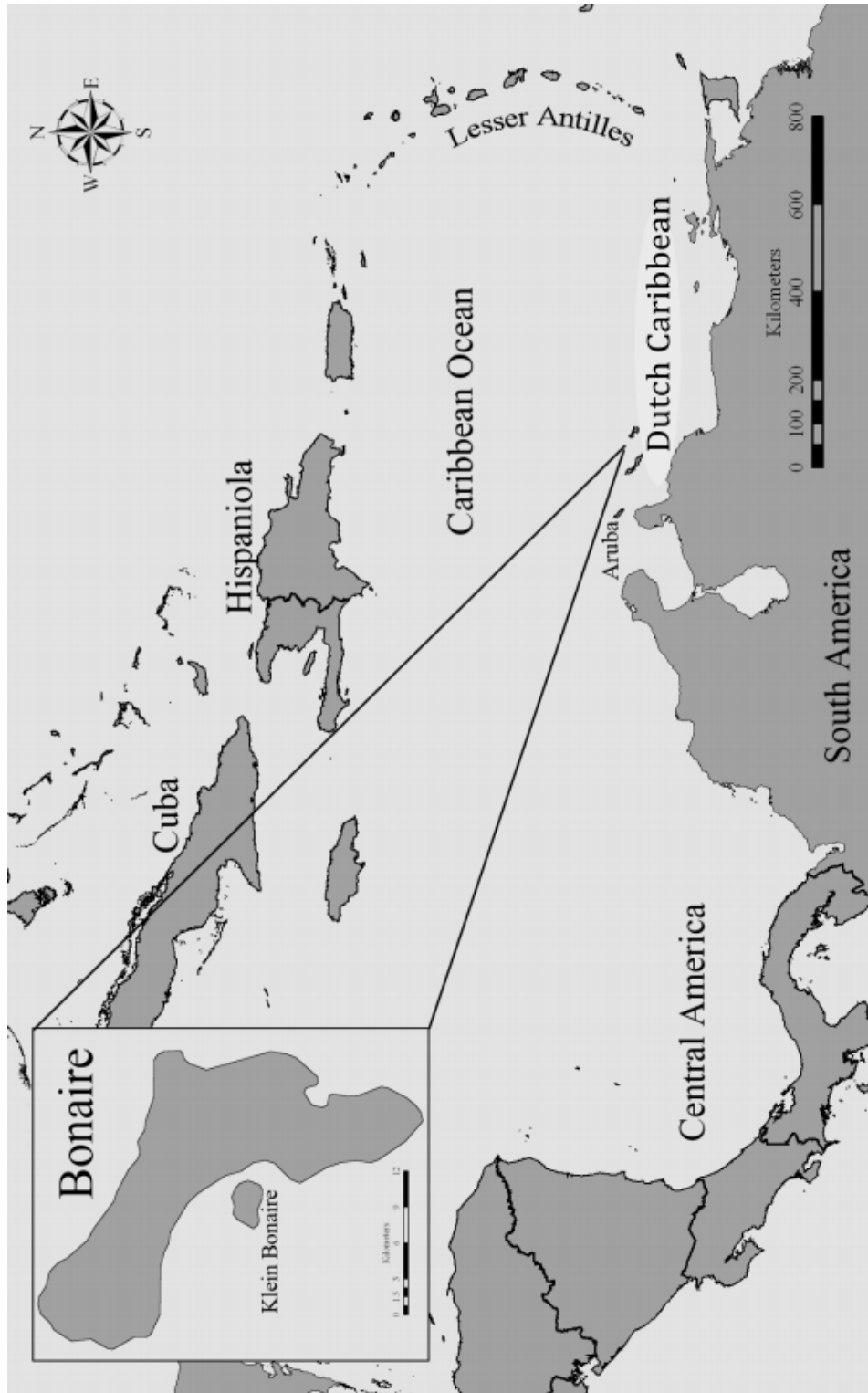
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REPORT

Helen Jarnagin • Occidental College • jarnagin@oxy.edu

Infection frequency and species identification of the black spot causing parasite found commonly on ocean surgeonfish (*Acanthurus tractus*) in Bonaire

Abstract Diseases, pathogens, and parasites in marine ecosystems are difficult to research and understand. Tracking the health of ecosystems, such as tropical coral reefs, is important for protecting these sensitive ecological areas. On the coral reefs surrounding Bonaire and other Caribbean islands, a dark spot ailment has been observed on ocean surgeonfish, *Acanthurus tractus*. This condition has been found to be a parasite, although its exact taxonomic identity is still unknown. The study of this parasite has become the point of interest for many researchers because dark spots have now been observed on other herbivorous fish in this region. The current frequency of the parasite on ocean surgeonfish and other species of surgeonfish is not known. These herbivorous fish are crucial to a healthy and sustainable coral reef ecosystem; a large change to the health of the population of these fishes could potentially affect the entire system. The purpose of this research was to find the prevalence of this parasite in species of surgeonfish through repetitive transects of counting infected individuals on the reefs of Bonaire. Additionally, collection and excision of parasites from their hosts allowed for a hypothesized genus of the infecting organism. The proportion of the density of ocean surgeonfish infected with this black spot causing parasite was 63% and it was found that the proportion of density for the degree of infection for ocean surgeonfish differed significantly among the population. Furthermore, through individual samplings of ocean surgeonfish, the lowest possible

taxonomic description of this parasite was found to be the genus *Paravortex*.

Keywords Surgeonfish • Black spot disease • *Paravortex*

Introduction

Tracking and studying marine diseases, parasites, and pathogens has proven to be a difficult, yet necessary, task for maintaining a healthy ecosystem, particularly on coral reefs (Vethaak and Rheinallt 1992). Marine ailments can threaten coral reefs as a decrease in abundance of one species could have a cascading effect on the entire ecosystem (Hayes and Goreau 1998). For example, white band disease in acroporid corals has caused wide-scale destruction and depletion of habitat for the fish within Caribbean reefs, threatening the endangerment of many different species and the system as a whole (Hayes and Goreau 1998). Studying diseases, parasites, and pathogens that affect the coral reef ecosystem is important for understanding how these various ailments spread and the various ways that the ecosystem can be damaged or changed by these pathogens.

Parasitic turbellarians are one type of marine affliction frequently observed in many different types of fish around the world (Williams and Mackenzie 2003). The transfer or reproduction of these parasites involves a complex multi-step process including multiple organisms. A turbellarian potentially requires the housing of a crustacean, fish, or a bird, or it

could be free-living (Whittington 1997). These rapidly reproducing worms can have significant impacts on their hosts and can quickly spread to the rest of the host-species population (Whittington 1997). Parasites of all types have caused a multitude of population collapses, and the rapid increase of the suspected transfer of parasites throughout marine ecosystems cause concern and a greater need to study these types of marine ailments (Marcogliese 2002; Williams and Mackenzie 2003). Turbellarians are of particular interest due to their complex lifecycles and potential to affect numerous hosts (Whittington 1997).

A turbellarian parasite has been observed on *Acanthurus tractus*, ocean surgeonfish, (previously classified as *A. bahianus*; Bernal and Rocha 2011), on the reef surrounding Bonaire, an island in the Dutch Caribbean. Herbivorous fish, such as an ocean surgeonfish, are an important member of the coral reef ecosystem because they graze on macroalgae that would otherwise outcompete corals. If this recently discovered parasite were to rapidly spread and harm these major grazers there could potentially be an increase in algae growth (Lewis 1986; Lawson et al. 1999). This increased algae growth could likely cause major harmful competition with corals for nutrients and sunlight (Hughes 1994). This turbellarian parasite was first noted in Bonaire because it causes black spots to appear on the scales of ocean surgeonfish. In 2013, a study was conducted in an attempt to discover the genus of the parasitic organism; while this study was unsuccessful in identifying the organism to the species level it left an open hypothesis for the organisms to be a parasitic turbellarian (Rodriguez 2013).

A disease resembling the black spots parasite on ocean surgeonfish in Bonaire has also affected many other species in saltwater aquaria. Kent and Olson (1986) studied the life cycle and possible treatment of these turbellarian parasites and proposed a possible genus, *Paravortex*, that could be causing the parasitic cysts in which the organism is commonly found. Given the importance of ocean surgeonfish and other herbivores on

Bonairean and Caribbean coral reefs, understanding the rate of infection for ecologically important herbivorous fish is an important step towards managing the spread of this harmful parasite.

The purpose of this research was to quantify the proportion of the ocean surgeonfish population currently afflicted with this parasite as well as to compare this frequency to a similar study completed in 2012 (Hoag 2012; Penn 2012). The frequency of this parasite was also evaluated in other species of surgeonfish commonly seen in Bonaire (*A. coeruleus* and *A. chirurgus*). The second objective is to find and confirm the proposed genus of parasite, *Paravortex*. With this information, this study aimed to provide data for future research on this potentially harmful parasite, in addition starting a basic understanding degree to which this parasite has infected the individuals and population of surgeonfish. With these objectives in mind field research was conducted to test the following hypotheses:

- H₁: The frequency of infection of ocean surgeonfish afflicted with the black spot parasite would have increased since 2012
- H₂: The genus of the parasite found would be *Paravortex*
- H₃: A wider variety of herbivorous fish related to ocean surgeonfish, with similar diets, would have been observed with the dark spots

Materials and methods

Study site

All surveys were conducted using SCUBA by swimming from Something Special Beach to Yellow Submarine on Bonaire in the Dutch Caribbean (N 12°09'40" W 68°17'1" and N 12°09'35" W 68°16'55" respectively; Fig. 1). These locations are on the western coast of Bonaire and north of the island's largest city, Kralendijk. The fringing coral reef around

these two sites is 50 m off of the shore, which has a sandy shelf leading up to the reef. The reef itself contains a high diversity of corals and fish that play an important role in the ecosystem of Bonaire (Sandin et al. 2008). Surveys were done at three different depths of 18 m, 12 m, and 5 m as to make a comparison to past studies of black spots on ocean surgeonfish. Fish were collected for parasite identification at about 5 m depth at Yellow Submarine.

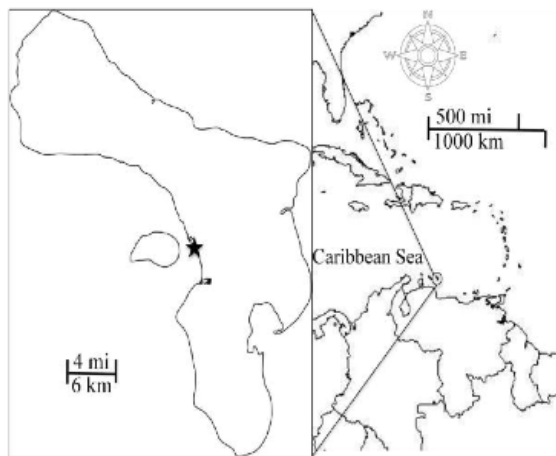


Fig. 1 Map of Bonaire located in the Dutch Caribbean. A small star represents the area between Something Special (N 12°09'40" W 68°17'1") and Yellow Submarine (N 12°09'35" W 68°16'55") (modified from Penn 2012)

Black spot affliction frequency in surgeonfish study methods

Methods for quantifying densities of ocean surgeonfish and other species of surgeonfish, *A. coeruleus* and *A. chirurgus* (blue tang and doctorfish respectively), affected by the black spot parasite were similar to a study by Penn (2012) so that a comparison could be made between 2012 and 2015. Divers entered the water at Something Special dive site and swam south for 250 m for each replicate. Swimming this prescribed distance and looking 10 m to each side of the surveyor achieved area surveys for a total of 250 m by 20 m for each depth until the surveyors reached Yellow Submarine dive site. Each replicate took place at 18 m, 12 m, and 5 m to compare the black spot prevalence at different depths. Individual surgeonfish were identified and split into four

categories based on the number of black spots on one side of the body: no spots, 1-4 spots, 5-10 spots, and greater than 10 spots. If another species was observed with similar black spots they were noted and placed into the same infection-level categories as surgeonfish.

Densities of surgeonfish were calculated in terms of individual per m² for each replicate to make a comparison against data from Penn in 2012 to see if there was a change in the prevalence of infection. Additionally, the differences between different species of surgeonfish and depths were analyzed in this study as well as noting additional species of fish with black spots.

Parasite identification and species comparisons

Four ocean surgeonfish with dark spots were collected along the Yellow Submarine site for closer examination of the dark spots. Collections took place in the late afternoon and fish were examined immediately after capture. Specimens were collected using an ELF (Eliminate Lionfish) device and were either immediately processed through examination or frozen until it could be processed.

Each specimen then went through a brief external examination to count the number of black spots on the body, then each black spot was carefully excised and either immediately stored or examined beneath a dissecting microscope (Roberts 2012). As described by Rodriguez (2013), the cysts were found directly under each black spot or directly next to it, precise removal of a thin layer of scales around the black spot revealed a semi-transparent cyst. Each successfully excised cyst was photographed. To ensure detection of the parasite inside of the cyst, in addition to proper removal of the organism from its housing, the removal and observation of the cysts had to occur directly after being caught, otherwise the worm was likely to have perished and degraded, which thus prevented it from being observed. Upon removing the worm from its casing, it was then compared visually to different turbellarian morphologies.

Data analysis

Data was analyzed using analysis of variance (ANOVA) tests comparing frequency of infection at depth and the frequency of different degrees of infection. Calculating density of the population and then the frequency of each density was used to better illustrate how many surgeonfish are affected with this black spot parasite within the whole population. To make an accurate representation of the proportion of infected individuals within a population, the density of infected individuals was achieved. This was achieved by taking the observed number of individuals and dividing by the total area surveyed per replicate for each denoted level of infection. This density per level of infection was then made into a proportion by the total amount of fish observed within each replicate, which was then averaged across all replicates at different depths. The average densities for ocean surgeonfish, blue tang, and doctorfish were all calculated separately while comparing different depths and stage of infection. These densities were calculated by taking an average of each replicate while also calculating the standard error. To compare to Penn's data (2012) the amount of individuals seen with any level of infection were divided by the total number of ocean surgeonfish individuals observed to get the frequency of infection within the ocean surgeonfish population.

A Chi-Squared test for association was completed to compare 2012 data to this study in order to see if the differences between the infection frequencies varied significantly. Two ANOVA tests were completed to see if there was variation between the frequencies of infection at different depths, and then to compare the differences between different levels of infection among each species of surgeonfish. The first ANOVA was to test if there was a significant difference between the proportions of total infected per depth. The second ANOVA test was to understand the difference between the proportions of the distinct levels of infection to see if these

variables differed significantly between each other.

Results

Infected fish analysis

A total of 378 fishes were studied over 360 minutes of observation in a total area of 5000 m². The number of spots on each individual varied, but was easily identified so that they could be placed into one of four categories of level of infection (0 spots, 1-4 spots, 5-10 spots, and >10 spots). Black spots were also observed on 11 species other than surgeonfish (Table 1). The other species represented different functional groups that could possibly be found with this parasite. Herbivorous fish and invertivores were the only types of fish observed with black spots, which include fish in the parrotfish, grunt, and snapper families.

Table 1 Non-surgeonfish species observed with black spots and their functional groups (eg. omnivores, carnivores)

Common Name	Scientific Name	Functional Group
Stoplight Parrotfish	<i>Sparisoma viride</i>	Herbivore
Princess Parrotfish	<i>Scarus taeniopterus</i>	Herbivore
Red-Band Parrotfish	<i>Sparisoma aurofrenatum</i>	Herbivore
Brown Chromis	<i>Chromis multilineata</i>	Herbivore, Invertivore
Bar Jack Schoolmaster	<i>Caranx ruber</i>	Invertivore
Snapper Mahogany	<i>Lutjanus apodus</i>	Herbivore, Invertivore
Snapper Blackbar	<i>Lutjanus mahogoni</i>	Invertivore
Soldier Yellowhead	<i>Myripristis jacobus</i>	Invertivore
Wrasse	<i>Halichoeres garnoti</i> <i>Haemulon</i>	Invertivore
French Grunt	<i>flavolineatum</i>	Invertivore
Smallmouth Grunt	<i>Haemulon chrysargyreum</i>	Invertivore

In this study, a total of 68% of the ocean surgeonfish population observed were infected at 5 m, 73% at 12 m and 62% at 18 m. Penn (2012) found that 93% of individuals were

infected at 5 m, 81% at 11 m, and 72% at 18 m. There was no significant level of differences between 2012 and 2015, meaning that the variation between these two data sets could be statistically random ($X^2=0.934$, $df=2$, $p=0.627$).

The analysis of this set of data was conducted in a different manner than both 2012 studies so that future comparisons can be made to track the overall spread and prevalence of the black spot causing parasite. The first ANOVA test was to determine the effect of depth on proportion of total infected for each species of surgeonfish. This test illustrates that there was no significant variance between infection and depth for the proportion of total infected fish of each species. The p-values illustrating this lack of variance were as follows: ocean surgeonfish: $p=0.52$, blue tang $p=0.074$, and doctorfish $p=0.250$. Due to the lack of differences at particular depths, the variance between the levels of infection at all depths combined for each individual species of surgeonfish was also testing using a one-way ANOVA. This ANOVA tested to see if the proportion of affected varies significantly over the level of infection. The p-values for these ANOVA tests showed statistically significant differences in the proportion of density at each level of infection for each species of surgeonfish. These data from the ANOVA tests can be visualized through the use of interval plots of the distinct proportions of density within each level of infection ($p<0.005$; Fig. 2). A Tukey test was also completed during this second round of ANOVA tests to show which level of infection for each species differed significantly from the rest (Fig. 2).

The densities for ocean surgeonfish, blue tang, and doctorfish were calculated to have a wide distribution of densities and different depths and stage of infection. Ocean surgeonfish have the highest density of infected individuals of 1-4 spots with a density with an average of 0.46 ± 0.054 fish per 100 m^2 (mean \pm SD, $n=134$). In blue tang and doctorfish populations there were a higher proportion of individuals who showed no signs of infection than any of the infection levels at any depth (Fig. 3).

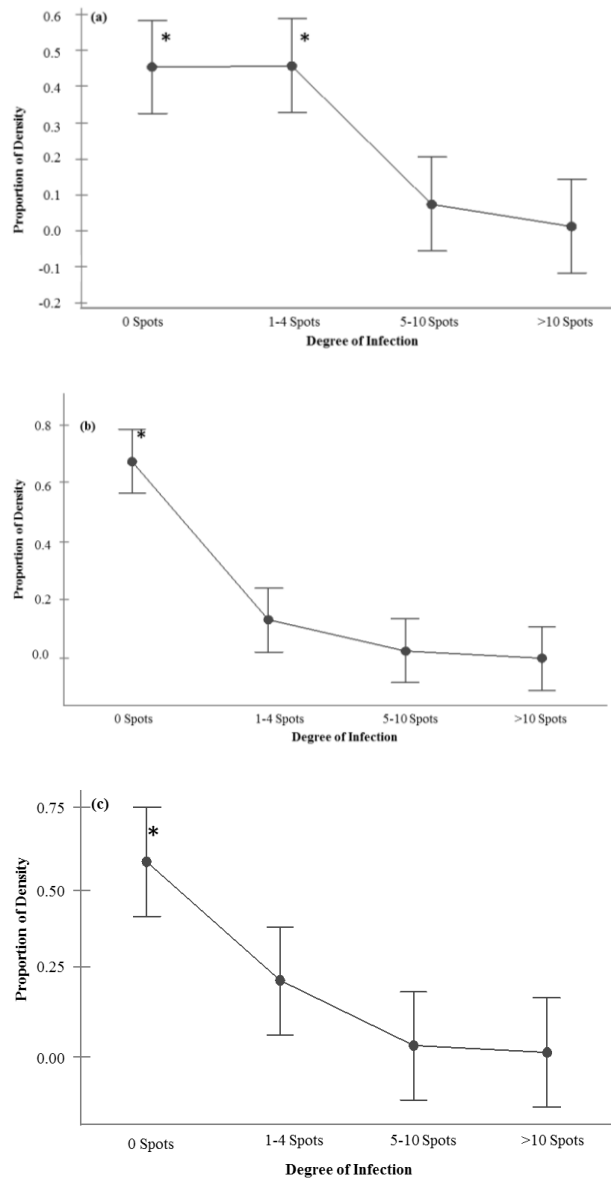


Fig. 2 ANOVA test comparing density of different degrees of infection for (a) ocean surgeonfish, (b) blue tang, and (c) doctorfish, across all depths, all p-values showed significant variation where $p<0.005$

* indicates groups that are statistically significant compared to the other group of data within that graph as calculated by a Tukey test

Parasite identification

Similar to Rodriguez's (2013; Fig. 4d) findings, a cyst was found around and within each black spot on collected individuals. On two particularly large spots, multiple cysts were found. Cysts were circular disks that had a rubbery coating that was difficult to penetrate (Fig. 4a, Fig. 4b).

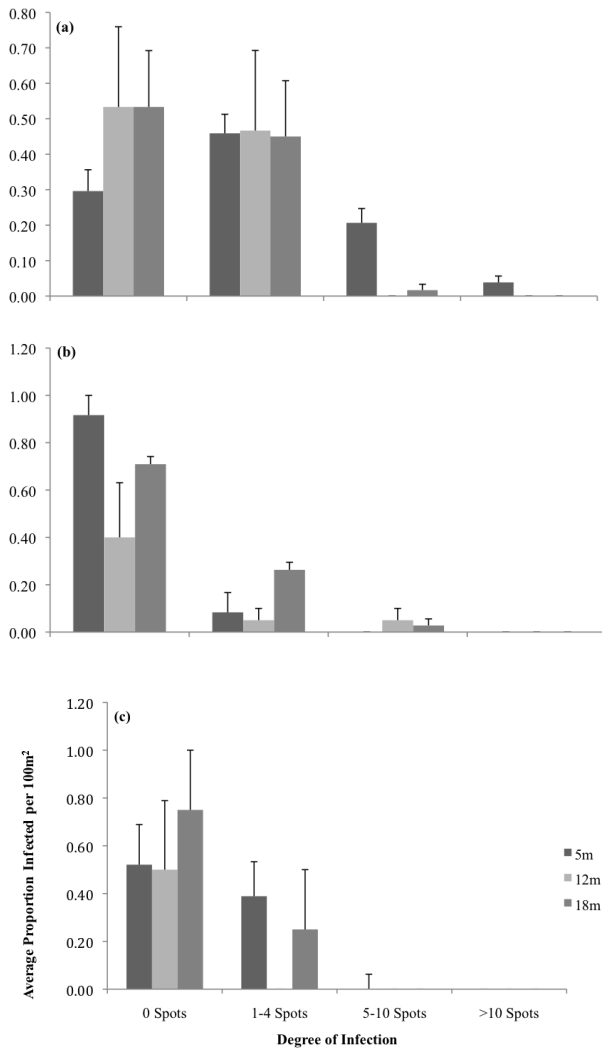


Fig. 3 Average proportion of infected individuals for each species comparing different degrees of infection to no infection of parasite for each depth in (a) ocean surgeonfish, (b) blue tangs, and (c) doctorfish. Different depths were also compared in these figures and can be observed as 5 m in dark grey, 12 m in the lightest grey, and 18 m in medium most grey. If all values for each depth within each category of degree of infection were added together, the sum would make 1, as each value is a proportion of the population density

No movement was observed in the cysts 3.5 hours past initial collection, or after any preservation methods were used on the fish or on the cyst. Black spots were difficult to observe once fish were removed from the water, but cysts were clearly embedded just under the first layer of scales on the fish. One successful removal of a live specimen showed the organism moving actively inside of its cyst. After cutting the cyst open, the organism quickly perished either from a wound caused by the excision or from exposure to the

environment. The morphology of this organism (Fig. 4c) was similar to the organisms found by Rodriguez (2013; Fig. 4d).

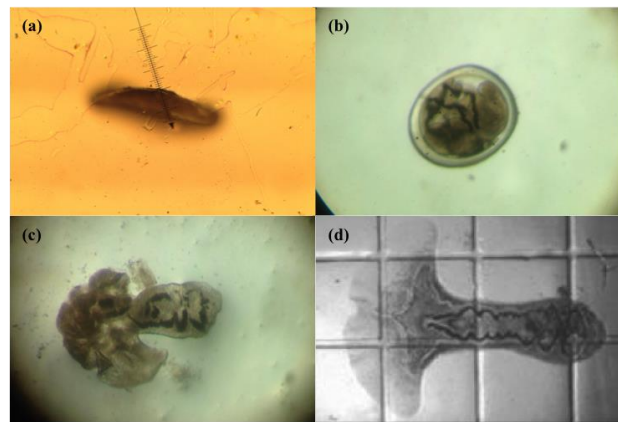


Fig. 4 (a) micrograph of a disk-shaped cyst placed on its side. (b) micrograph of a cyst with a live parasite inside. Parasite was moving when picture was taken, and is surrounded by a thick membrane of the cyst. (c) parasite after removing it from the encasing, slightly damaged in removal process. (d) parasite extracted by Rodriguez (2013)

Discussion

The frequency of black spot infection continues to be observed primarily within ocean surgeonfish, and this infection is most commonly in a lesser stage of advancement. In previous studies it was found that depth correlates to the frequency of infection for the ocean surgeonfish population (Hoag 2012), but through ANOVA tests it was found that there was no association between depth and the amount of individuals infected in this study. From another set of ANOVA tests for each species of surgeonfish it was observed that there were significant differences between each stage of infection. Evidence of variation between each stage of infection is biologically significant, within each black spot 1-3 cysts were found, indicating the more black spots that are observed on individuals further implying a progression of the disease. Finding the proportion of the density of individuals found in an area allowed for a better idea of how the population itself is afflicted with this parasite as well as the ability to repeat this study in the same manner and track the spread

of this dependent organism. Though the frequency of observed individuals in 2012 seemed to demonstrate a higher prevalence of the disease than today, the methods and small sample size of Penn's study could account for the differences in these proportions. Furthermore, the differences between these two studies emphasize the need for more studies to shed light on infection frequency of ocean surgeonfish.

This study determined that the parasitic cause of the black spots observed on ocean surgeonfish in Bonaire is likely turbellarian worms from the genus *Paravortex*. This was concluded by research done on the two primary genera of possible turbellarian parasites that can be found on fish, *Ichthyophaga* sp. and *Paravortex* spp., (Cannon and Lester 1988). It was determined that this particular parasite was *Paravortex* primarily due to the morphology of the turbellarian and past research by Kent and Olson (1986), which cited a similar organism of this genus. No eye-spots were observed on the excised individuals in 2013 (Rodriguez) and in this study the presence of a fan-like tail indicated that the parasite could also be identified in the class Cestoidea (Roberts 2012). However, despite these two morphological differences, the cyst that these organisms were found in addition to their ciliated bodies, and their flat bodies indicated that they were indeed *Paravortex* (Kent and Olson 1986; Ogawa 2011). Another indication that this parasite was within this genus was the fact that only one organism was found within each cyst. These observations were in agreement with Rodriguez (2013). In contrast, within *Ichthyophaga* sp., it is more common to find multiple individuals inside each cyst. The fact that the parasites found in this study are also hypothesized to be on other types of herbivorous fish indicate a lack of host specificity which also suggests the *Paravortex* genus (Justine et al. 2009). Lastly, the material of the outside of the cyst cavity was found to be a thick rubbery substance, different than the loose fibrous tissue that typically contain *Ichthyophaga* individuals (Cannon and Lester 1988). In fact, it was found that the black spots

on parrotfish are most often caused by turbellarians from the *Ichthyophaga* genus, indicating that this particular turbellarian parasite could not be spreading as rapidly to other species as it was once thought (Cannon and Lester 1988).

While observing the black spots on collected specimens of ocean surgeonfish under a dissecting microscope, it was noted that the black spots themselves were not a separate entity within the scales. The spots appeared to be an excess of skin pigmentation instead of a separate formation. There are many possible explanations for black spot causing parasites on marine organisms; for instance, black spots have been observed on unicorn fish in the Pacific Ocean that are likely caused by a species within the *Ichthyophaga* genus. Furthermore, monogean parasites found on surgeonfish in the British Virgin Islands also cause black spots (Justine et al. 2009; Sikkell et al. 2009). The black spots may be an underlying immune response, an idea that stemmed from research in sea urchins, which has demonstrated an innate immune response in their larval stage that is also found in the DNA of invertebrate and vertebrate animals including fish (Hibino et al. 2006). This innate immunity implies that the bodies of these animals react to an outside pathogen. Other research has suggested that fish and other vertebrates can secrete hormones that lead to pigment production as an immune response (Yada and Nakanishi 2002). Based on this research, the assumption that all black spots on all fish species on Bonaire are caused from these turbellarian parasites cannot be made as there are many other viable causes. From the data on multiple similar cysts found in this study and by Rodriguez (2013), further research can be based on the idea that ocean surgeonfish are likely infected with this particular parasite; however, black spots on most fish species could be a number of infections, parasites, or pathogens.

The results of this study were not entirely conclusive as the basis of identification was just from one individual. Additionally, the method used in this study for analyzing the

proportion of infection within the surgeonfish population led to an inability to confirm if there had been a true increase in the amount of individuals infected with this parasite. However, this study lends itself nicely to future potential research on this particular black spot causing parasite. The first priority of future researchers would be to continue surveys to discover infection frequency and to gain an accurate depiction of the population affected with this parasite. Secondly, further research into the taxonomic identification of this parasite to find the species would allow for further understanding of its potential population impact and spread to other organisms. The visibility of the response to this parasite makes it ideal for future infection frequency studies even though working with the encysting parasites can be cumbersome. Studying diseases, parasites, and pathogens in marine organisms and ecosystems can be difficult, but understanding infection frequency and what is infecting these species and systems is an important step to protect the environment.

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REPORT

Bianca Zarrella • Vassar College • bizarrella@vassar.edu

Effects of pumping efficiency on the antibacterial properties of sponges *Aplysina archeri* and *Aplysina lacunosa* on Bonaire

Abstract The filter feeding mechanism of marine sponges exposes them to water-borne toxins and bacteria, forcing them to evolve immune systems effective in fighting these pathogens. Therefore, antibacterial properties of the sponge's defense system are effective tools that can be used in medicinal therapies. By modeling sponges' response to pathogens, advances can be made in human medicine. This study analyzed how the pumping efficiency of the species *Aplysina archeri* and *Aplysina lacunosa* affected the antibacterial properties of the sponge. Sponges were sampled from depth ranges of 10 – 12 m, and 16 – 18 m. The pumping efficiency of each sponge was tested using water sampling (In-Ex), determined by comparing the turbidity of water before it entered and as it exited the sponge. Variation in antibacterial properties was analyzed by assembling antibiotic assays from sponge extracts. Using this method, sponges showed no bacterial inhibition. Both *A. archeri* and *A. lacunosa* filtered water more efficiently in shallow water, but this trend was not significant. This study sought to introduce information that could be useful when determining what sponge to use in pharmaceutical testing. With such knowledge, pharmaceutical companies can continue to compile qualities to formulate an ideal sponge species they should research for medicinal cures.

Keywords Antibacterial assay • Inhalant-exhalant • Pharmaceutical

Introduction

The pharmaceutical industry is constantly discovering new organisms with medicinal properties, and coral reef environments have proven to be a large contributor of these therapeutic cures (Newbold et al. 1999). Infectious diseases are constantly in an arms race with the antibiotics that researchers derive. This continual evolution makes it important for the scientific community to discover new remedies (Laport et al. 2009). Sponges are sessile organisms that inhabit a variety of marine habitats (Yahel et al. 2005). Sponge compounds are found in over 5,300 pharmaceutical products, and every year over 200 new sponge metabolites are discovered (Laport et al. 2009).

Sponges have the ability to promote the growth of antibacterial compounds that help them control bacterial attachment to their exteriors (Newbold et al. 1999). Environmental conditions such as aggressive competition and water pollution level expose sponges to different stressors that they must defend themselves against accordingly. Sponges that are exposed to the highest level of toxins are located in the highest stress environments (Proksch 1994). Frey (2014) found that colonies of *A. archeri* expressed increased antibacterial properties when exposed to polluted waters on Bonaire; the bacterial inhibition of *A. archeri* was 1.46 times greater in highly polluted waters than in the areas of low pollution.

To identify the properties of a sponge ideal for pharmaceutical use, the filtering efficiencies of sponges must be considered.

Higher filtration efficiencies remove more particles from the surrounding water, exposing the sponge to more toxins. The sponge should produce effective resistances to those toxins in order to remain healthy. Olson and Gao (2013) sampled sponges along a depth gradient and found that sponge species displayed different pumping efficiencies at a range of depths. Fundakowski (2014) found similar results when investigating the pumping efficiency of *A. archeri* and *A. lacunosa* sponges in shallow and deep water (6 – 12 m and 20 – 26 m, respectively) on Bonaire. The pumping efficiency of *A. archeri* was found to be higher in deeper waters, while *A. lacunosa* was more efficient in shallow waters (Fundakowski 2014). With this information, the two species of sponges can be tested at both depths to discern whether the pumping efficiencies influence the antibacterial properties of the sponges.

No research has been conducted on how the pumping efficiency of the sponge influences its antibacterial properties; however, a good deal is known about the sponges' filtration mechanisms. Sponges are filter feeders; their pumping efficiency quantifies how they filter the water that passes through them, and therefore how much bacteria they come into contact with (Riisgard and Larsen 1995). Their suspension feeding mechanism allows sponges to filter large volumes of water (Yahel et al. 2005). In this way, sponges can remove nutrients, particles, and other food items, such as free-living bacteria and phytoplankton from the water column (Newbold et al. 1999). It has been found that sponges express plankton-eating productivities extending from 75 – 99% (Pile et al. 1996; Pile et al. 1997). This direct exposure to water-borne toxins may have an affect on the bacterial inhibiting properties of sponges.

This study aims to investigate the antibacterial properties found in the sponge species *A. archeri* and *A. lacunosa* by examining whether increased pumping efficiency at shallow and deep depths increases the sponges' inhibition of bacteria. By demystifying the optimal conditions for which

sponges produce the most antibacterial compounds based on depth and pumping efficiency, we can find under what circumstances sponges' antibacterial compound production should be explored for medicinal remedies. Ideally, pharmaceutical companies should seek to research sponges that have a high efficiency of filtering, because there will be a better chance of harvesting medicines from these sponges.

H₁: Sponges with higher filtering efficiencies would inhibit bacteria more due to increased exposure to polluted water, requiring sponges to heighten their internal defenses

H₂: *A. archeri* would inhibit more bacteria at depth than in shallow water, while *A. lacunosa* would inhibit more bacteria in shallow waters

Materials and methods

Study site

This study was carried out on Bonaire, Dutch Caribbean, a small island (~288 km²) situated ~80 km north of Venezuela. The study site, Yellow Submarine dive site, is located on the western side of Bonaire (12°09'36.5" N, 68°16'55.2" W), just north of the capital, Kralendijk. The sandy flat is replaced by reef habitat 50 m from the shoreline 10 m below the surface. The fringing reefs on Bonaire are known for their high diversity, with both soft and hard corals, as well as sponges and algae, on site.

Study organisms

Two species from the *Aplysina* genus, known for their inhibition of bacterial attachment to their external surfaces, were chosen for research. Commonly known as the stovepipe sponge because of its tube shape, *Aplysina archeri* is found frequently throughout Caribbean reefs. *Aplysina lacunosa*, commonly referred to as the convoluted barrel sponge,

resembles a wide, vase shape that tapers out closer to the base. Both species are found mostly in the tropical Atlantic Ocean (Newbold et al. 1999). Both species have proven to be effective in negatively influencing the growth of bacteria (Frey 2014; Kelley et al. 2003). Fundakowski (2014) found that *A. lacunosa* filtered water more efficiently in shallow waters, while the filtering efficiency of *A. archeri* was higher in deep water.

Methodology

The focus species for this research, *A. archeri* and *A. lacunosa*, were chosen for the feasibility of sampling, with both species populating the shallow (10 – 12 m) and deep (16 – 18 m) areas of the site. The two depths were sampled to see if there would be a correlation between the increased pumping efficiency and the antibacterial properties of the sponge. Samples were taken by cutting several small pieces of sponge that were placed in plastic containers with water from the collection site. Samples were labeled with species and depth. After collection, the samples were frozen in a -20°C freezer until further testing could be done.

Bacteria panel

Sponge samples were thawed and diced into small sections. Following the methodology in Frey (2014), each sample reached a total volume of 15 ml by saturating the pieces of sponge in a 10 ml 100% methanol solution. Sponges soaked for 48 hours. The solvent was extracted from the sponge into a separate flask. The solvent was left to evaporate completely at room temperature, and to reach the volumetric concentration of the original tissue, the extract was redissolved in 5 ml of 4:1 100% methanol:water solution.

Antibacterial assay

The sponge solution was pipetted onto discs cut from filter paper, and the solvent was left to evaporate. To generate the antibacterial assay, methods from Frey (2014) were followed.

Filter paper circles were placed on agar plates with bacteria, then were incubated at 30°C for 72 hours. The bacteria cultures were taken from the human mouth using a q-tip, and added onto the agar plate. The human mouth has proven to be an appropriate source for bacteria due to its diverse array of fauna, containing Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, Synergistetes, and Tenericutes (Wade 2012). Ten plates were created, with each plate containing discs for both sponge species at deep and shallow depths, and the controls. The positive control on each plate was antibacterial dish soap, and the negative control was methanol. After the agar plate containing bacteria and sponge extract was incubated for 72 hours at 30°C, the diameter of the region of inhibition was measured directly with a ruler in millimeters for each sample's circular filter paper disc. The region of inhibition refers to the ring where bacteria did not grow on the disc around the sponge extract.

Inhalant-exhalant (In-Ex) water samples

To quantify the filtration rate of sponges, an In-Ex method as outlined by Fundakowski (2014) and Yahel et al. (2005), was used. The method consists of collecting water from the inhalant and exhalant openings (ostial and osculum, respectively) of each individual, expecting that water turbidity would decrease upon being filtered by the sponge (see Turbidity analysis section below). Water samples were taken by two SCUBA divers at the same time using 50ml syringes. Samples were labeled with the depth, species, and collection location on the sponge (ostial or osculum) and were processed immediately once back in the laboratory.

The filtration efficiency of sponge samples was evaluated with a Turner Designs Trilogy Laboratory Fluorometer, calibrated using 1, 10, 100 and 1000 nephelometric turbidity unit (NTU) solutions. The solutions were made from a 1000 NTU stock solution from serial solutions. Turbidity of the water samples was measured using the Fluorometer by placing the samples in plastic cuvettes.

Data analysis

Bacterial inhibition

An average of the region of inhibition was calculated for each species at each depth.

Turbidity

For each sponge, percent reduction in turbidity was calculated between the inhalant sample and the exhalant sample. The reduction in turbidity was handled as a percentage for each sample, and then was averaged for each species at each depth. To assess the effect of species and depth on percent filtration rate, two-way analyses of variance (ANOVA) was calculated. Unpaired t-tests were performed to test the significance of the effect of depth on filtration rate.

Results

Bacterial inhibition

To test if the filtering mechanisms of sponges influenced their antibacterial effects, sponges *A. archeri* and *A. lacunosa* were used. Seven samples of *A. lacunosa* were taken from shallow depths (10 – 12 m), and seven samples were taken from deep depths (16 – 18 m), for a total of fourteen samples. Six samples of *A. archeri* were taken from shallow depths, and seven samples were taken from deep depths, for a total of thirteen samples. A total of 27 assays were made, one for each of the sponge samples. Microbiological assays were performed on nutrient agar plates with their correspondent positive and negative controls. No bacterial inhibition was found in any of the 27 sponge samples (Fig. 1).

Turbidity

The inhalant and exhalant (In-Ex) water samples from the 27 sponge samples were analyzed using a Fluorometer. The difference of In-Ex turbidity values was not statistically

significant between species ($F = 0.00$, $p = 0.96$), or between depths ($F = 0.83$, $p = 0.37$; Fig. 2, Tables 1 and 2). A two-way ANOVA found that percent reduction in turbidity was not significantly influenced by species ($F = 0.50$, $p = 0.48$) or depth ($F = 0.49$, $p = 0.48$; Fig. 3, Tables 1 and 2).



Fig. 1 Negative results of the antibacterial activity of the secondary metabolite extracts of sponge species *Aplysina archeri* and *Aplysina lacunosa*. From left to right, filter paper soaked extracts of 1) *A. archeri* shallow, 2) *A. archeri* deep, 3) negative control (methanol), 4) positive control (antibacterial soap), 5) *A. lacunosa* shallow, 6) *A. lacunosa* deep

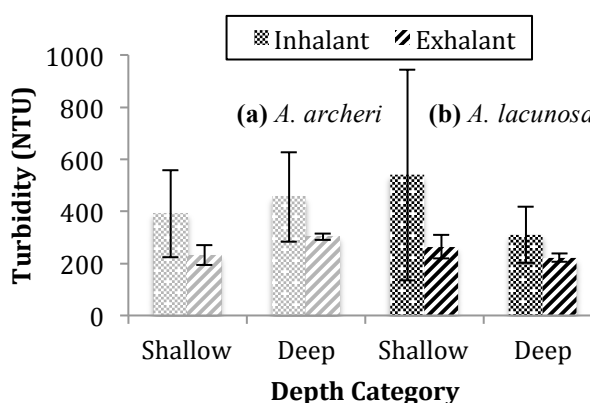


Fig. 2 Turbidity (NTU) of both inhalant (spotted) and exhalant (striped) water samples for two species of sponge, (a) *Aplysina archeri* (unshaded) and (b) *Aplysina lacunosa* (shaded), at two depth categories. Error bars represent standard error of the mean (SEM). (For *A. archeri* shallow $n=6$, and for all others $n=7$)

Table 1 Comparison of Inhalant and Exhalant turbidity measurements for species *Aplysina lacunosa* at shallow and deep depths, measured in nephelometric turbidity units (NTU). %In-Ex is defined as the percentage of filtered water

Depth	Turbidity Analysis		
	Inhalant	Exhalant	%In-Ex
shallow	339.41	320.11	5.69
shallow	282.74	257.40	8.96
shallow	319.93	271.17	15.24
shallow	255.09	194.88	23.60
shallow	363.20	262.54	27.71
shallow	921.05	315.19	65.78
shallow	1296.35	226.71	82.51
deep	216.77	208.11	4.00
deep	216.77	186.38	14.02
deep	348.14	294.97	15.27
deep	205.93	157.36	23.59
deep	332.44	241.48	27.36
deep	344.34	209.92	39.04
deep	506.31	250.84	50.46

Table 2 Comparison of Inhalant and Exhalant turbidity measurements for species *Aplysina archeri* at shallow and deep depths measured in nephelometric turbidity units (NTU). %In-Ex is defined as the percentage of filtered water

Depth	Turbidity Analysis		
	Inhalant	Exhalant	%In-Ex
shallow	214.11	180.11	15.88
shallow	257.95	204.47	20.73
shallow	344.58	257.17	25.37
shallow	428.58	237.62	44.56
shallow	413.83	221.79	46.41
shallow	684.11	287.78	57.93
deep	287.74	238.27	17.19
deep	344.43	283.24	17.77
deep	727.17	513.60	29.37
deep	364.88	234.42	35.75
deep	673.03	410.46	39.01
deep	420.22	237.28	43.53
deep	372.03	197.32	46.96

In shallow water, *A. archeri* exhibited a higher percent reduction in turbidity than in deep water (shallow: $35.15 \pm 16.79\%$; deep: $32.79 \pm 11.86\%$; $t = 0.38$, $p = 0.712$). The same was found in *A. lacunosa* (shallow: $32.79 \pm$

29.67% ; deep: $24.81 \pm 15.86\%$; $t = -2.73$, $p < 0.05$; Fig. 3), although these differences were not statistically significant for either species. Negative turbidity values were not included, because this would indicate that the sponge increased the turbidity of its exhaled water samples.

Discussion

This study sought to determine if the pumping efficiencies of marine sponges *A. archeri* and *A. lacunosa* influenced their antibacterial properties. Neither sponge species inhibited bacterial growth in any of the trials. This was contrary to the findings of Frey (2014), which discovered that *A. archeri* inhibited bacteria growth. An inconsistency that must be accounted for is the origin of the bacteria used for the bacteria panel. Though this study and Frey (2014) obtained bacteria from swabbing a human mouth, the individual donors differed, which could result in a discrepancy between the types of bacteria grown in the bacterial panel. This is due to the diverse collection of bacteria that constantly colonize the human mouth. A study by Bowden and Hamilton (1998) found that the oral bacteria in humans depends on the values of oxygen, pH, and carbohydrate concentration, as well as other microbial interactions and salivary components. Though the human mouth has an average range for these variables, slight variations have major effects on the types of bacteria that grow in the human mouth. Additionally, the level of dental plaque formation has a direct influence on the type of bacteria that can adhere and grow in the mouth. Individuals with varying levels of dental plaque formation harbor different types of bacteria in their mouth (Bowden and Hamilton 1998). This could support the fact that *A. archeri* and *A. lacunosa* may only inhibit certain types of bacteria, such as the bacteria specimen used by Frey (2014).

Though there is evidence that sponges are exposed to toxins in the surrounding water, it may not be the case that sponges must defend

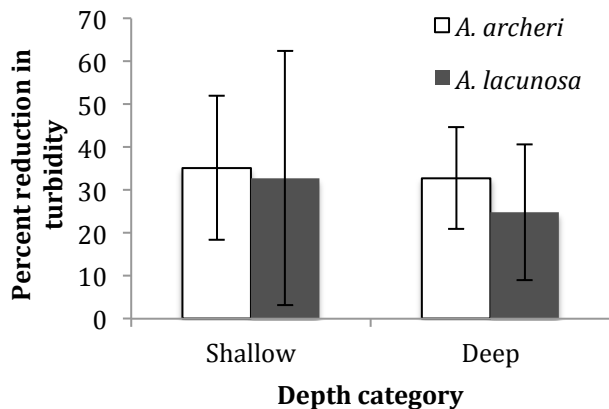


Fig. 3 Percent reduction in turbidity of *Aplysina archeri* (unshaded) and *Aplysina lacunosa* (shaded), at two depth categories. Error bars represent standard error of the mean (SEM). (For *A. archeri* shallow n=6, and for all others n=7)

themselves against all bacteria. Müller et al. (2004) confirmed that the filter feeding mechanism of sponges creates currents, exposing the sponge body to bacteria from surrounding water. However, they also found that sponges retain some bacteria and form mutualistic relationships. The metabolites produced by the sponge are used by symbiotic bacteria as a source of energy. In return, some bacteria create bioactive compounds to safeguard their host from other bacterial invaders (Müller et al. 2004).

However, previous studies have supported the fact that sponges are exposed to pathogens in their surrounding seawater, and also that sponges utilize the bacteria to their advantage (Kelley et al. 2003; Laport et al. 2009; Frey 2014). Therefore, methodological error is the most likely the cause for the discrepancy between the findings of this study and previous research, because the antibacterial property of sponges is a topic that is widely supported across scientific literature.

Extraction of the sponges' secondary metabolites proved to be the most convoluted aspect of the methods. Perhaps future studies could focus on more reliable ways to extract the secondary metabolites from the sponge accurately each time. Extraction by pressing the sponge could have left secondary metabolites behind. Instead of squeezing extract out of the sponge samples, protocol

involving the grinding of sponges should be followed. For example, Pawlik et al. (2007) utilized rotary evaporation to extract sponge secondary metabolites. This method would ensure that a sufficient amount of secondary metabolites were extracted, and not left in the sponge itself.

Though differences in the In-Ex turbidity values were not statistically significant between species or between depths, slight trends were observed. Similar to results found in Fundakowski (2014), *A. lacunosa* filtered more efficiently at shallower depths. However, contrary to Fundakowski (2014), *A. lacunosa* also displayed higher filtration rates in shallow water. A study by Riisgard et al. (1993) found that temperature and pumping rate in marine sponges are positively correlated. This could support the finding that sponges filter water better in warmer, shallow water, where sunlight penetrates more. However, the depths sampled in this study would have to be expanded in order to observe a significant temperature change to come to this conclusion. Overall, further investigation could be done on the filter feeding mechanism of sponges. The method of suspension feeding and the factors that impact the standard pumping method are not well understood (Bell 2008). Information on this topic could provide insight on the types of compounds the sponges expose themselves to during feeding, and whether this would affect the antibacterial properties of sponges.

This study provides a framework for the use of sponges in pharmaceutical remedies. Due to the high level of variance between sponge individuals, additional studies could assess how the antibacterial properties of sponges are influenced by their surroundings. Environmentally related variables such as water salinity, depth and competition could have an effect on antibacterial properties. For example, sponges are known to produce secondary metabolites effective in reducing the photosynthetic potential of nearby algae, but other effects regarding how competition influences the sponges' antibacterial properties are not known (Pawlik et al. 2007).

The main focus of future studies should be on solving the various correlations between the antibacterial properties of sponge individuals, and the variables relevant to the individual's environment, such as the depth, surrounding water composition, or presence of nearby species. Studies could also be expanded to test other sponge species. Understanding how antibacterial properties vary in an assortment of sponge species in relation to an assortment of variables could offer practical knowledge on the ideal sponge individual that should be tested for pharmaceutical research.

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REPORT

Benjamin E.R. Foxman • University of Colorado Boulder • befo9704@Colorado.edu

Distribution and abundance of spatial competition between scleractinian corals and sessile aggressive invertebrates on the west coast of Bonaire

Abstract Processes affecting reef ecosystems have three levels of organization: macroscale, mesoscale and microscale. These processes are conducive to interspecific competition amongst various coral and aggressive invertebrate species. Surveys of these organisms' distribution, abundance and ecological description of their intra/interspecific competition have been conducted throughout the Caribbean. Previous research has found that scleractinian corals in reef slope ecosystems are frequently outcompeted by sessile aggressive invertebrates, such as Clionid sponges, encrusting bryozoans, encrusting gorgonians and overgrowing mat tunicates. Furthermore, interspecific spatial competition between corals and aggressive invertebrates has been observed to increase in frequency with depth. This project analyzed the distribution and abundance of coral-aggressive invertebrate spatial competition along a fringing reef ecosystem on the west coast of Bonaire. Belt transects were laid out between 200 m north and south of the GPS coordinates N 12°09.6 12' W 068° 16.9 15', at two depths (10 and 15 m) along the reef slope. Instances of spatial competition involving individuals at least 10 cm in length were photographed for further analysis. Five coral species and 25 aggressive invertebrate species were encountered in a total of 216 coral-aggressive invertebrate interactions. Quantitative data analysis showed that the orange lumpy encrusting sponge (*Scopalina ruetzleri*) was the most abundant aggressor at 15 and 10 m, although mean total area covered by coral-aggressive invertebrate interactions and their frequency did not increase with depth. Findings

could be used as a baseline for future scientific marine research, potentially on growth rate of competing species and the underlying mechanisms responsible for their interspecific spatial competition.

Keywords *Scopalina ruetzleri* • Spatial competition • Bonaire

Introduction

Processes affecting reef ecosystems

Reef ecosystems are affected by macroscale, mesoscale and microscale processes. Macroscale processes contribute to whether reefs found globally are considered keep-up, catch-up, or give-up reefs. Mesoscale processes, such as temperature, salinity and wave energy are either directly or indirectly connected to macroscale and microscale processes (Hubbard 1997). Microscale processes include environmental factors that can vary on a local scale, such as light irradiance, sedimentation, nutrient input and antecedent topography (Hubbard 1997). These abiotic processes are integral for creating conditions conducive to the biotic processes that determine reef community composition (i.e. spatial competition) between a diverse array of sessile aggressive invertebrate and coral species (Zea 1993; Glynn 1997).

Bioerosion through decalcification, allelopathy as well as physical interactions, such as sweeper tentacles, boring and growing over skeletal structures are all examples of biological mechanisms employed by aggressive

invertebrate and coral species in order to compete with each other (Jackson and Buss 1975; Porter and Targett 1988; Vogel 1993; Aerts and van Soest 1997; Rützler 2002). Allelopathy may not simply decalcify skeletal structures of coral species, but may also lower their defenses against epizootic recruitment “fouling” (Jackson and Buss 1975). Mucous secretion of many sponges provides a medium for allelochemical concentration at the sponge-competitor interface with minimal dilution and/or interference by ocean currents (Porter and Targett 1988). Previous qualitative observations in a study of coral-sponge interactions on Jamaican reefs revealed that sponges overgrew corals more often than corals overgrew sponges (Jackson and Buss 1975). The occasional observations of corals outcompeting sponges for space showed no signs of damage to sponge tissues adjacent to these corals, while the common observations of sponges overgrowing corals included salient examples of bare pavement and dead coral skeleton directly adjacent to sponge tissue. Bare pavement and dead areas of coral in direct proximity to overgrowing sponge tissues strongly suggest the involvement of allelochemical mechanisms from reef sponges (Jackson and Buss 1975).

Competitive mechanisms differ between coral species and competitive hierarchies of intraspecific competition between corals only tend to apply when competing species use similar mechanisms of competition (Aerts and van Soest 1997). Competitive hierarchies can break down when competing species are able to employ alternative competitive mechanisms, such as sweeping tentacles, which cause tissue necrosis in competitors in close proximity (Sebens and Miles 1988). Overgrowth, boring and allelopathy are additional competitive mechanisms that may be employed by sponges. A study of a fringing reef community in NE Colombia under stressful conditions showed that aggression from sponges of various species was not equal in terms of overgrowth (Aerts and van Soest 1997). However, boring and allelopathic mechanisms employed by sponges and other aggressive invertebrates may be more

effective at competing for hard substratum space than coral species.

Research has also found that spatial competition increases in frequency with depth (Suchanek et al. 1983). The deeper the spatial competition, the less light is available for zooxanthellae within corals to provide corals with essential energy for growth. Regardless of depth, sponges are typically one of the most abundant “cryptic” or hidden sessile components of reef ecosystems. Scleractinian corals, hydrocorals, and crustose coralline algae are typically dominant in non-cryptic “open” space (Zea 1993). Where disturbance is low and space/light resources are limited, sponges and other aggressive invertebrates play an important role in open space and cryptic reef community dynamics (Zea 1993).

Sponges may have a predominant role in spatial competition in the Caribbean Sea, where there are approximately 600 species (Diaz and Rützler 2001). On many reefs, sponge biomass exceeds scleractinian (including hermatypic) coral biomass, although many surveys used point-count and projected-area methodology, which underestimate the abundance and distribution of both sponges and corals in reef ecosystems by simply giving an estimate for sponge biomass in cryptic habitats. Among these sponges are the substratum boring Clionid sponges, which are often overlooked by divers because of their cryptic nature and competitive mechanisms (Diaz and Rützler 2001).

Sessile aggressive invertebrates and corals in the Caribbean Sea

A large quantity of research has been conducted on the abundance and distribution of sessile reef community assemblages, particularly on non-scleractinian components and their contribution to reef diversity and dynamics throughout the Caribbean (Bak and Engel 1979; Rose and Risk 1985; van Veghel et al. 1996; Hill 1998; Humann and DeLoach 2003). The distribution, frequency, and ecological description of competition dynamics between corals and aggressive invertebrates are

covered by a variety of case studies. The encrusting gorgonian *Erythropodium caribaeorum* was found in cumulative abundances of 54.23% in a high-latitude southern Floridian reef system (Moyer et al. 2003). In a study conducted on reef ecosystem dynamics in the United States Virgin Islands, *E. caribaeorum* was the most frequently spotted aggressive invertebrate at depths of 3.05 m (Suchanek et al. 1983). Typically the most effective reef competitor in fringing reef ecosystems, the white encrusting zoanthid (*Palythoa caribaeorum*), typically the most effective sessile competitor in fringing reef ecosystems, was found to be outcompeted by *E. caribaeorum* (Suchanek et al. 1983).

Red boring sponges (*Cliona delitrix*), another one of the Caribbean's most effective reef space competitors, rapidly overtook great star coral heads (*Montastraea cavernosa*) on Grand Cayman fringing reefs in the 1980's (Rose and Risk 1985). This aggressive sponge species, along with other *Cliona* species (*C. langae*, *C. varians* and *C. caribbaea*), thrive on generous levels of organic pollutant input from sewage discharge (Rützler 2002). Where *Cliona* sponge colonies thrive, *M. cavernosa* colonies are inadequate in spatial dominance, let alone coral recruitment (Rose and Risk 1985; Chaves-Fonnegra and Zea 2010).

Certain competing invertebrate species are a particularly predominant component of reef community dynamics throughout the Caribbean. A sponge commonly known as the Caribbean chicken liver sponge (*Chondrilla nucula*) has been observed interacting with sessile and motile marine organisms in 30-50% of Caribbean reef ecosystems (Hill 1998). During a 15-year monitoring period, *Trididemnum solidum* abundance had increased 900%, covering all available hard substrata, primarily *Orbicella annularis* (Bak et al. 1996). These findings were believed to be indicative of the current distribution and abundance of sessile aggressive invertebrates on Bonairean fringing reef ecosystems.

Hypotheses

- H₁: Mean size of each aggressive invertebrate would be larger at 15 m than at 10 m
- H₂: The average total area covered by aggressive invertebrates in competitive interactions would be larger at 15 m than at 10 m
- H₃: Spatial competition between corals and aggressive invertebrate species would be observed with greater frequency at 15 m than at 10 m
- H₄: *Cliona* spp. would be the most frequently observed aggressors

Relevance of study

This study primarily focused on the distribution, abundance and ecological description of coral-aggressive invertebrate interactions along the west coast of Bonaire. There has been limited research conducted on the community assemblages of scleractinian corals, sponges, sessile cnidarians, encrusting gorgonians and overgrowing tunicates in Bonairean fringing reef ecosystems. Gathering quantitative and qualitative data regarding these interactions can provide essential empirical evidence for future scientific studies on spatial competition dynamics on tropical fringing reef ecosystems, nutrient cycling on tropical fringing reef ecosystems and a baseline for sessile benthic community composition studies in Bonaire.

Materials and methods

Study site

Research was conducted on the west coast of Bonaire, an island approximately 81 km east of Curaçao and 80 km north of the coast of Venezuela (Fig. 1), at various sections of the reef slope within 200 m north and south of the dive site *Yellow Submarine* (N 12°09.6 12' W 068° 16.9 15'). The site displays signs of diver

impact as well as boat traffic congestion, particularly around the reef crest (pers obsv). It has a sandy reef crest with gobies, anemones and other benthic dwelling creatures, covered with mooring blocks serving as small oases of sessile and motile marine life. The reef slope area, ranging from 9-30 m, has good visibility, with sunlight penetrating the ocean water to the bottom of the reef slope. Scleractinian and hermatypic coral colonies as well as sponge and invertebrate assemblages are abundant on the fringing reef slope.



Fig. 1 Geographical location of Bonaire within the Western Atlantic Ocean basin. The five-point star marks the location of study site

Field experiment and observations

Seven SCUBA dives were made over a 21-day period between 7 and 28 March 2015, to collect quantitative and qualitative data on the community composition of hermatypic/scleractinian corals and encrusting, boring or otherwise competing sessile invertebrates.

For each SCUBA dive, a secondary surveyor laid out two 10 m belt transects: the first one at 15 m and the second one at 10 m. All transects were approximately parallel to the shoreline. Once each transect tape was laid out, a T-bar was used to measure a belt transect, including spatial competition interactions observed within 2 m on each side of the tape. Within each 10 m by 4 m belt transect, photos were taken with a GoPro Hero 4 camera of each colony/individual using the following procedure: (1) Close up picture of small slate with the Individuals/Colony: “Ind/Col#” numbered. (2) A picture of the individual/colony itself, with a small slate marked every centimeter, positioned next to the individual/colony. Isolates that were located in immediate proximity of each other on the same

colony were deemed to be parts of the same individual. Data collection only included individuals that were 10 cm or greater in length.

The objective of each data acquisition period was to observe and count the number of open space, non-cryptic reef colonies, and individuals of coral species and aggressive invertebrate species within those colonies, that exhibited spatial competition between each other. The competition was quantified regardless of whether the aggressive invertebrate and coral species were dead or alive as long as each species could be identified. Variables including competing species mortality, recency of mortality, and presence of bare pavement or turf algae overgrowing surrounding substrate were all taken into account for this study.

Research methods did not take certain parameters into consideration. Due to logistical reasons, quantifying spatial competition between coral and cryptic aggressive invertebrate species was not possible. Quantifying cryptic aggressive invertebrate abundance may have given a more accurate assessment of distribution and abundance of aggressive invertebrates (Zea 1993).

Data analysis

Minitab 17 software was used to run a one-way analysis of variance (ANOVA) to find the mean frequency of coral-aggressive invertebrate competitive interactions (per transect), specifically pertaining to *Cliona* spp. and *S. ruetzleri*. These values were obtained by calculating the mean and the standard error of the mean of the total amount of coral-aggressive invertebrate interactions within the belt transects (n=14). Microsoft Excel software was used to run a Student’s t-test in order to determine the mean frequency of all observed coral-aggressive invertebrate competitive interactions, derived from the average of interaction frequency at 10 and 15 m (n=7 at each depth). Microsoft Excel and ImageJ software were used to calculate the standard deviation and standard error of the means of (1)

competitive interactions between *S. ruetzleri* and other species derived from the belt transects (n=14), (2) total area covered by aggressive invertebrates (m²) per depth (n=7 at each depth), and (3) size of each aggressive invertebrate species surveyed (m²).

Results

There was no significant difference between the mean area covered by each individual aggressive invertebrate species observed at 15 m (Table 1a) and 10 m (Table 1b). Average total area covered by all observed aggressive invertebrates at 15 m was 0.31 ± 0.13 m² (mean \pm SE), slightly higher than the mean observed total area of 0.26 ± 0.04 m² (mean \pm SE; Fig. 2), but these findings were not significantly different. Competitive interactions between corals and aggressive invertebrates were greater at 10 m at 15.86 ± 2.22 interactions (mean \pm SE) than at 15 m at 15 ± 1.45 interactions (mean \pm SE; Fig. 3). However, frequencies of these coral-aggressive invertebrate competitive interactions at each depth were not significantly different. Five coral species and 25 aggressive invertebrate species were found within 2 m on each side of the belt transects (n=14). Of the 14 transects laid on the reef (7 at each depth of 15 m and 10 m), 216 coral-aggressive invertebrate interactions were observed. Of these 25 aggressive invertebrates, *S. ruetzleri* was found to be involved in 51.85% of all observed competitive interactions, while *Cliona* spp. were found to be involved in 4.17% of all observed competitive interactions (Fig. 4). A Student's t-test found mean aggressive invertebrate-coral competitive interaction frequency involving *Cliona* spp. and *S. ruetzleri* to be significantly different ($p=1.49 \times 10^{-10}$; $F=103.46$; Fig. 4). Mean frequency of spatial competition interactions against *S. ruetzleri* was observed to be most frequent with *Orbicella faveolata* at both depths, yet differences in spatial competition frequency were not found to be significantly different (Fig. 5)

Table 1 Average size of each aggressive invertebrate species observed at 15 m (a) and 10 m (b). Average size was tabulated in meters squared (m²). "SD" denotes the standard deviation for each aggressive invertebrate and "SE" denotes the standard error of the mean for each aggressive invertebrate

(a) Species	Average size (m ²)	SD	SE
<i>Agelas clathrodes</i>	0.400	0.033	0.023
<i>Erythropodium caribaeorum</i>	0.041	0.029	0.012
<i>Trematooecia aviculifera</i>	0.035	0.000	0.000
<i>Palythoa caribaeorum</i>	0.031	0.000	0.000
<i>Siphonodictyon coralliphagum</i>	0.024	0.021	0.011
<i>Holopsamma helwigi</i>	0.015	0.000	0.000
<i>Monanchora barbadensis</i>	0.014	0.001	0.006
<i>Halisarca</i> sp.	0.013	0.008	0.002
<i>Niphates erecta</i>	0.011	0.009	0.004
<i>Scopalina ruetzleri</i>	0.011	0.007	0.001
<i>Trididemnum solidum</i>	0.009	0.005	0.004
<i>Cliona varians</i>	0.007	0.002	0.001
<i>Zoanthus pulchellus</i>	0.007	0.000	0.000
<i>Clathria</i> sp.	0.007	0.000	0.000
<i>Hippoporina verrilli</i>	0.007	0.000	0.000
<i>Cliona langae</i>	0.006	0.000	0.000
<i>Displastrella</i> spp.	0.005	0.004	0.002

(b) Species	Average size (m ²)	SD	SE
<i>Agelas clathrodes</i>	0.067	0.073	0.052
<i>Erythropodium caribaeorum</i>	0.063	0.000	0.000
<i>Trematooecia aviculifera</i>	0.053	0.020	0.014
<i>Palythoa caribaeorum</i>	0.027	0.000	0.000
<i>Siphonodictyon coralliphagum</i>	0.020	0.014	0.006
<i>Holopsamma helwigi</i>	0.019	0.015	0.007
<i>Monanchora barbadensis</i>	0.017	0.018	0.011
<i>Halisarca</i> sp.	0.016	0.000	0.000
<i>Niphates erecta</i>	0.015	0.024	0.003
<i>Scopalina ruetzleri</i>	0.015	0.000	0.000
<i>Trididemnum solidum</i>	0.014	0.010	0.007
<i>Cliona varians</i>	0.013	0.009	0.005
<i>Zoanthus pulchellus</i>	0.010	0.000	0.000
<i>Clathria</i> sp.	0.007	0.005	0.002
<i>Hippoporina verrilli</i>	0.007	0.002	0.002
<i>Cliona langae</i>	0.007	0.000	0.000
<i>Displastrella</i> spp.	0.006	0.004	0.002
<i>Ectyoplasia Ferox</i>	0.003	0.000	0.000

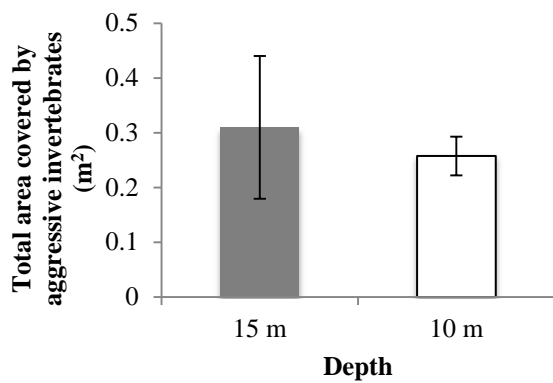


Fig. 2 Average total area in meters squared (m²) covered by all of the aggressive invertebrates (each individual >10cm) observed at 15 m and 10 m (n=7 at each depth). The error bars represent standard error of the mean (SE)

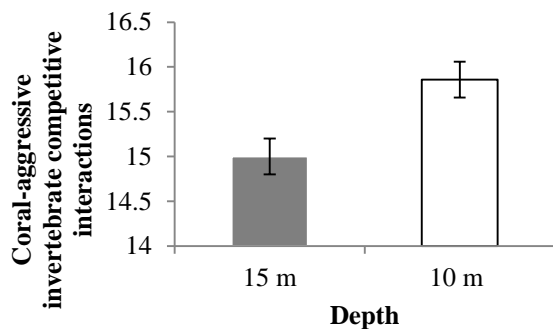


Fig. 3 Average coral-aggressive invertebrate competitive interactions per transect at depths of 15 and 10 meters. Error bars represent the standard error of the mean (n=7 for each depth)

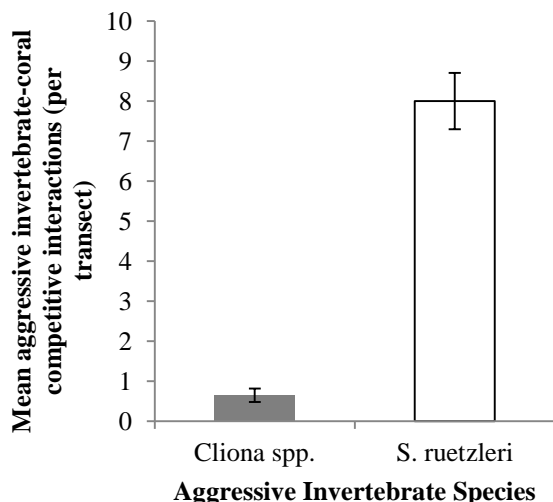


Fig. 4 Average *Cliona* spp.-coral and *Scopalina ruetzleri*-coral interspecific competitive interactions observed per transect. Error bars represent the standard error of the mean at both depths (n=14 for each species)

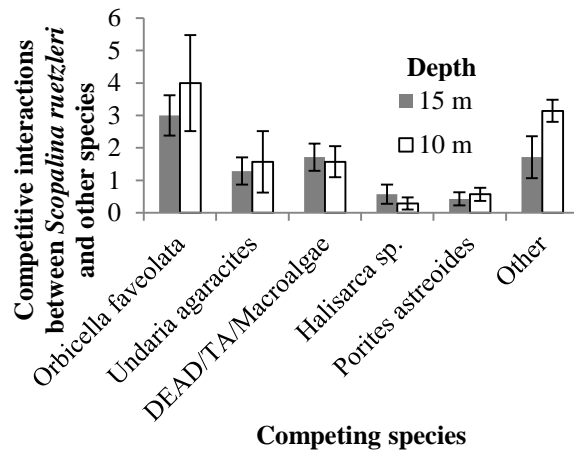


Fig. 5 Average frequencies of spatial competition between orange lumpy encrusting sponge (*Scopalina ruetzleri*) and corresponding sessile invertebrates (n=14). “Other” corresponds to individual competitive interactions between *S. ruetzleri* and the following species, seen at a frequency lower than 2%: *Cliona varians*, *Diplastrella megastellata*, *Ectyoplasia ferox*, *Monanchora barbadensis*, *Montastraea cavernosa*, *Mycetophyllia* sp., *Palythoa caribaeorum*, *Niphates erecta*, *Siderastrea siderea*, White Calcareous Sponge

Discussion

Elaboration on quantitative analysis

Previous research suggested that competitive interactions between corals, sponges and other aggressive invertebrates would be found in greater abundance at greater depths in Caribbean reef communities (Suchanek et al. 1983). Yet the average total area, distribution, frequency and abundance of spatial competition on Bonairean fringing reef ecosystems was not found to be significantly different between 10 and 15 m (Fig. 2; Fig. 3; Fig 5). These findings did not support H₁, H₂ and H₃, which all suggested that the mean size of each aggressive invertebrate (n=7 for each depth), average total area covered by each aggressive invertebrate species (n=7 for each depth), and the frequency of spatial competition observed between *S. ruetzleri* and other predominantly competitive species (n=14) would be greater at 15 m than at 10 m (Fig. 2; Fig. 3; Fig. 5). An explanation for this inconsistency may lie in the nutrient input from Bonaire’s septic tank system (Rousmaniere 2006). This non-point source nutrient pollution

may be frequent and intense enough to flood Bonairean reef ecosystems with excess nutrients, creating an environment conducive for ubiquitous aggressive invertebrate growth, regardless of depth.

It was also hypothesized that *Cliona* spp. would be the most frequently observed aggressors on Bonairean fringing reef ecosystems, based on previous research involving a sharp increase in abundance of *Cliona delitrix* on Grand Cayman fringing reef ecosystems in response to a sharp increase in nutrient pollution (Rose and Risk 1985). This hypothesis (H₄) was not supported, as *S. ruetzleri* was the most frequently observed aggressor. An explanation for this discrepancy could lie in the previous distribution and abundance of *S. ruetzleri* on Bonairean fringing reef ecosystems. If the distribution and abundance of *S. ruetzleri* was already predominant, then with other biotic and abiotic variables remaining constant, *S. ruetzleri* would be expected to remain in large abundances.

Prevalence of the orange lumpy encrusting sponge (*Scopalina ruetzleri*)

The distribution and abundance of *S. ruetzleri* on Bonairean west coast fringing reef ecosystems was not expected to be as frequent as the study's hypotheses predicted. However, based on previously published research, *S. ruetzleri* was mentioned as a predominant aggressive invertebrate in spatial competition among open space reef ecosystems; namely the abundance of *Dictyonella ruetzleri* (synonymous for *S. ruetzleri*) on reefs around Venezuela, Cuba, Jamaica, Florida and Santa Maria (Zea 1993). However, local abundances of this and other sponge species may vary between fringing reef ecosystems. A large abundance of *S. ruetzleri* was observed among the mangroves of Lac Cai on Bonaire's east coast. Their abundance could also be related to nutrient input from external sources (e.g. terrestrial sewage and agricultural runoff) as well as naturally occurring sedimentation

accumulation around mangroves' aerial prop roots (Diaz et al. 2004).

Underlying mechanisms influencing spatial competition

Certain abiotic factors could have influenced spatial competition amongst sessile organisms. Sedimentation rates from marine organisms or nutrient input from septic tank systems on the west coast (Rousmaniere 2006) may have influenced spatial competition between corals and aggressive invertebrates, similar to a study on the Grand Cayman fringing reefs (Rose and Risk 1985). Competitors that were closer to the source of sedimentation, at 10 as opposed to 15 m, may have conducted primary sedimentation uptake.

Environmental pressures, including organic and/or inorganic pollution via anthropogenic means, are considered processes that can be categorized as a macroscale, mesoscale or a microscale process (Hubbard 1997). Sessile reef organisms (e.g. corals and aggressive invertebrates) are particularly susceptible to the effects of environmental pressures (Nava and Carballo 2013). Scleractinian, including hermatypic coral species, are relatively more reliant on reef ecosystem stability than other aggressive invertebrates. Ocean temperature, water level, currents and chemical composition can adversely affect scleractinian coral and aggressive invertebrate species' health. Increasing ocean temperatures have been positively correlated with an increase in global coral and sponges bleaching (Fang et al. 2013). With rises in sea level, little photosynthesizable light will be available for *Symbiodinium* present within the sponge and coral species largely occupying Bonairean reef ecosystems. Excessive nutrient input within a reef ecosystem can create conditions more beneficial for aggressive invertebrate growth than coral growth. Nutrient input via wastewater (i.e. Bonaire's septic sewage system wastewater) can be a vector in disease transmission to coral reef ecosystems (Voss and Richardson 2006). Disease may play a crucial role in coral and aggressive invertebrate

(i.e. sponge) species assemblages in Caribbean reef ecosystems (Wulff 2006). If conditions for organismal growth are favorable towards aggressive invertebrates while corals' immune responses are preoccupied by diseases created by wastewater, then aggressive invertebrates are most likely going to outcompete corals in spatial competition on reef substrata. Pollution from the septic tank system lining the west coastline of Bonaire may have a synergistic effect with water pollution from boating traffic moving over Bonairean reef ecosystems and may contribute to the ecological description of sessile benthic invertebrate community assemblages in Bonaire (Rousmaniere 2006). If certain hermatypic coral species (e.g. *Orbicella annularis*, *Orbicella faveolata*, etc.) die off due to these environmental pressures, other scleractinian coral species' resistance to bioerosion from aggressive invertebrate species and population resilience against high mortality rates will be significantly hindered (Bak and Engel 1979; Fang et al. 2013).

Broader implications

Environmental pressures can also affect scleractinian corals' immune system defences. Microbiotic communities found in the surface mucous layer of scleractinian corals contain representatives from all three taxonomic kingdoms—Bacteria, Archaea, and Eukarya, as well as numerous viruses (Rosenberg et al. 2007). Bacterial and viral predation by sponges is a recently discovered nutrient-flow pathway in reef ecosystems. Sponges are one of the only known filter feeders that are able to capture microscopic particles at such high efficiencies, and can ingest bacterioplankton and virioplankton, incorporating them into their physical structure (Hadas and Marie 2006). This innate filter feeding ability, in addition to biotic (i.e. biological competitive mechanisms employed by sponges) as well as abiotic environmental pressures (i.e. temperature rise, ocean acidification), can have a significant effect on nutrient uptake and disease-resistance in corals (Rosenberg et al. 2007).

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REPORT

Christina Mielke • Oregon State University • 2percentmielke@gmail.com

An analysis of abundance, ecology and life history of the lettuce sea slug *Elysia crispata* on the island of Bonaire

Abstract With the exception of *Elysia crispata*, shell-less Sacoglossa species (Order: Mollusca), have been widely studied. Within the Caribbean, these small bodied organisms occur in low population densities, making them hard to find and, in turn, difficult to study. This project served to assess *E. crispata* abundance, ecology and life history traits on the island of Bonaire. Data collected for this study was conducted by observations from ten 40 m² transects located at depths of 2.2 m, 7.9 m and 10.7 m. A pair of surveyors recorded the number of individuals found, size of individuals, substrate individuals were located on, and color intensity of individuals within each transect. Overall abundance of individuals found at the study sites were much higher than anticipated. Of the 275 individuals found, the highest population densities were found in shallow transects. The average size of individuals was between 3.0 ± 2.6 cm to 5.0 ± 3.8 cm (mean \pm SD) in length, with no correlation between size of individual and coloration. Overall abundance of smaller individuals found on shallower transects could indicate higher densities of preferred substrate within these areas. Roughly 94% of individuals were found on various compositions of turf algae. These results implied that *E. crispata* were biased towards occupying substrates with turf algae compositions as opposed to other available substrates.

Keywords Opisthobranch • Fringing coral reef • Size distribution

Introduction

Information on ecology, life history and abundance of a species are important components for understanding details of unusual organisms, such as select members of the sea slug group, Sacoglossa (Phylum: Mollusca). Shell-less Sacoglossa species, such as *Elysia crispata*, are known for their ability to perform a unique life history trait called kleptoplasty, defined as the act of harvesting and utilizing plants cells within the body of an animal (Dorrell and Howe 2012). Ranging from Jamaica to Venezuela (Gavagnin et al. 2000), this small bodied species (1-10 cm) often occurs in low population densities, making them a difficult species to study in the field (Jensen 1997). As a result, little is known about *E. crispata*'s ecological traits.

Other comparable kleptoplastic species, such as *Elysia chlorotica* and *Elysia viridis*, have been extensively studied (Rumpho et al. 2000), but despite occurring throughout the Caribbean, basic information about the life history of *E. crispata* has not been reported. Thus far, it has been assumed that different species of kleptoplastic slugs are functionally the same, however, additional information is required to support that belief (Pelletreau et al. 2011). Dorrell and Howe (2012) suggest that different Sacoglossa species selectively choose which algae to harvest plastids, hinting at relevant differences between kleptoplastic slugs. Species-specific preference for select algae plastids is so particular that in some cases the slug cannot metamorphose into an adult without their specialized algal component (Rumpho et al. 2010). Jensen (2008) proposed

that plastid preference could be derived from different morphologies of radula (feeding apparatus of slug) among Sacoglossa species, indicating that the alga preference per species depends on the size and shape of their radula. Within a given species, Jensen (2008) also proposed that sacoglossan slugs may also alter their algal preferences during their life cycle, as their radula are smaller during adolescence than adulthood. Information of algae preference during all life stages for *E. crispata* has been debated since the discovery of the species and has yet to be resolved (Cruz et al. 2013), like other details about their life history.

Since it is not known what *E. crispata* consumes, it is difficult to evaluate their nutrient needs or identify health indicators. *Elysia crispata*'s kleptoplastic habits allow them to puncture an algae cell with a specialized radula and siphon the contents into its digestive tract (de Vries et al. 2014). The process incorporates ingested plastids into the cell membrane of the organism's digestive canal within the parapoda (ruffled edges of body). These plastids continue to function inside of the organism, using photosynthesis to produce sugars, amino acids and other nutrients that directly benefit the host slug (Dorrell and Howe 2012), which extends periods between feedings for the host *E. crispata* for up to 10 months (Händeler et al. 2009; Rumpho et al. 2010; Christa et al. 2013). Although it is unknown how many plastids a slug would need to survive a period of starvation, the number of ingested plastids may be linked to the overall health of the host organism.

Since many details of *E. crispata*'s ecology have not yet been determined, one aspect of this study was to assess which substrate they preferred to occupy, which could relate to the organisms' grazing preference. Preferred substrate of *E. crispata* could also potentially indicate a distribution of depths they occur at, given that as depth increases, light availability decreases, thus affecting and potentially limiting, algae composition. Additionally, larger individuals may have larger radula and therefore access to a wider range of food sources, potentially indicating that they have

more success in acquiring food and plastids. This aspect of their morphology may lead to higher abundances of larger individuals than smaller individuals due to larger organisms potentially having more resources available to them. Cruz et al. (2013) speculated that the coloration of *E. crispata* was connected to the number of viable plastids within the body of the slug, which, in turn, could be related to the health of an individual. Therefore, larger individuals may be healthier (i.e. more vibrant or colorful) or more robust than smaller individuals. With these key features in mind, this study proposed three hypotheses:

- H₁: The population density of *E. crispata* would be greater at shallower depths than deeper depths
- H₂: The abundance of larger individuals would be higher than smaller individuals
- H₃: Larger individuals would express more vivid coloration than smaller individuals

This study serves to gather information about *E. crispata*'s lifestyle, average organism size, substrate preferences, and population density. This study was considered an initial analysis of *E. crispata*'s ecology and life history that has thus far been previously undocumented. Though this study does not focus on the mechanisms of kleptoplasticity in *E. crispata*, life history traits and ecological details of the organism in question could be the key to enlightening aspects of future kleptoplastic research, helping us understand the enigma behind how an animal can actively utilize living plants cells within its own body.

Materials and methods

Study site

An observational survey was conducted at Eighteen Palms dive site (12° 8' 18.058" N 68° 16' 36.654" W) on the island of Bonaire, located in the Dutch Caribbean (Fig. 1).

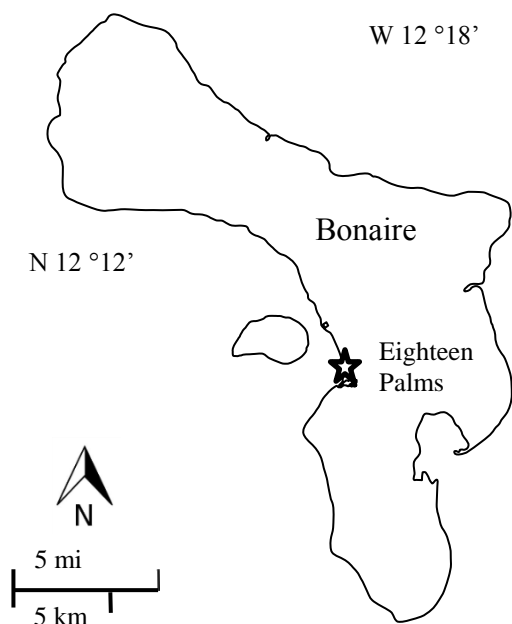


Fig. 1 Map of Bonaire indicating where Eighteen Palms dive site is located with a star ($12^{\circ} 8' 18.058''$ N $68^{\circ} 16' 36.654''$ W)

The dive site, located ~2.7 km south of downtown Kralendijk on the western coast of the island, is adjacent to Plaza Resort, whose beach is the point of entry for the site. Eighteen Palms is home to a fringing reef system with easy shore access that is a popular area for SCUBA diving and other marine activities. Lining the intertidal area, from the shore to 2 m deep are large, flat plates of pavement covered in various algae and a few heads of fire coral (*Millepora complanata*). Shallow areas between 3-7 m mainly consist of sandy substrate dotted with isolated heads of live coral colonies. The reef crest runs parallel to the shoreline and starts ~8-9 m deep, continuing down an intermediately steep slope that ends with a sandy, flat bottom (max depth: 25 m). Along the crest and slope, coral colonies dominate the macro epibenthic landscape, interspersed with moderately large assemblages of sponges and smaller portions of algae and sandy areas. This site has historically had a noticeable population of *E. crispata* and was an appropriate area to collect data due to ease of access to the water as well as the organism in question.

Observational methodology

Observations of *E. crispata* density (individuals/40 m²), organism size, and substrate preferences were made by SCUBA diving and snorkeling. Two divers collected data concurrently on either side of a transect tape, estimating a 1 m² area surveyed on their respective side of the tape. The study was consistently conducted at two times during the day, 09:00 hrs and 13:30 hrs, once a week for a five week period (27 March - 1 April 2015). The survey took place in areas where coral cover was consistent (i.e. the reef crest and slope and the intertidal pavement area), thus omitting sandy substrate areas with sparse coral communities. Standardized 40 m² (20 m by 2 m) belt transects were laid at depths of 2 m, 8 m and 11 m parallel to the shoreline, with three transects for the two deeper depths and four transects in the shallowest depth. Data collected within these transects included number of individuals per area, length of each individual, type of substrate individuals were located on, and an estimation of the individuals' health through evaluations of color vividness.

Measurements for individuals' length were to the nearest centimeter using a ruler and health of organism was determined by a scale of vibrancy in coloration of the parapoda. The determination of vibrancy in individuals was a three point scale with gray (G) as the lowest vibrancy, medium contrasts (M) ranging from white to some color, and vivid (V) equating to highest vibrancy with distinct, bright coloration. The substrate the organism was found on was identified on site into generalized categories of *Dictyota* spp. (DIC), turf algae (TA), crustose coralline algae (CCA), sand (SAND), and a combination of substrates, such as *Dictyota* spp. and turf algae (DIC/TA). Codes and categories of substrate were as per Atlantic and Gulf Rapid Reef Assessment (AGRRA) benthic version 5.4 protocol (Lang et al. 2010).

Data analysis

Density and abundance

To estimate population densities of *E. crispata* within a given area (40 m² per transect), the number of individuals observed in all transects (n=10) from both surveyors was calculated into the means of individuals observed per depth and averaged into a mean of individuals found within a 40 m² area per depth (n=3). To determine an average length for *E. crispata*, total density of *E. crispata* was calculated into different lengths of individuals recorded to evaluate population differences in overall size of organism. The average sizes and abundance of individuals in each size category was calculated from the total number of individuals observed from both surveyors in all transects. The means of individuals in each size category found in a 40 m² area per depth were calculated. To assess life history and ecology details of *E. crispata*, an analysis of variance (ANOVA) test was used to compare densities of individuals found at different times of day were also considered for organism association with active times of the day.

Color vibrancy

The scale of vividness of individuals was compared to length of individual organisms to assess relationships between health and size of *E. crispata*. A comparison of the occurrences between color expression, size of individuals and time of day was also made. This study assumed that the sex of the organism could be considered negligible when investigating the size and coloration of individuals, and therefore *E. crispata* were not separated by gender.

Substrate analysis

Data for all ten transects surveyed was evaluated for the total number of times individuals were observed on each substrate category. Categories of substrate were from AGRRA version 5.4 protocol (Lang et al.

2010). The total number of times individuals were observed on substrates was separated by depths of these occurrences. It was assumed that the substrate *E. crispata* was most frequently located on was a preferred location as opposed to random association.

Results

Density and abundance

A total of 275 individuals were found along ten 40 m² transects surveyed. Of that amount, 85.01% of all individuals were found at 2.2 m (n=275). In contrast, 2.54% and 7.23% of individuals were found at 7.9 and 10.7 m, respectively. Average densities per depth also indicated a trend of individuals being more abundant on shallower transects (Fig. 2). Densities at 2.2 m, 7.9 m and 10.7 m were 31.0 ± 12.82 (n=4), 1.17 ± 2.04 (n=3), and 3.33 ± 2.42 (n=3) individuals per 40 m², respectively (mean density \pm SD).

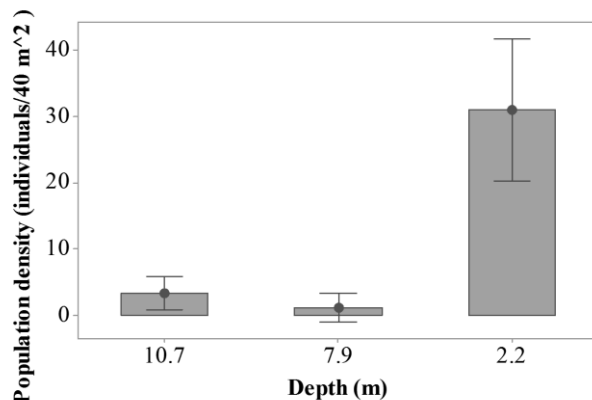


Fig. 2 The average density of total individuals found in each transect per depth (n=10). Error bars are the SD of the mean

The highest average abundance of individuals ranged from 3.0 ± 2.6 cm to 5.0 ± 3.8 cm (mean \pm SD) in length (Fig. 3a). On average, the highest densities of both larger and smaller individuals occurred in the shallowest transects (Fig. 3b). Smaller individuals that were 1.0-5.0 cm, occurred in densities that ranged from 0.3 ± 0.5 to 6.1 ± 3.9 individuals

per 40 m² (mean ± SD). Larger individuals that were greater than 5.0 cm occurred in densities that ranged between 0.1 ± 0.3 and 4.1 ± 2.6 individuals per 40 m² (mean ± SD; Fig. 3b).

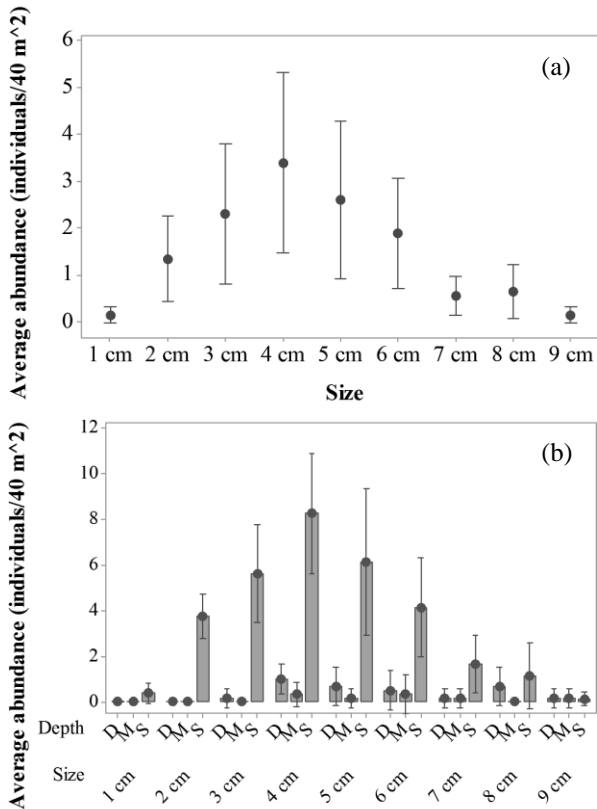


Fig. 3 (a) Average abundance of individuals per size category. Densities per size were calculated with total number of individuals of different lengths per transect averaged over transect (n=10), with each transect being 40 m². (b) Average abundance of individuals per depth index. Depth index correlates to transects with the following abbreviations: D= deep, or 10.7 m; M= middle, or 7.9 m; and S= shallow, or 2.2 m

An analysis for the differences between average abundances at different times of the day yielded no correlation between variables. Averages for abundance in the afternoon and morning were 14.9 ± 19.2 and 12.6 ± 14.3 individuals per 40 m² (mean ± SD), respectively (Fig. 4). ANOVA results indicated that there was no difference between abundances of individuals during the afternoon or morning hours (p=0.765, F=0.09).

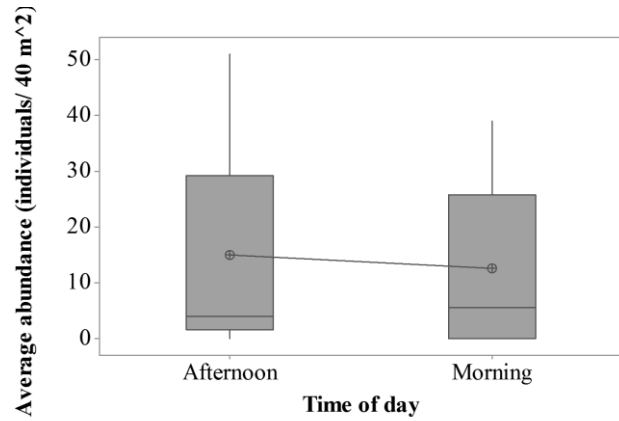


Fig. 4 Mean and median abundances of lettuce slugs at different times of day. Data was compiled from all transect depths into two categories. Morning surveys were conducted at 09:00 hrs, while afternoon surveys were conducted at 13:30 hrs

Color vibrancy

Average size of individuals was a weak indicator of coloration expression (Fig. 5a). There was no correlation between vividness expressed in individuals, average size of individuals, and what time of day they were found at (Fig. 5b).

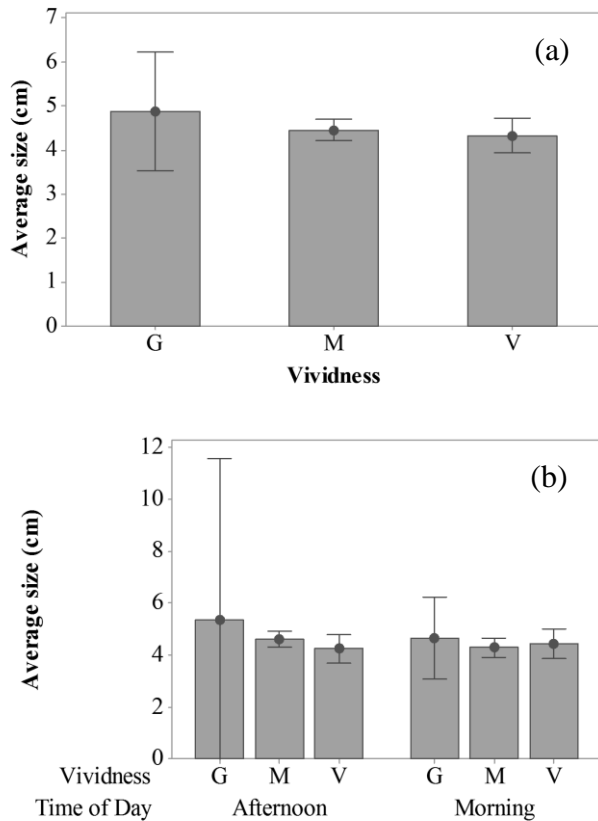


Fig. 5 (a) Average size of individuals found at all transects in relation to the vividness of color displayed

(n=10). (b) Average size of individuals and vividness at different times of the day. Color scale was split into three categories: G= gray or no coloration, M= medium/white coloration, and V= vivid/very noticeable coloration

Substrate analysis

The substrate individuals were found on throughout all transects surveyed was predominantly turf algae, accounting for 94.2% of the surfaces they were found on, with all other substances accounting for less than 5.8% of all occurrences (Fig. 6a). Total number of individuals found at shallower transects (2.2 m) was 248, while 27 individuals were found at deeper transects (10.7 and 7.9 m) combined. Three individuals were observed moving around live coral polyps and migrating to or from dead coral surfaces that were covered in turf algae. The number of times individuals occurred on each substrate per depth showed a strong pattern of turf algae being the most prominent substrate individuals occurred on at shallower transects (Fig. 6b).

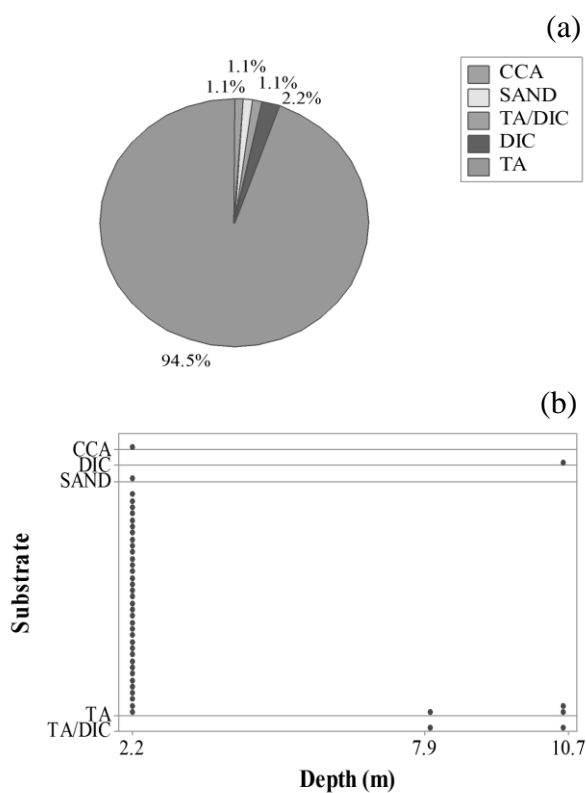


Fig. 6 (a) Substrate individuals were found on (n=275). (b) Total number of substrate observations per depth of

transects. A large majority of turf algae observations occurred within the 2.2 m transects (n=4). CCA= crustose coralline algae SAND= sand, TA= turf algae, DIC=*Dictyota* spp., and TA/DIC= a combination of turf algae and *Dictyota* spp

Discussion

H₁: The population density of *E. crispata* would be greater at shallower depths than deeper depths

With densities of *E. crispata* significantly higher in shallower transects, hypothesis one was strongly supported. One explanation for a higher abundance of *E. crispata* at shallower transects could correlate to an abundance of potential food sources available within those areas. Transects at 2.2 m had noticeably more instances of individuals observed on turf algae than at deeper transects surveyed, which could indicate a higher preference for that particular substrate among *E. crispata* populations. This scenario could also lead to shallower transects being able to support a higher population density of *E. crispata* than deeper transects by having a higher composition of preferred substrate available for consumption. Higher densities of turf algae were observed within shallower transects than at deeper transects, with the dominant algae largely unidentified but not being a member of the *Dictyota* genus.

Though H₁ was supported with higher densities of individuals occurring at shallower depths, the overall high abundance of *E. crispata* found at Eighteen Palms was surprising. Population densities of *E. crispata* observed at other dive sites outside of this study located further north on Bonaire were either similar to that of deeper transects at Eighteen Palms, such as at Tolo (12° 12.689' N 68° 19.651' W) and Karpata (12° 13.171' N 68° 21.118' W), or were largely absent from a dive site, such as at Yellow Submarine (12° 9.578' N 68° 16.937' W). Considering the predicted low densities of sacoglossan slugs in the field, the large gradient in observed population distributions was unexpected.

Given that Eighteen Palms is further south than other sites *E. crispata* have been identified at, a hypothesized explanation for a heightened population density could be that Eighteen Palms might serve as an active breeding ground for this organism. Ocean currents on Bonaire run from south to north close to the shoreline and could provide a connection between populations of *E. crispata* at Eighteen Palms and other sites, potentially allowing individuals to migrate further north on the island. Additionally, reproductive patterns could be linked to the relative size of individuals, implying that smaller individuals are younger adults than larger individuals. The higher abundance of smaller sized individuals observed at Eighteen Palms potentially indicated that at least shallower portions of the site induced favorable conditions for *E. crispata* propagation. However, additional studies are required in order to confirm this hypothesis as well as to further identify what these drivers may be.

H₂: The abundance of larger individuals would be higher than smaller individuals

Given that the highest average abundance of individuals found corresponded to size classes 3 and 5 cm in length, the results of this study weakly supported hypothesis two. Again, reproductive patterns could be linked to these results. Assuming that all individuals found were adults, the pattern displayed in Fig. 3a shows a normal curve for distribution of size classes among *E. crispata*. Again, little is known about their life history so additional information is needed to confirm whether or not this pattern is an accurate representation of *E. crispata*'s size class distribution.

In regards to their life history, these results could also potentially indicate that *E. crispata* displays a Type I survivorship curve, where the highest mortality rate occurs during the later portions of their lives (Nakaoka 1998). Assuming that *E. crispata* produces large amounts of larvae, this alternative hypothesis could be a driver for population densities

favoring smaller (i.e. younger) individuals and help provide insight on the organisms' reproductive events. Since minimal information is known about their life history, if *E. crispata* has a high older adult mortality rate, it would be important to know if they obtain their kleptoplastic abilities at a later stage or all stages of their life. Though it seems unlikely that slugs would only perform kleptoplasty later in life due to the fact that the basic structure of their radula does not change over time, it only increases in size, lending to smaller slugs still being able to harvest organelles from algae. Regardless, additional information is necessary to determine at what time in *E. crispata*'s life cycle they acquire their kleptoplasticity and whether or not they display a type I survivorship curve.

H₃: Larger individuals would express more vivid coloration than smaller individuals

Given that there was little to no correlation between coloration expressed in individuals and average size of individuals, hypothesis three was not supported. Results from this study indicated the opposite; larger individuals showed less coloration overall than smaller individuals. A possible explanation of these results could be that as an individual becomes larger, it might be more difficult to harvest ample plastids to retain vivid coloration and result in the overall decline of the individuals' health. Assuming that larger individuals are generally located at the end of *E. crispata*'s life cycle, older individuals might naturally display less coloration because of the large amount of energy required to obtain necessary plastids.

An additional explanation of these results could lie in the methods of estimating coloration of individuals used in this study. Ranges of individual vividness and coloration were far more complex than we had anticipated and required a more detailed quantitative color scale for surveyors to reference while in the field. Had such a detailed scale been utilized, the recorded data might have shown a stronger association. This aspect of analyzing coloration

and vividness would be a beneficial component to draw connections to plastid retention and overall health of *E. crispata* and deserves further assessment in future studies.

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REPORT

Sami Chase • Colorado State University • samichase@hotmail.com

The effect of social status and species on habitat preference of grunts, Haemulidae, on the fringing coral reef surrounding Bonaire

Abstract Many fish prefer to live in certain habitats based on protection, shade cover, rugosity, and foraging return. Haemulidae, also known as grunts, are a family of fishes that are prevalent on Bonaire and can be found throughout tropical coral reef ecosystems. This study utilized *in situ* observations to investigate the preferred habitats of solitary grunts compared to colonial grunts based on the amount of the of protection and shade cover that the habitat provided. Three types of grunts were focused on in this study: blue striped grunts (*Haemulon sciurusnch*), French grunts (*Haemulon flavolineatu*), and smallmouth grunts (*Haemulon chrysargyreum*). The results of this study demonstrated that solitary smallmouth grunts utilized habitats that provided the most protection. Further, solitary smallmouth grunts were found in habitats shaded by coral more than the other grunt species. French grunts were found in overall shade cover more than both smallmouth grunts and blue striped grunts. This study showed that blue striped grunts rely mainly on their size for protection, smallmouth grunts on schooling, and French grunts on habitat. A general knowledge of grunts habitat preferences is beneficial because many reefs worldwide are currently degrading. If the reefs continue to degrade, grunts could be in danger because of their specific habitat preferences, based on both their species and social status. Preserving the complexity of coral reef habitats is crucial for some grunts, such as French and smallmouth grunts, but it is not crucial for other grunts, such as blue striped grunts.

Keywords Haemulidae • Habitat preference • Coral reef ecosystem

Introduction

Habitat choice is very important for marine species because not only does a habitat provide protection from predators, but also it often provides food sources for the organism(s) that utilize it (Lowe et al. 2003). Past studies have demonstrated that fishes have preferences for the type of habitat that they live in (Gristina et al. 2014), and given a choice, a fish will often choose to live in one type of habitat over another (Munday 2001; McCormick et al. 2013). This theory is supported by a study conducted by Sale and Dybdahl (1975) which showed that many of the fish studied preferred to live on *Acropora pulchra* (branching coral), as opposed to pieces of equally sized eroded coralline rock. Habitat preference is further illustrated by a study conducted by Williams and Sale (1981) that considered the recruitment of juvenile fishes to different coral species. They found that many of the fish species observed established a marked preference among the different coral species as settling sites (Williams and Sale 1981). Furthermore, the notion that fishes demonstrate preferences when it comes to habitat choice is highlighted by a study done by Shulman (1984) showing that juveniles of most of the fish species observed preferred to live in branching corals because of the large number of available crevices. The height of the habitat structure is often a predictor of total fish abundance in that specific structure (Gratwicke and Speight 2005). Habitat preference is further demonstrated by fish because some fish choose to live in hard structures, while some prefer to live in soft structures, while others prefer to live in shaded structures (Harasti et al. 2014).

The habitat that a fish chooses to live in is often based on the ability of the fish to escape predators, the amount of protection that the structure provides, the amount of sunlight that reaches the structure, the number of hiding places that are provided by the structure, and the depth of the structure (Lamouroux et al. 2001). Habitat choice can also be based on factors such as species-specific and age-specific behavioral characteristics, but ultimately, a habitat is chosen based on the ability of that habitat to protect a fish from predators (Werner et al. 1983; Bohnsack 1989). When choosing habitats, animals chose structures that fluctuate in qualities such as foraging return, otherwise known as the ability of a fish to obtain food, and mortality hazard (Gilliam and Fraser 1987).

This study focused on the habitat preferences of species within the Haemulidae family, commonly known as grunts. In particular, this study examined how the species and social status of grunts related to habitat preference. A study conducted by Pattengill-Semmens (2002) illustrated that the grunt population off of Klein Bonaire, a small island off the west coast of Bonaire, is smaller than the grunt population in Lac Bay, a sheltered area on the east coast of the island. The data collected by Pattengill-Semmens (2002) study supports the idea that the difference in grunt populations might be due to a difference in the available habitats in Lac Bay compared to Klein Bonaire. This trend may have been observed because mangroves and sea grasses can be found in Lac Bay, but not on Klein Bonaire, and juvenile grunts often utilize mangroves before migrating to the reef (Pattengill-Semmens 2002). Grunts live in many different habitats, and not all grunt species prefer to live on the same substrata. A study conducted by Shulman (1984) looked at different types of fish and their survivorship on coral reefs in the US Virgin Islands. This study emphasizes the idea that grunts have preferred habitats by showing that *Haemulon flavolineatum*, commonly known as the French Grunt, were found most abundantly in sand and rubble habitats.

Many different types of grunt are abundant off the coast of Bonaire, and this study explored how the grunts' species and social status relate to

the habitat being utilized. This study looked at the habitat preference of grunts as well as the relationship that habitat choice might have to the social status of the grunt. Social status, for the purpose of this study, refers to if an observed grunt is solitary or in a school. The term colonial was also used to describe schooling situations. Many different species of fish can be found both solitary and schooling (Jackson 1977). Some grunts, for example, can be seen on the reef in large schools, while other grunts tend to be solitary. Schooling has positive effects for fish because it increases protection and foraging rate (Marras et al. 2014). Further, habitat choice can often be influenced by the schooling status of fish. When a fish is part of a school, it has a greater amount of protection than if it is solitary and, therefore, does not need as much protection from its habitat (Neill and Cullen 1974). When a fish is solitary it is more vulnerable and therefore searches for habitats that provide more protection (Jackson 1977). It is beneficial to a fish to have coverage on the top and sides so that predators have less opportunity to reach the fish and the fish is able to be better prepared for approaching predators. Schooling fish have protection from the other fish around them because the other fish in the school react to a predator so that the fish is able to pick up on this behavior and anticipate when predators are approaching; therefore, schooling fish do not require as much protection from a habitat as solitary fish do (Seghers 1981). Furthermore, large groups of fish often cannot fit into habitats that are protected on the sides and top, which are more protected from predators; therefore, colonial fish often utilize more open habitats (Marras et al. 2014).

Another factor that plays a significant role in habitat choice of fish is shade cover. While shade cover does not affect food intake or growth of fish, many species still prefer to live in habitats that provide overhead cover as opposed to habitats that are in direct sunlight (Orpwood et al. 2010). A source of shade might be beneficial to a fish because overhead cover might be better for avoiding predators from above. Also, fish living in shaded areas may appear to be more hidden in their surroundings and also be able to see greater distances, because the shade cover

over their eyes acts as a shield (Cocheret et al. 2004). Overall, many fish choose habitats based on factors including shade cover, amount of protection, and its social status.

The purpose of this study was to examine what effect social status had on the habitat preference of grunt fish. In order to achieve this objective, this study focused on three different types of grunts: the blue striped grunt (*H. sciurusnch*) the French grunt (*H. flavolineatu*), and the smallmouth grunt (*H. chrysargyreum*). The information obtained from this study is significant to both scientific researchers and the general public because it highlights the habitat types that are most important to conserve in order to keep the grunt population thriving in Bonaire as the maintenance of the grunt population contributes to the overall health of the reef. Conserving the grunt population on Bonaire is imperative because the Haemulidae family makes up a large portion of the coral reef community. Grunts are omnivores, and therefore forage on plants and other small fish. Without the large population of grunts, the entire coral reef ecosystem would be affected because a large link in the food chain would be missing (Sale and Williams 1984). On a larger scale, this information is useful because it can be used to make generalizations about preferred habitats and how that habitat compares to the social status of many different species of fish living in numerous environments worldwide. Additionally, this research is also useful for scientific researchers because it yielded results that tied together the relationship between shade cover, social status, and habit preference of grunts, which can then be used to make further hypotheses about the relationship of those factors in other economically important marine species.

The research conducted for this study found results that highlight the connection between shade cover, social status, and habitat preference of different types of grunts found on the coral reef surrounding Bonaire. My hypotheses were as follows:

H₁: Schooling grunts would be found more often in habitats that provide less protection to the fish as compared to

solitary grunts that would be found more often in habitats that provide more protection

H₂: Solitary grunts would be found in shaded areas more often than colonial grunts, which would not be found in shaded areas as often

H₃: Blue striped grunts, French grunts, and smallmouth grunts would each demonstrate different habitat and shade cover preferences

Overall, the question that was explored by this study is what the relationship is between social status, species, and habitat preference.

Materials and methods

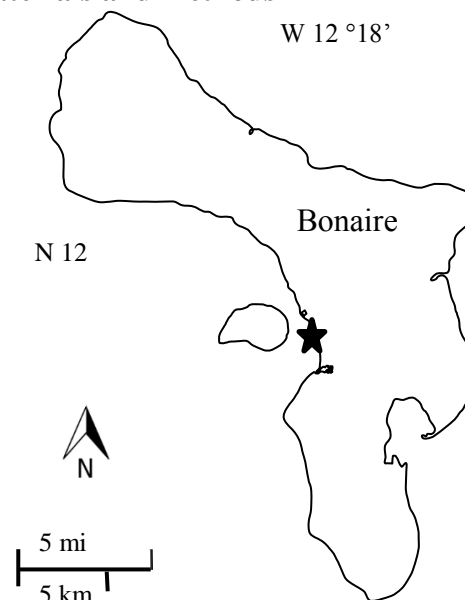
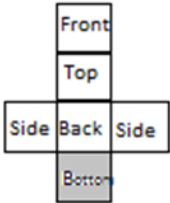
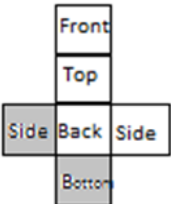
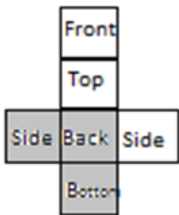
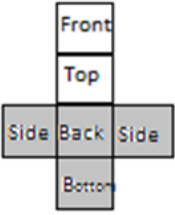
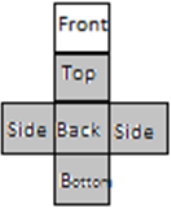
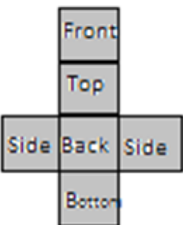


Fig. 1 Yellow Submarine dive site (indicated by a star), where all of the dives conducted for the data collection part of this study took place. The site is comprised of a complex coral reef with high benthic cover and an abundance of grunt fish can be found there

Study site

All of the dives for this research study were conducted at Yellow Submarine dive site (N 12°09'35", W 68°16'55") during the month of February 2015. Yellow Submarine is located on the island of Bonaire (Dutch Caribbean) in the southeast region of the Caribbean (Fig. 1). This site had many boats anchored out front that provided shade coverage for much of the fringing

Table 1 The images that were used for the methods section of this study to describe the amount of protection that was provided by the habitat the grunt was found in

Amount of protection	Diagram relating to habitat
Bottom protection: 1 side total	
Bottom + 1 side: 2 sides total	
Bottom + 2 sides: 3 sides total	
Bottom + 3 sides: 4 sides total	
Bottom + 4 sides: 5 sides total	
Bottom +5 sides: 100% Protection	

reef around the site. At this site, there was mainly sand stretching from the shore until the reef crest, and then a complex coral reef began that stretched both north and south of the site. The reef slope begins approximately 40 m off shore at a depth of ~9 m and progresses slowly downwards to ~30 m. This site was chosen because there is a high abundance of grunts (Haemulidae) present. Blue striped grunts, French grunts, and smallmouth grunts can all be found at both shallow depths and deep depths. The abundance of potential predators is very low because fish that prey on mature grunts are not very common near the study site and apex predators, such as sharks, are extremely rare throughout the area. Occasionally larger sized fish that prey on grunts, such as the occasional barracudas, lionfish, and larger sized groupers, can be found lurking around the site.

Study organism

The Haemulidae family is made up by several different types of grunts that inhabit coral reefs throughout the Caribbean. Specifically to this study, blue striped grunts (*Haemulon sciurusnch*), French grunts (*Haemulon flavolineatu*), and smallmouth grunts (*Haemulon chrysargyreum*) were focused on. Grunt fish are abundant on coral reefs and feed primarily on benthic invertebrates within the reef and nearby grass beds (Burke 1995). Grunts come in a large range of sizes and all the grunts observed during this study were between 15 and 33 cm. Grunts can be found in the sandy shallows as well as in deeper depths along the reef slope.

Data collection and analysis

In order to collect the data for this study, *in situ* observational methods were used while SCUBA diving. All dives were done between 12 and 18 m along the reef slope, and the part of the reef that was surveyed varied throughout each dive. Observational data on every grunt or group of grunts seen during the dive was recorded. The sample size of each type of grunt is as follows: solitary blue striped grunts (n=19); colonial blue

striped grunts (n=0); solitary French grunts (n=43); colonial French grunts (n=15); solitary smallmouth grunts (n=15); colonial smallmouth grunts (n=26). The data collected included the type of grunt, the total length of the grunt (cm), amount of protection that was provided by the habitat that the grunt or group of grunts was found in, and the type of shade coverage over the grunt (if the grunt was in a shaded habitat). The different types of habitats that were found in the field were classified and recorded using the images expressed in Table 1. The relationship between the social status of the grunt and the amount of protection that was provided by the habitat was compared using a Student's t-test as well as a one-way analysis of variance (ANOVA; $\alpha = 0.05$) and a Tukey pairwise comparison post-hoc test of response variables (Tukey 95% CI). The relationship between the shade coverage of grunts and social status was analyzed and compared using Pearson Chi Square tests.

Results

Amount of protection

The first factors examined were the relationship between the social status of the grunt and the amount of protection that was provided by the habitat in which the grunt was found. The results showed that, on average, solitary grunts were protected by more sides of coverage than colonial grunts (Fig. 2). Solitary smallmouth grunts had the most protection out of all the three types of grunts that were studied (2.4 sides \pm 1.1 SD; Fig. 2). There was not a significant difference in the amount of protection provided by the habitat for solitary French grunts when compared to colonial French grunts ($t=0.74$, $df=1$, $p=0.446$; Fig. 2). In contrast, there was a significant difference in the amount of protection provided by the habitat for solitary smallmouth grunts compared to colonial smallmouth grunts; solitary smallmouth grunts had more protection than colonial smallmouth grunts ($t=-3.39$, $df=1$, $p=0.003$; Fig. 2).

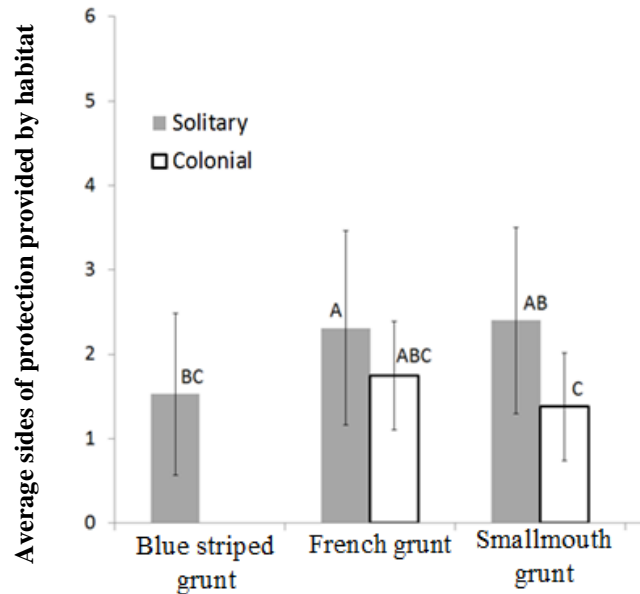


Fig. 2 Social status compared to the sides of protection provided by the habitat that the grunt was found in. This graph displays the type of grunt as well as if the grunt is solitary (grey) or colonial (open box) to the average amount of protection that was provided by the habitat on a one to six sided scale. The error bars represent the standard deviation. The letters above the bars represent if each group is similar to another group or if each group is different as defined by a post-hoc test (Tukey HSD, $\alpha=0.05$)

Shade cover

There was a difference in the amount of shade coverage observed between blue striped grunts and French grunts ($X^2=5.975$, $df=1$, $p=0.015$; Fig. 3) as well as a difference in the shade coverage observed between French grunts and smallmouth grunts ($X^2=5.760$, $df=1$, $p=0.016$; Fig. 3). There was no difference in the behaviors of blue striped grunts and smallmouth grunts ($X^2=0.368$, $df=1$, $p=0.544$; Fig. 3).

Solitary French grunts were found in habitats that were shaded by a boat significantly more than solitary blue striped grunts or solitary smallmouth grunts (Fig. 4a, Table 2a). Colonial French grunts were found in habitats shaded by coral significantly more than colonial smallmouth grunts (Fig. 4b, Table 2b). Finally, solitary smallmouth grunts were found in habitats that were shaded by coral significantly more than colonial smallmouth grunts. (Fig. 4b, Table 2b).

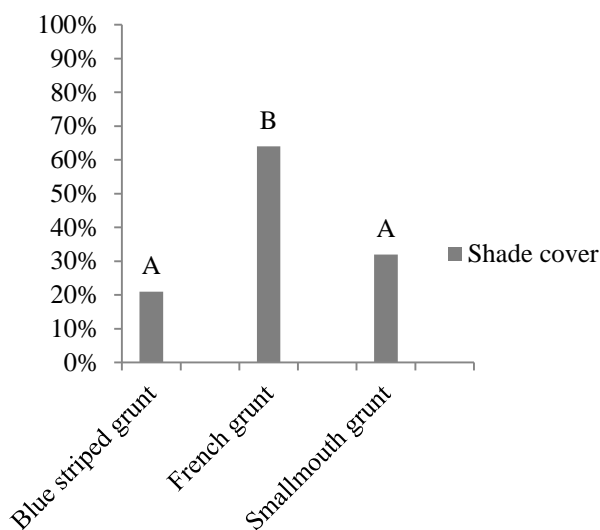


Fig. 3 The type of grunt compared to the percentage at which each specific type of grunt was found in shade cover; the percentage of shade cover combines grunts shaded by a boat and grunts shaded by coral. The letters above the bars refer to groups that are significantly different from each other as defined by a Pearson Chi Square test. The numbers on the bottom of the bars represent the sample size of each grunt type

Table 2a Pearson Chi Square values between grunts that were shaded by a boat

Type of grunt	Colonial French grunt	Colonial smallmouth grunt	Solitary French grunt	Solitary smallmouth grunt
Solitary blue striped grunt	$X^2=2.667(p=0.102)$	$X^2=0.224(p=0.636)$	$X^2=3.928(p=0.047)$	$X^2=0.155(p=0.694)$
Colonial French grunt	N/A	$X^2=1.789(p=0.181)$	$X^2=0.012(p=0.913)$	$X^2=3.333(p=0.68)$
Solitary smallmouth grunt	*See above	$X^2=0.675(p=0.411)$	$X^2=4.432(p=0.035)$	N/A

All of the degrees of freedom for every Pearson Chi Square value calculated are 1. *Boxes with this star indicate that the Pearson Chi Square value is also recorded in another column of the table. Bolded results indicate that there is a significant difference expressed by the p-value

Table 2b Pearson Chi Square values between grunts that were shaded by coral

Type of grunt	Colonial French grunt	Colonial smallmouth grunt	Solitary French grunt	Solitary smallmouth grunt
Solitary blue striped grunt	$X^2=2.524(p=0.112)$	$X^2=0.172(p=0.679)$	$X^2=1.055(p=0.304)$	$X^2=1.093(p=0.296)$
Colonial French grunt	N/A	$X^2=6.322(p=0.012)$	$X^2=0.760(p=0.383)$	$X^2=0.000(p=1.00)$
Solitary smallmouth Grunt	*See above	$X^2=4.498(p=0.034)$	$X^2=1.093(p=0.296)$	N/A

All of the degrees of freedom for every Pearson Chi Square value calculated are 1. *Boxes with this star indicate that the Pearson Chi Square value is also recorded in another column of the table. Bolded results indicate that there is a significant difference expressed by the p-value

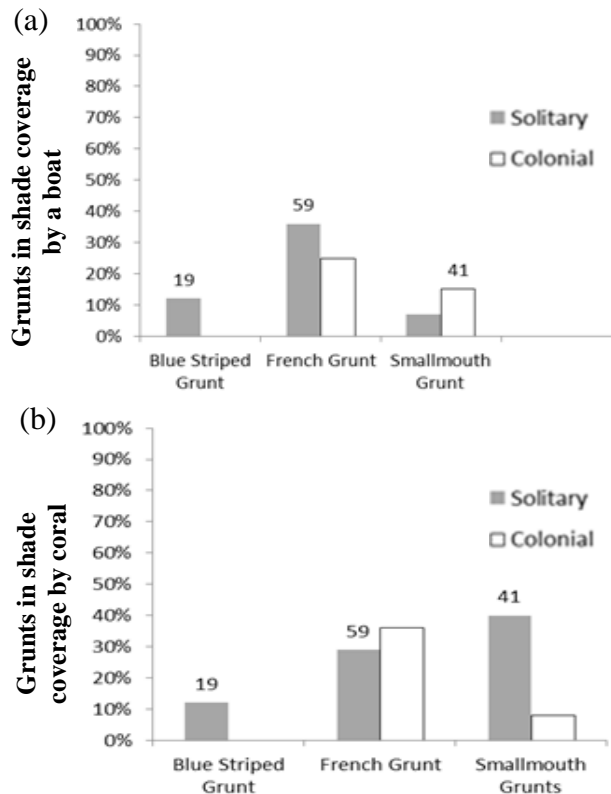


Fig. 4 The proportion of grunts shaded by boats (a) and the proportion of grunts shaded by coral (b). The grey bars are used to indicate solitary grunts and the white bars are used to indicate colonial grunts. The numbers above the bars represent the sample size of each type of grunt

Discussion

Solitary smallmouth grunts were found in habitats that had significantly more protection than the habitats in which colonial smallmouth grunts were found. In contrast, the habitats of both solitary and colonial French grunts did not differ significantly in the amount of protection that was provided. Further, the first hypothesis that was tested by this study, stating that schooling grunts would be found in habitats with more protection than colonial grunts, is partially supported by the results of this study. Solitary smallmouth grunts were found in habitats that were shaded by coral significantly more than colonial smallmouth grunts. This result supports the second hypothesis made by this study stating that solitary grunts would demonstrate shade cover more often than colonial grunts. Colonial French grunts were found shaded by coral significantly more than

colonial smallmouth grunts. Overall, French grunts were found in habitats that were shaded significantly more often than both blue striped grunts and smallmouth grunts.

Solitary smallmouth grunts may have been found in habitats with more protection than colonial smallmouth grunts because the habitat itself may be replacing some of the benefits of being in a school. It is known that being in a school is beneficial to a fish primarily because it offers more protection to the fish (Seghers 1981; Marras et al. 2014). Therefore, when a fish is solitary, it may utilize habitats with more with more sides so that the habitat provides more protection to the fish that is not being provided by a school of other fish (Werner et al. 1983). French grunts may prefer using shelter for protection, whereas smallmouth grunts prefer using schooling behavior for protection. Further, solitary smallmouth grunts may prefer habitats with the highest amount of sides between all the grunts focused on during this study because of their small size. Small fish are at a higher risk of predation (Werner et al. 1983), therefore, if the fish is not in a school, it must seek protection from the habitat that it utilizes (Lamouroux et al. 1999).

Solitary smallmouth grunts may have been found in habitats that were shaded by coral more often than colonial smallmouth grunts because coral offers a direct overhead shade source that can also serve as protection (Orpwood et al. 2010). Based on the results of this study, it is possible that the grunt population in the Caribbean now might be lower than it was when the branching coral *Acropora palmata* was abundant in the Caribbean because *A. Palmata* was a coral that provided lots of crevices and overhead protection for fish (Lirman 1999), and was therefore an ideal habitat for smallmouth grunts. Moreover, the decline in *A. Palmata* may have led to a lower grunt population throughout Caribbean coral reefs. Further, fish are able to see further when than are in the shade, which could be beneficial for a small, solitary smallmouth grunt (Bohnsack 1989). Shade coverage allows a fish to see greater

distances because the fish's eyes are covered by shade, which acts as a visor. This is beneficial because a fish that can see farther can see an approaching predator sooner, and have more time to hide or escape if necessary; this ability is beneficial to a solitary fish because a solitary fish is lacking protection from a school. A coral shade source could also help a fish appear darker and blend in better with its surroundings; this could also be beneficial to solitary fish and could be part of the explanation for why solitary smallmouth grunts were shaded by coral more often than colonial smallmouth grunts (Cocheret et al. 2004). French grunts were found in overall shade cover more often than both blue striped grunts and smallmouth grunts. A possible explanation for this result may be because French grunts are often solitary and therefore sought opportunities that provided the highest amount of protection; being in shade may help the grunt blend in as well as anticipate approaching predators better than a sunny habitat.

On a larger scale, the results of this study demonstrate that, often times, solitary grunts utilize habitats with more sides of protection than colonial grunts. Further, both solitary grunts and French grunts in general have a high preference for shade covered habitats. Therefore, in order to keep the grunt population thriving in Bonaire, it is crucial that habitats that provide many sides of protection as well as direct overhead coral shade sources are preserved rather than degraded. This study showed that solitary grunts prefer habitats with more protection, solitary smallmouth grunts prefer habitats shaded by coral, and French grunts have a high preference for shade covered habitat in general. Moreover, the complex habitats and abundant coral shade sources as well as the rugosity of the reef must be maintained in order for the grunt population to thrive and remain stable for many years to come (Hewitt et al. 2005). Further, this study demonstrated that blue striped grunts rely mainly on their size for protection, smallmouth grunts rely mainly on schools for protection, and French grunts rely mainly on habitat type and structure for protection. Based on their

habitat preference, it is likely that French grunts will be affected the most by a degrading ecosystem. Moreover, fish that prefer to be solitary rather than expressing schooling behaviors are going to undergo many problems if coral reefs continue to degrade at the current rate.

Future studies that could be done in order to enhance and build upon the results of this study could widen the study to include other types of grunts such as sailor's choice grunts, white grunts, Spanish grunts, and Caesar grunts, and also other families of fish. This would be beneficial because it would demonstrate the preferred habitats of both larger and smaller grunts than the grunts focused on for this study as well as other types of fish that might demonstrate similar behaviors. Future studies could also focus on the specific type of habitat, such as coral type, sponge type, etc., that grunts prefer. These results would further aid in demonstrating the crucial habitats that must be preserved in order to keep a sustainable grunt population throughout tropical coral reefs.

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REPORT

Cathryn Morrison • Villanova University • catmorrison@bellsouth.net

The role of mimicry within congruencies amongst herbivorous and carnivorous fish in Bonaire

Abstract Mimetic behavior signifies organisms evolving to share behaviors and a common resemblance despite different phylogeny. Relationships in mimicry rely on the characteristics of mimics and models: appearance and size, vertical and geographical distribution, mimic to model abundance, behavioral shifts of the mimic and observed benefits. Using these criteria, species from the *Hypoplectrus* genus (hamlets) were analyzed as potential aggressive mimics. Using a visual census, the distribution of each mimic and model were surveyed and behaviors of individuals within the mimetic pairs were video recorded. There was 80-94% difference between the population densities of two potential mimetic pairs: *Hypoplectrus nigricans* (black hamlet) and *Stegastes adustus*, (dusky damselfish) and *Hypoplectrus unicolor* (butter hamlet) and *Chaetodon capistratus* (foureye butterflyfish). Data collected for the potential mimetic pair, *Hypoplectrus chlorurus* (yellowtail hamlet) and *Microspathodon chrysurus* (yellowtail damselfish) does not support the hypothesis because the population density of the supposed mimic was higher than that of the potential model. In addition, for all studied pairs, no notable behavioral shifts were observed, and therefore whether the studied pairs are cases of mimicry is still a question.

Keywords *Hypoplectrus* • Mimetic criteria • Aggressive mimicry

Introduction

The theory of evolutionary biology connects species to a common ancestral organism (Huelsenbeck et al. 2001). Species sharing similar phylogenetic origins are more likely to possess similar characteristics in appearance, behavior, habitat, etc. Several cases where color patterns are similar between multiple species across trophic levels can be accounted for by the presence of similar selective pressures between two species. Such scenarios where congruencies amongst species are found but are unrelated generally result from coincidental or convergent evolution. Convergent evolution involves organisms evolving independently from one another yet developing similar characteristics due to similar niches. However, behavioral association between two physically similar species is hypothesized as being mimetic. The mimicry hypothesis proposes that natural selection produced resemblance in a species (mimic) that closely matches that of a different species (model) (Robertson 2013). Varying interactions between mimic and model species describe differing forms of mimicry. Aggressive mimicry exists in scenarios when a predatory species resemblances a harmless species in order to increase opportunities for foraging by deception (Moland et al. 2005). Batesian mimicry exists when a harmless species avoids predation by mimicking a predatory species (Robertson 2013). Mullerian mimicry occurs when multiple hazardous species share a common appearance thus reinforcing predator deterrence (Moland et al. 2005). Lastly, social mimicry exists when a

species gains protection from predators through association with similarly colored individuals (Moland et al. 2005). Theories on mimicry were developed around insects and terrestrial organisms, however the same principles can also apply to marine organisms (Moland et al. 2005). Approximately 60 cases of mimicry have been reported for oceanic environments (Moland et al. 2005). Of those reports, the most abundant type of mimetic relationships was aggressive mimicry (Moland et al. 2005). In order to be categorized as a mimic, five main criteria are required: (1) Resemblance: similar features on mimic/model include form of body and fins, size, and coloration (Robertson 2013); (2) Geographic overlap: close proximity of mimic and model distributions; generally mimics should occupy the same habitat or geographical range as model species (Randall and Randall 1960); (3) Relative Abundance: the mimic species must be rare in comparison to their model species (Bates 1862); (4) Evidence of behavior change: mimetic species generally alter their behavior to better resemble their models (Moland et al. 2005); and (5) Evidence of benefit: any direct evidence of benefits to the mimic as a result of its resemblance to the model (Robertson 2013). Although mimetic cases in marine environments exist, few have been substantiated using the specific criteria. This study aims to provide observational data to support the claim of mimicry in relation to the following possible mimetic pairs: *Hypoplectrus unicolor* (butter hamlet) and *Chaetodon capistratus* (foureye butterflyfish); *Hypoplectrus chlorurus* (yellowtail hamlet) and *Microspathodon chrysurus* (yellowtail damselfish); lastly *Hypoplectrus nigricans* (black hamlet) and *Stegastes adustus* (dusky damselfish). Using mimetic criteria the following hypotheses were determined,

- H₁: *Hypoplectrus* spp. would mimic Pomacentridae and Chaetodontidae
 H₂: Pomacentridae populations would be larger than those of *Hypoplectrus* spp, making Pomacentridae more likely the model

Materials and methods

Study site

All research was conducted at Yellow Submarine dive site located on the western coast of Bonaire (Fig. 1). The reef crest extends approximately 60 m from the coast line and transitions to the reef slope at a depth of approximately 7.6 m. Data was collected over a five-week period from March to April 2015.

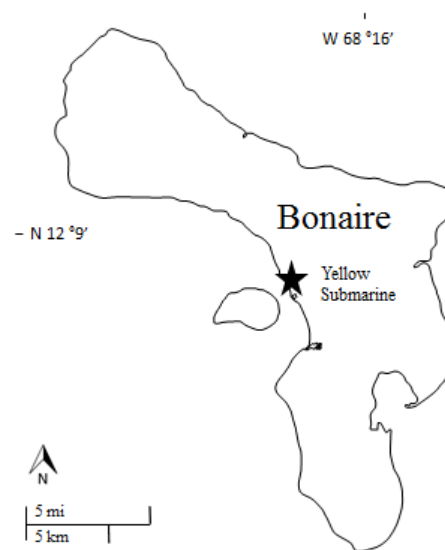


Fig. 1 Map of the island of Bonaire, marking the study site Yellow Submarine (12°9'36.32"N 68°16'54.91"W) as a black star

Study organisms

This study focused on potential mimetic *Hypoplectrus* spp, members of the family Serranidae. The specific study species were *H. nigricans*, *H. chlorurus*, and *H. unicolor*, which are tropical reef fish commonly found in the Caribbean (Humann and DeLoach 1989). The model species to which the *Hypoplectrus* spp. was compared included two species from Pomacentridae and one from Chaetodontidae, all of which are also prominent throughout the Caribbean.

Hypoplectrus nigricans and *Stegastes adustus*

Hypoplectrus nigricans is primarily found near the bottom of the reef slope at depths ranging from 3 to 12 m (Humann and DeLoach 1989). The species of comparison, *S. adustus*, prefers rocky reef areas and is commonly found at depths ranging from 1.5 to 12 m (Humann and DeLoach 1989). Both *H. nigricans* and *S. adustus* appear to be primarily dark brown to blueish black, however *S. adustus*' coloration commonly includes a gradient pattern (Humann and DeLoach 1989).

Hypoplectrus chlorurus and *Microspathodon chrysurus*

The species of the potential mimetic pair *H. chlorurus* and *M. chrysurus*, share similar dark brown to bluish black coloration. Both species remain within their own respective territory at depths ranging from 3 to 12 m, however *H. chlorurus* frequents the bottom of the reef while *M. chrysurus* inhabit reef tops (Humann and DeLoach 1989). The size range for *H. chlorurus* is 76.2-114.3 mm. The size range for *M. chrysurus* is 101.6-165.1 mm (Humann and DeLoach 1989).

Hypoplectrus unicolor and *Chaetodon capistratus*

Hypoplectrus unicolor often has a black dot on the snout and has grayish white to yellow coloration (Humann and DeLoach 1989). More prominent on *H. unicolor* is the large black saddle on the base of the tail. *Chaetodon capistratus* appears silver gray with thin, dark lines radiating diagonally from midbody (Humann and DeLoach 1989). The size range for *H. unicolor* is 76.2-114.3 mm. For *C. capistratus* the size ranges from 76.2-101.6 mm (Humann and DeLoach 1989).

Data collection

Heading south from the a midpoint between the dives sites locally known as Yellow Submarine and Something Special (12°9'38.79"N,

68°16'57.41"W), a visual census of the abundance of the study species in terms of density (individuals/100 m²) was conducted at both 12 and 9 m using SCUBA. After 45 minutes of surveying, the surveyors swam directly east to the shore. The exit and entry point were marked on Google Earth® and from those two points the length of the transect was calculated. The transect width was approximately 1 m (half a meter on each side of each surveyor). Species were observed during three transects in order to decrease bias and human error. To compare the behavior of mimics and models, on each dive two individuals were recorded per species (*H. unicolor*, *H. chlorurus*, *H. nigricans*, *C. capistratus*, *M. chrysurus*, and *S. adustus*) for a length of five minutes per individual. To decrease effects of diver presence on the behavior of the studied individuals, an appropriate distance was maintained between the subject and observer. During video recordings, observations outside of the frame were noted in order to aid footage analysis and comparisons. Two video recordings were taken at 11:45 hrs and one dive for video recordings occurred at 18:15 hrs. Surveying transects approximately an hour before sunset provided a control for analyzing behavioral data. *Hypoplectrus* spp. activity increases approximately an hour before sunset, and an overall increase in predatory fish activity equated to a consequential decrease in herbivorous fish activity (Fischer 1980). This subsequent decrease in herbivorous fish means a decrease in prominence of model species. Therefore the mimetic *Hypoplectrus* spp. will hypothetically be under less pressure to behave similarly to its model species.

Data analysis

Data derived from the two surveyors observing the study species were averaged. For a depth of 12 m, the means for the two transects were used to determine a more standardized average abundance of studied species. For the population densities of studied species at 12 m standard error was calculated. Total size of the

species was calculated by using measurements of a fixed item in the video recordings of the studied species. All sizes recorded for each specific study species were averaged and compared against their potential mimetic companion to find the difference in size (%).

Results

Hypoplectrus nigricans and Stegastes adustus

Both *H. nigricans* and *S. adustus* co-occurred at the two studied depths at Yellow Submarine. The ratio of *H. nigricans* to *S. adustus* at 12 and 9 m was 1:12 and 1:15 respectively (Fig. 2). Of the studied fish species, *S. adustus* were most abundant; for the three transects a mean of 34.7 ± 5.4 *S. adustus* were recorded (mean \pm SD, $n=3$). At 12 m the population density of *S. adustus* was 91% higher than the population density of *H. nigricans*, and at 9 m the proportion of *S. adustus* to *H. nigricans* had a difference of 94%. *Hypoplectrus nigricans* was the most conspicuous of the studied species and therefore the pair of *H. nigricans* and *S. adustus* were not included in the video analysis for behavioral changes.

Hypoplectrus chlorurus and Microspathodon chrysurus

Hypoplectrus chlorurus occurred at both depths with a population density of 2.8 ± 1.6 individuals per 100 m^2 at 12 m (mean \pm SD, $n=2$) and from the one surveying of 9 m the calculated population was 3.8 individuals per 100 m^2 ($n=1$). There were no sightings of *M. chrysurus* at 12 m (Fig. 3a). However at 9 m, 0.6 individuals per 100 m^2 of *M. chrysurus* were recorded (Fig. 3b). At a depth of 12 m *H. chlorurus* was the only species of this possible mimetic pair to be recorded. At 9 m, the proportion of *H. chlorurus* was 85% higher than the population density of *M. chrysurus*.

The size of the *H. chlorurus* surveyed during video observations had an average of 102.6 ± 17.7 mm per individual (mean \pm SD, $n=4$). The recorded *M. chrysurus* had a mean

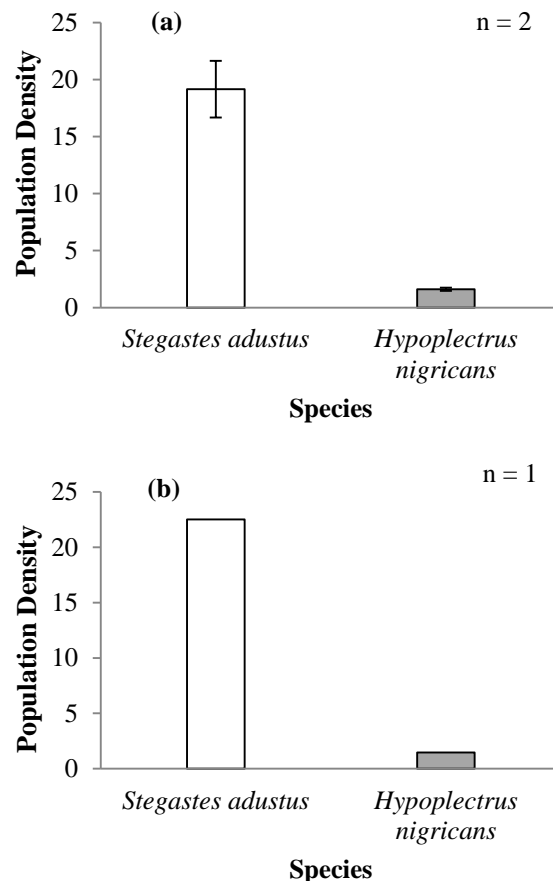


Fig. 2 Population density estimates of individuals per 100 m^2 for *H. nigricans* and *S. adustus* at Yellow Submarine, Bonaire. n = number of transects. (a) Mean population density of two transects at 12 m. Error bars are standard errors. (b) Population density from abundance collection at 9 m

size of 136.8 ± 32.3 mm per individual (mean \pm SD, $n=3$). *Microspathodon chrysurus* have a relative size approximately 25% larger than that of *H. chlorurus*.

Video recordings of *H. chlorurus* at dusk displayed a pair swimming around the reef at a depth of 9-12.8 m. The pair of *H. chlorurus* behaved in a calm manner, not easily frightened by large fish or the presence of divers. One individual was chased off by a graysby, however upon being separated, its mate followed the *H. chlorurus* and they continued swimming together. The *H. chlorurus* swam with their pectoral and caudal fins. The pelvic, pectoral, spinous dorsal, and anal fins were extended. *Hypoplectrus chlorurus* utilized primarily their pectoral, caudal, and pelvic fins. The *H. chlorurus*

remained steady, moving in a calm, cautious manner.

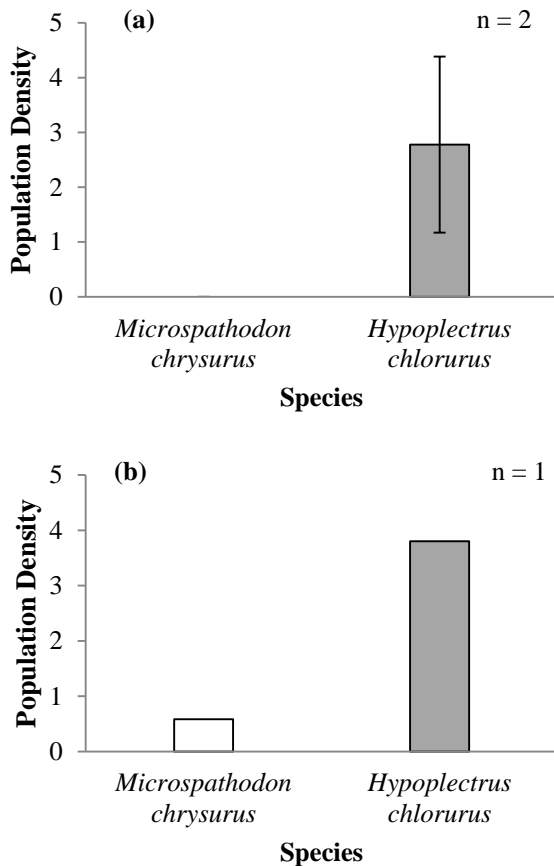


Fig. 3 Population density estimates per 100 m² for *H. chlorurus* and *M. chrysurus* at Yellow Submarine, Bonaire. n = number of transects. (a) Mean population density calculated from two transects at a depth of 12 m. Error bars are standard error. (b) Population density from one transect at 9 m

The only behavioral data collected on *M. chrysurus* was taken midday at a depth of approximately 6 m. The first of the two recorded individuals was observed eating algae off mooring blocks and swimming into holes of the blocks. The second individual also frequented the holes of a mooring block. This Pomacentridae primarily utilized its pectoral fins for swimming. It was also observed foraging on algae, occasionally switching between mooring blocks. *M. chrysurus* did not appear territorial and was not frightened by larger fish or the presence of divers.

Hypoplectrus unicolor and *Chaetodon capistratus*

Both *H. unicolor* and *C. capistratus* co-occurred at depths of 12 and 9 m (Fig. 4). Within the pair of *H. unicolor* and *C. capistratus*, *C. capistratus* were more abundant. The proportion of *C. capistratus* to *H. unicolor* had little variance amongst the depths of 12 and 9 m. At a depth of 12 m the population density of *C. capistratus* was 83% higher than the population density of *H. unicolor*. The ratio of *C. capistratus* to *H. unicolor* at a depth of 9 m was a 79% difference.

The mean total size of the recorded *H. unicolor* was 101.4 mm (SD=21.8, n=3) For *C. capistratus* the mean size was 99.5 mm (SD=23.3, n=6). The difference in size between *H. unicolor* to *C. capistratus* was only a 2% increase.

At dusk, *H. unicolor* swam in a pair at a depth of 9.5 m. Two *H. unicolor* were seen mating, breaking apart, and intertwining again. When separated the hamlet acted sporadic, turning frequently from side-to-side. The pectoral and pelvic fins of the *H. unicolor* remained extended throughout the time of surveying. The footage taken midday of *H. unicolor* displayed a solitary hamlet. This hamlet primarily utilized its pectoral, pelvic, and caudal fin for movement. The fish was continuously in motion and made deliberate, quick movements, constantly speeding up and slowing down in a seemingly random manner. The hamlets from both recordings (dusk and midday) were taken at the same habitat, which consisted of scattered pore rope sponge. At dusk the recorded *C. capistratus* swam frantically, showing no apparent preference in habitat or depth. The video recording began with the *C. capistratus* at a depth of 7.3 m and ended around 4.6 m. At dusk, the recorded *C. capistratus* were only solitary individuals. The butterflyfish were easily frightened by

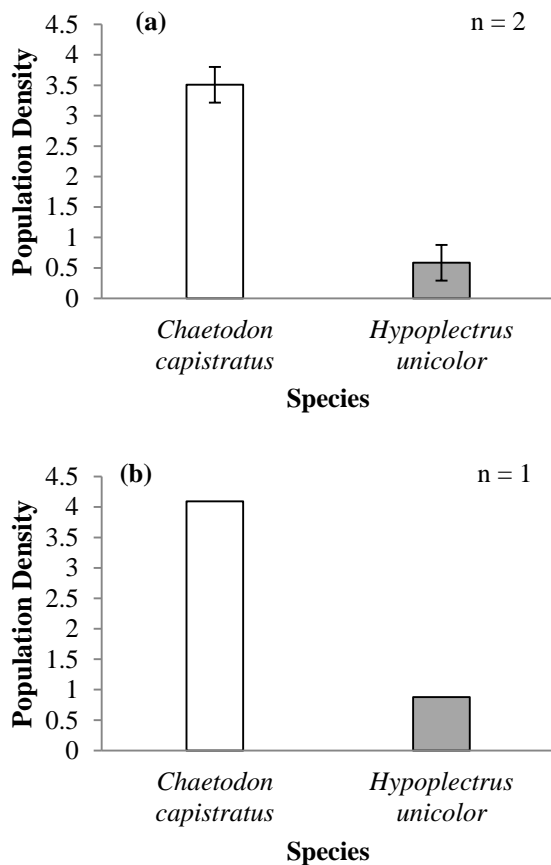


Fig. 4 Population density estimates per 100 m² for *H. unicolor* and *C. capistratus* at Yellow Submarine, Bonaire, with n = number of transects. (a) Mean population density from two transects taken at a depth of 12 m. Error bars are standard error. (b) Population density from one transect at 9 m

approaching fish and remained cautious throughout the collected video data. Of the recorded *C. capistratus* midday, the two surveyed individuals were solitary. During the video of the first individual, the *C. capistratus* was recorded swimming in a circle, rubbing against a rock, and being frightened by several larger fish. The *C. capistratus* relied on its caudal fin for movement and appeared to utilize its pectoral fins for directionality and balance. The *C. capistratus* was not recorded eating, however it did rejoin its mate near the end of the video recording. The second *C. capistratus* was observed rubbing against coral and remaining almost stationary on the bottom of the seafloor. When approached by large fish, the *C. capistratus* was unreactive.

Discussion

Hypoplectrus nigricans and *Stegastes adustus*

Although collected data on population density suggests a relative abundance of both *H. nigricans* and *S. adustus*, there is little weight in the findings on *H. nigricans* sightings. Therefore this potential mimetic pair was removed from any further behavioral study.

Hypoplectrus chlorurus and *Microspathodon chrysurus*

Appearance

The potential mimetic pair of *H. chlorurus* and *M. chrysurus* are both distinguished by their dark body and yellowtail (Humann and DeLoach 1989). Body shape of *H. chlorurus* differs from that of *M. chrysurus* primarily in the angled slope of the head, *Hypoplectrus* spp. have a flatter slope than that of Pomacentridae spp. Size also differs amongst this pair, *H. chlorurus* ranges from 76.2 to 114.3 mm, while *M. chrysurus* is relatively larger, ranging from 101.6 to 165.1 mm (Humann and DeLoach 1989).

Vertical distribution and relative abundance

Within an area of 100 m² both *H. chlorurus* and *M. chrysurus* were sighted, however, the depth of the surveyed transect was a contributing factor to the *M. chrysurus* population density. The depth range for both species is supposedly 3 to 12 m, however at this maximum depth no *M. chrysurus* were sighted. Consequently, focus on this potential mimetic pair was moved to shallower depths of approximately 9 m.

Evidence of behavioral shifts and benefits

The recordings taken of this studied pair were inconclusive due to a lack of data sets. Both *H. chlorurus* and *M. chrysurus* were unreactive towards divers and larger fish, remaining calm throughout the time of observations.

Hypoplectrus unicolor and Chaetodon Application capistratus

Appearance

Of the studied pairs, *H. unicolor* and *C. capistratus* have appearances with the most incongruences, meaning the coloration and patterns were not as closely matched. Although both species have primarily a white to yellow coloration, *C. capistratus*' body coloration consists of a black spot near the upper base of the tail, several black lines radiating diagonally from its midbody and a black bar running across the eye (Humann and DeLoach 1989). *Hypoplectrus unicolor* has a large black saddle on the base of the tail, similar to that of *C. capistratus*, but unlike *C. capistratus* it has blue markings on the head and gill cover (Humann and DeLoach 1989). Both *H. unicolor* and *C. capistratus* have a size range of 76.2 to 114.3 mm (Humann and DeLoach 1989).

Vertical distribution and relative abundance

Both species co-occurred at the surveyed transects of both 12 and 9 m. *Hypoplectrus unicolor* are more common at the bottom of the reef while *C. capistratus* are more frequent at reef tops (Humann and DeLoach 1989). At both surveyed depths, there was a higher abundance of the model species, *C. capistratus* to the mimic species, *H. unicolor*.

Evidence of behavioral shifts and benefits

Video analysis of behavior for *H. unicolor* and *C. capistratus* was inconclusive. Observations of each species' behavior noted variance in swimming patterns. The footage taken at dusk of *H. unicolor* displayed two individuals mating, which involves irregular behavior, thus the behavioral baseline for *H. unicolor* was not accurately established. Additionally, no direct benefits to a potential mimetic relationship was distinguished.

In the case of *H. chlorurus* and *M. chrysurus*, the evidence does not support both hypotheses. The abundance of *H. chlorurus* is greater than that of the proposed model species, *M. chrysurus*. A further study hypothesizing *M. chrysurus* as the mimetic species would be beneficial to gain a better understanding of mimetic relationships. The evidence collected from observations of *H. unicolor* and *C. capistratus* supports mimetic claims. Although the appearance of the two species have a larger variance than other mimetic pairs, the impacts of varying appearance is dependent on the target of the mimetic resemblance (Cheney and Marshall 2009). For stronger support of mimetic claims, expanding the time period of observations to different periods throughout the year would eliminate behavioral outliers. Also increasing the number of data sets would decrease bias in the results. Future studies would benefit from focusing on distinguishing the benefits and behavioral shifts between potential mimetic pairs.

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REPORT

William Duritsch • University of Dayton • duritschw1@udayton.edu

Finding a correlation between zooplankton abundance and the aggregation of *Abudefduf saxatilis* (sergeant major damselfish) beneath boats

Abstract *Abudefduf saxatilis*, or sergeant major damselfish, are a common reef fish in the Caribbean and western Atlantic that form large feeding aggregations. *Abudefduf saxatilis* are primarily planktivorous, with zooplankton making up over 50% of their diet. Zooplankton are known to have diel movements to avoid predation, which have been shown to be triggered by the presence of ultra violet radiation. Beneath boats along the coast of Bonaire, aggregations of *A. saxatilis* have been observed, but why they prefer these areas over the open water column had not previously been examined. The abundance of zooplankton was estimated beneath boats as well as in the open water, up-current from the boats. Both the abundance and bite rates of *A. saxatilis* were also estimated beneath the boats that corresponded to the estimates of zooplankton abundance. In addition, the bite rates of *A. saxatilis* were estimated in the open water. It was found that the zooplankton abundance ($p < 0.001$) and the bite rate of *A. saxatilis* ($p < 0.001$) were both significantly greater beneath boats than in the open water. Also, a significant correlation was found between increasing abundances of zooplankton and *A. saxatilis* ($p < 0.01$). These results demonstrate that one of the main drivers for the aggregation of *A. saxatilis* beneath boats is likely to feed on the zooplankton, which are in high abundance. In turn, this could alter community structure on the reef due to a decrease in the amount of algae grazing by *A. saxatilis*.

Keywords *Abudefduf saxatilis* • Boat effects • Zooplankton abundance

Introduction

Understanding fish behavior is a key aspect in understanding how a coral reef ecosystem works. A behavior seen in reef fish is aggregating, or gathering of fish into groups. There are three main types of aggregations that occur within fish species: schooling, spawning, and feeding. Schooling aggregations most likely occur because they help reduce predation on the individuals in the schooling group (Brock and Riffenburgh 1960). Spawning aggregations occur when individuals of a species of fish gather in large groups in order to release their eggs and sperm into the water column to mate (Tucker et al. 1993). Some common species that form spawning aggregations include Nassau groupers (Tucker et al. 1993) and blue tangs (Deloach and Humann 1999), as well as cubera, mutton, and dog snappers (Heyman and Kjerfve 2008). Feeding aggregations are less common among fish species, but some examples of these can be seen in some whale sharks (Heyman et al. 2001) and various species of surgeon fish, such as the blue tang (Deloach and Humann 1999).

Off the western coast of Bonaire, under boats moored above the reef crest, large aggregations of *Abudefduf saxatilis*, also known as sergeant major damselfish, can be seen. *Abudefduf saxatilis* are damselfish that tends to spend the majority of its time in the water column from 0-20 m deep (Feitoza et al. 2003). *Abudefduf saxatilis* form large feeding aggregations of up to 150-200 individuals (Fishelson 1970), but there was no research as to why they would form large aggregations primarily in the shade found under boats rather

than out in the open water column. One explanation for why *A. saxatilis* may prefer locations beneath boats could stem from its diet, which is mainly composed of zooplankton, plus a small portion of algae and fish and invertebrate larvae (Randall 1967). In fact, unlike other damselfish, *A. saxatilis* rarely take food from hard surfaces, but rather spend most of their time feeding in the water column (Fishelson 1970).

Behaviors exhibited by zooplankton could possibly be linked to why *A. saxatilis* are found primarily in the shade under boats. There has been a great deal of research done on the migration of zooplankton throughout the water column (Zaret and Suffern 1976; Ohman et al. 1983; Forward 1988; Speekmann et al. 2000; Leech et al. 2005; Zengling et al. 2013). Zooplankton tend to change their depth in the water column depending on the time of day and exhibit what is referred to as diel vertical migration. It is believed that this behavior evolved so that zooplankton could avoid predators by moving deeper in the water column during the day (Zaret and Suffern 1976; Ohman et al. 1983). Multiple studies found that one of the main factors that determines the depth at which zooplankton can be found in the water column is the intensity of ultra violet, or UV, radiation (Forward 1988; Speekmann et al. 2000; Leech et al. 2005). Presence of UV radiation alerts the zooplankton that it is time to take part in their diel vertical migration in order to avoid the predators that forage for them during the day. Since the research has shown zooplankton tend to migrate vertically in the water column in the presence of UV radiation, it can be reasoned that it is also possible for them to migrate to shaded areas such as under boats (Zengling et al. 2013).

The purpose of this study was to determine whether there was a correlation between the abundance of zooplankton under the boats off the coast of Bonaire and the presence of *A. saxatilis*. The hypotheses were as follows:

H₁: Aggregations of *A. saxatilis* were more likely to be found in areas where zooplankton was abundant

H₂: During the day, zooplankton would be found in a higher concentration in shaded areas, such as beneath boats

This is an important topic to study for multiple reasons. First, there is very little to no research on this behavior that is exhibited by *A. saxatilis*. Most of what is known about *A. saxatilis* concerns their breeding habits, so this could provide an excellent opportunity to gain new insight into their behavior. Secondly, *A. saxatilis*, like other planktivorous fish, are a link between the energy produced by the plankton in the water column and the rest of the reef trophic structure, which makes them an important component of the reef ecosystem (Marcus and Boero 2003).

Materials and methods

Study site

Data collection took place on the island of Bonaire in the Dutch Caribbean. The study site was the diving location known as Yellow Submarine (12°16'02.44"N, 68°28'19.74"W), which is located just north of the capital city of Kralendijk. The study site was a series of ten, approximately 10 m long boats that were moored approximately 40 m from shore above the reef crest. There was a space of approximately 10 m between each boat. The site has a large population of *A. saxatilis*, which can primarily be found beneath the boats, with a few individuals also found on mooring blocks guarding eggs and in the open water column above the reef crest. An average of 25 individuals was found beneath each boat. The site generally had very little current or wave action, but when a current is present it was typically moving from south to north, parallel to the shoreline.

Data collection

Three different data sets that were collected: zooplankton abundance, *A. saxatilis* abundance, and bite rate of *A. saxatilis*. The abundance of zooplankton was estimated under randomly selected boats throughout the study site and in the open water 5 m up current from each boat chosen. Of the ten boats located at the study site, five boats were tested twice, but never on the same day of data collection. The abundance and bite rate of *A. saxatilis* were estimated underneath boats that corresponded with zooplankton estimates. In addition, the bite rate of *A. saxatilis* was determined in locations in the open water, also corresponding with the sampling locations of zooplankton. Data collection always occurred around midday on days with minimal cloud cover.

Zooplankton was captured using a 20-micron plankton net with an opening that was 30 cm in diameter and a cod end that could hold 400 ml. A rope with a knot tied at 7 m was attached to the net to facilitate the collection of the sample.

Zooplankton collection: beneath boats

A research team of two individuals submerged to a depth of 2 m adjacent to one of the boats. At one end of the boat, one researcher held the net while the other swam, with the rope, along the bottom of the boat until they had reached a distance of 7 m from the net. The first researcher proceeded to release the net while the second quickly pulled it towards themselves with the rope. The opening of the net was quickly pinched off to prevent the escape of any zooplankton and the process was repeated so that there were two passes underneath each boat, equivalent to a 14 m pass. The opening of the net was once again quickly pinched off and the research team

swam to the surface so that the sample could be placed into a labeled 100 ml sample bottle.

Zooplankton collection: open water

The team resubmerged 5 m up current from the location of the boat, above the reef crest. If there was no detectable current present, the research team would submerge on the south side of the boat. Once the team reached a depth of 2 m, they performed two more 7 m tows, parallel with the boat just sampled, with the same process as was performed beneath the boats. In total, 15 samples were taken from under the boats and 15 were taken from the open water, up current from the boats, over the course of the experiment.

Zooplankton in the laboratory

The collected samples were taken to the laboratory at CIEE Research Station Bonaire. Each sample bottle was shaken in order to ensure that any zooplankton that had settled to the bottom would be re-suspended in the water. A 30 ml portion of a sample was measured out and poured into a beaker. The zooplankton were euthanized using 5 ml of 95% ethanol. A 1 ml portion of the solution from the beaker was pipetted onto a petri dish with gridlines, which was then placed under a dissecting microscope. The number of zooplankton, which included copepods and the larvae of other arthropods, were observed and recorded. This was repeated nine additional times in order to create ten replicates for each sample. The ten replicates were then averaged to give the estimated abundance of zooplankton per ml of the sample. This average was then entered into the following equation in order to determine the number of zooplankton per liter of sea water:

$$\frac{\text{number of zooplankton}}{1 \text{ ml of solution}} \times \frac{35 \text{ ml of solution}}{30 \text{ ml of sample}} \times \frac{400 \text{ ml of sample}}{1 \text{ tow}} \times \frac{1 \text{ tow}}{\pi(0.15 \text{ m})^2 \times 14 \text{ m}} \times \frac{1 \text{ m}^3}{1000 \text{ L}}$$

Abundance of *Abudefduf saxatilis*

The abundance of *A. saxatilis* was determined through the use of photography. Before the collection of zooplankton occurred, a photo was taken with a Panasonic Lumix DMC-FT5 waterproof camera under the corresponding boat at a distance as to include all visible *A. saxatilis*. Each photo was later analyzed by visually counting to determine the total number of individuals present under each boat. The total sample size for the abundance of *A. saxatilis* beneath boats was 15.

Bite rate of *Abudefduf saxatilis*

The bite rate of *A. saxatilis* was quantified two separate ways. One researcher observed a single, random individual for one minute and recorded every time they would bite at the water. A bite was denoted by anytime that the mouth would open in such a way that the lips would quickly extend forward and return back to the starting position. The second researcher had the camera and would video a randomly selected individual for a minute. Each of the videos was then visually analyzed in order to determine the bite rate of that individual. The bite count obtained from each researcher was then averaged to give one number for under each boat and in each open water location. The sample size for underneath boats and in the open water was eight.

Data analysis

The mean zooplankton abundances from underneath the boats and in the open water were compared through the use of a Student's t-test. The data for the abundance of *A. saxatilis* was transformed using the equation $\text{Log}_{10}(x+1)$, where x denotes the number of *A. saxatilis*, in order to create a linear relationship rather than exponential. This relationship was then tested with a linear regression, with zooplankton abundance as the explanatory variable. The mean bite rates of *A. saxatilis*

from underneath boats and in the open water were compared using a Student's t-test. The relationship between the bite rate of *A. saxatilis* and the abundance of zooplankton for both data sets were tested with a linear regression, with zooplankton abundance as the explanatory variable.

Results

Zooplankton abundance and location

For zooplankton abundance, there was a total of 30 samples taken, 15 from beneath the boats and 15 from the open water column 5 m up current from each boat. Zooplankton were more abundant under boats than in open water ($t=38.19$, $df=1$, $p<0.001$; Fig. 1).

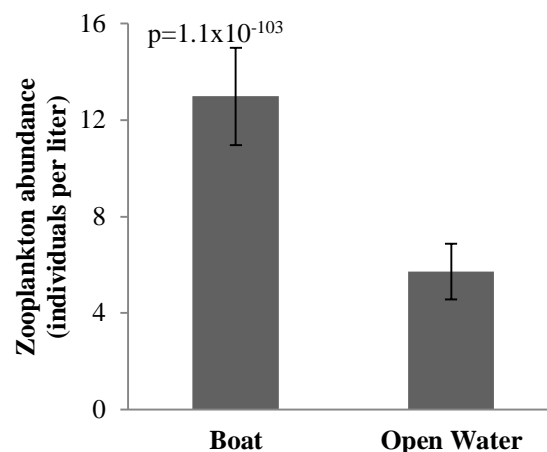


Fig. 1 Comparison of the mean zooplankton abundance, shown in individuals per liter, between two locations, beneath boats and in the open water column above the reef crest ($n=15$ per treatment). Error bars denote the standard deviation from the mean zooplankton abundance for each location

Abudefduf saxatilis and zooplankton abundance

The number of *A. saxatilis* beneath each boat ($n=15$) was compared to the abundance of zooplankton and a significant correlation was found between a higher number of individual *A. saxatilis* and a higher zooplankton abundance ($R^2=0.5609$, $df=14$, $p<0.01$; Fig. 2).

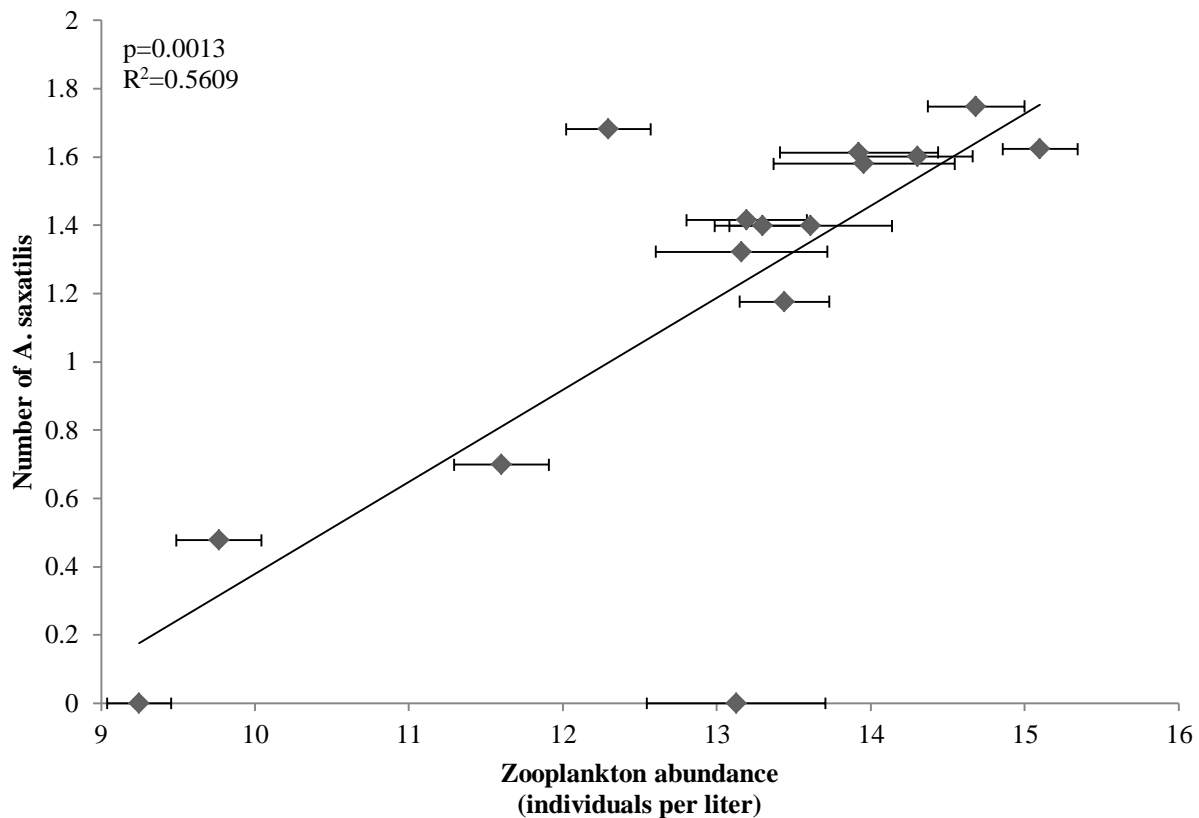


Fig. 2 Relationship between the number of individual *A. saxatilis* and mean zooplankton abundance, shown in individuals per liter, beneath each boat (n=15). The number of *A. saxatilis* has been entered into the equation $\text{Log}_{10}(x+1)$, where x is the number of individual *A. saxatilis*, in order to show a linear relationship rather than an exponential. The error bars denote the standard error from the mean zooplankton abundance beneath each boat

Bite rate and location

For the bite rate of *A. saxatilis*, measured in bites per minute, there were a total of 16 samples taken; eight beneath the boats and eight from the open water column 5 m up current from each boat. Each sample was an average of two observations; one observation in the field and one video observation. The overall average bite rate of *A. saxatilis* in the open water was significantly less than beneath boats ($t=5.45$, $df=1$, $p<0.001$; Fig. 3).

Bite rate and zooplankton abundance

The bite rate of *A. saxatilis* in open water (n=8) was compared with the abundance of zooplankton and no significant correlation was found ($R^2=9 \times 10^{-5}$, $df=7$, $p>0.05$; Fig. 4a). The bite rate of *A. saxatilis* beneath boats (n=8) was also compared to the zooplankton abundance and there was a significant correlation found

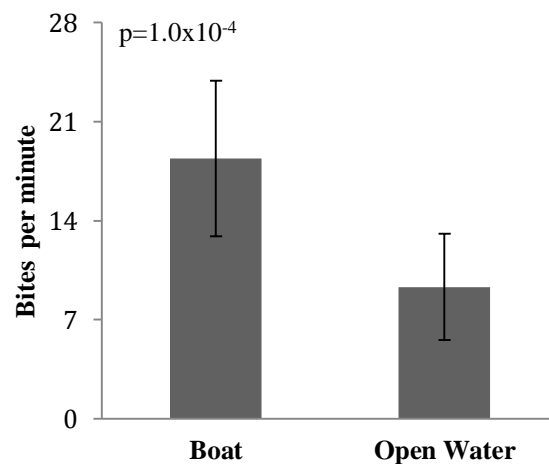


Fig. 3 Comparison of the mean bites per minute of *A. saxatilis* between two locations, beneath boats and in the open water column above the reef crest (n=8 per treatment). The error bars denote the standard deviation from the mean bite rate

between a higher number of bites and a higher abundance of zooplankton ($R^2=0.7565$, $df=7$, $p<0.05$; Fig. 4b).

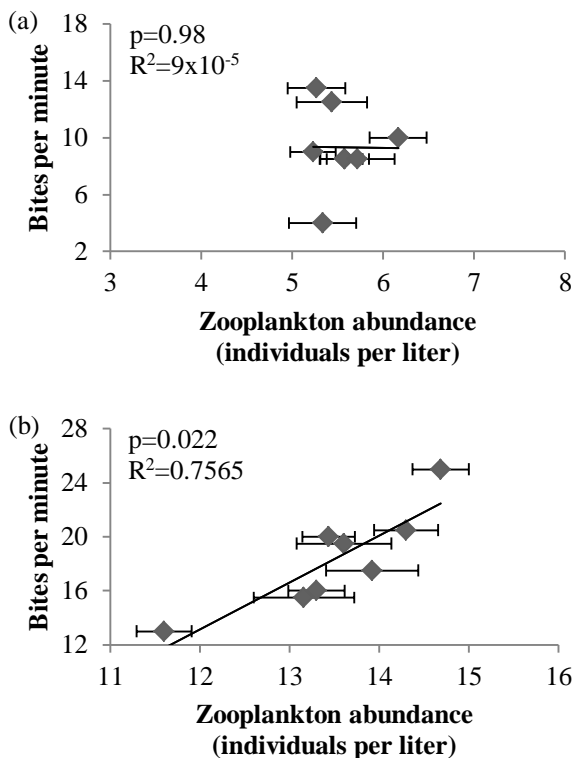


Fig. 4 Relationship between bites per minute of *A. saxatilis* and mean zooplankton abundance, shown in individuals per liter (a) shows this relationship in the open water column above the reef crest, (b) shows this relationship beneath boats. The scale for both for both bite rate and zooplankton abundance is different for (a) and (b) due to great differences in value in the data sets. The error bars denote the standard error from the mean zooplankton abundance beneath each boat

Discussion

The results of this study showed all of the following relationships were significant: (1) the abundance of zooplankton was higher in the shaded areas beneath boats than in the open water 5 m up current (2) as the abundance of zooplankton increased under boats, the number of individual *A. saxatilis* increased exponentially (3) the bite rate of *A. saxatilis* was higher in the shaded areas beneath boats than in the open water (4) as the zooplankton abundance beneath boats increased, so did the bite rate of *A. saxatilis*. The only relationship that did not show any significant trend was that of zooplankton abundance compared to the bite rate of *A. saxatilis* in the open water, which

was expected.

All of the results gained through this study supported the hypotheses that *A. saxatilis* aggregate in areas of high zooplankton abundance and during the day, a higher abundance of zooplankton is found in shaded areas, such as beneath boats. All of the previously stated relationships provide support for these hypotheses, including the relationship between the zooplankton abundance and bite rate of *A. saxatilis* in the open water. This is the case since this shows that the individuals in the open water have various levels of feeding not consistent with the abundance of zooplankton, but when the individuals are found beneath boats, they are there primarily for feeding purposes, and, therefore, have a bite rate proportional to the amount of zooplankton present.

The higher abundance of zooplankton in the low light conditions beneath boats is consistent with the findings of Zengling et al. (2013), that zooplankton exhibit a horizontal migration to shaded areas in conditions of high light intensity.

The results of this study show how large numbers of boats moored above a reef can change the ecosystem structure. The high abundance of zooplankton present beneath the boats provides a large food source for planktivorous fishes during the day. This large abundance of food provides the means for a population increase for species of planktivorous fish. This also means that species, such as *A. saxatilis*, that have more than one food source, would be eating less of their other food source since their primary source of food, zooplankton, is in high abundance. It could be expected that, since *A. saxatilis* is not feeding on algae as much as in a system without high zooplankton abundance, the algae abundance on the reef would also likely see an increase when compared to an area with fewer boats. This means that the herbivores that would normally be in competition with *A. saxatilis* for algae would have less competition and there would also likely be a population increase within these species. Since there would likely be an increase

in the population size among the lower trophic levels, of the reef ecosystem it can also be inferred that an increase could also be seen in the populations of piscivores. This would lead to an increase in the overall biomass of fish in the ecosystem.

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REPORT

Kayley You Mak • Barnard College • kyoumak@gmail.com

Aggressive nest guarding behavior of sergeant major damselfish (*Abudefduf saxatilis*) in association with guarding male and nest size

Abstract Among fish that lay their eggs on the ground (demersal) and have external fertilization (oviparous), typical parental care includes building a nest, then cleaning and guarding it as an investment in the fitness and survival of their offspring. Male Caribbean sergeant major damselfish, *Abudefduf saxatilis*, take on a darker coloration when they prepare a nest and engage in mating rituals, then guard and aerate their nest. Aggression of the guarding *A. saxatilis* could be linked to a number of factors. Nest size, size of the guarding fish is, or age of the nest may all affect nest guarding territoriality. Based on observational data collected in Bonaire in the Dutch Caribbean, there was no association between aggression (attack rate or average response distance) the guarding fish size (fork length), nest area, number of eggs, or age of the nest. Aggression was found to correlate with time of day, which may have been due to higher abundance of egg predators later in the day. Nest guarding aggression and parental care are complex behaviors that are largely species and context specific, making it difficult to make true predictions about aggressive behavior.

Keywords Territoriality • Nest guarding aggression • Sergeant major damselfish

Introduction

Parental care is an investment in the fitness of an organism (Gilbert 2013). Providing parental care is energetically costly; a cost which organisms are willing to pay for the long-term

benefit of increased offspring survival (Steinhart et al. 2008). Each parent acts in a way to maximize the number of young produced in its entire lifetime. Fishes typically have uniparental care by males, which greatly enhances offspring survival, with biparental care having little further impact on offspring survival (Krebs 1977). Fish adjust their investment in parental care based on the number of offspring in their nest, past investment, and alternative mating opportunities since these variables affect the value of current offspring relative to future offspring (Gross 2005).

For fish that lay their eggs on the ground (demersal) and have external fertilization (oviparous), typical parental care consists of nest building, cleaning, aerating, and guarding. In most demersal species, the male builds the nest and attracts the female with some form of signaling or courtship (Baxter 2001). Demersal oviparous fishes exhibit a wide range of parental care, from simply depositing the eggs on a suitable substrate to more extensive protection of the egg (Hempel 1979; Gross and Shine 1981).

More extensive parental care involving egg guarding is associated with territoriality, a behavioral mechanism used to establish and regulate social contact (Kaya and Burgess 2007). The costs and benefits of territory defense change with incremental alteration in territory size (Thresher 1976). A smaller territory or nest may be easier to patrol because the guardian remains closer to the eggs, thus increasing the odds of being near enough to protect the eggs from predators (Souza and Ilarri 2014). However, a larger territory or nest

could confer an advantage for attracting mates, as seen in Western Meadowlarks (Aweida 1995), therefore increasing the fitness of the male and making individual losses less important overall.

In order to maximize reproductive output, organisms need to attract mates. Female fish have sometimes shown a preference for laying their eggs with larger males and/or in nests with eggs already present, resulting in more eggs in a nest (Sargent 1989; Forsgren et al. 1996). Females may prefer nests with more eggs because in some species, more individuals survive from larger clutches (Sargent 1988; Lindström 1998) and larger clutches receive more parental care (van Iersel 1953; Rohwer 1978; Coleman and Fischer 1991; Forsgren et al. 1996; Lindström 1998; Manica 2003). Females might also prefer larger males, which may be beneficial for females because in some species, there is a positive correlation between male size and egg-hatching success (Downhower and Brown 1980; Bisazza and Marconato 1988; Côté and Hunte 1989; Knapp and Warner 1991). For males, large size may be advantageous for securing space preferred by females and defending the nest, particularly against conspecifics (Downhower and Brown 1980; Bisazza and Marconato 1988; Bisazza et al. 1989). Larger males that attract more mates may also display higher aggression, as both types of behavior may be controlled by androgenic hormones (Snekser et al. 2008).

The aggression of guarding males when defending their nest may also correlate with the age of the nest or daytime versus nighttime. Both Downhower and Brown (1980) and DeMartini (1987) found that eggs deposited later (the youngest eggs) in a nest had higher mortality rates, which could be due to the preferential filial consumption of newer eggs. With higher risk for newer eggs, a guarding male may guard the nest more aggressively when the eggs are younger. The perceived aggression of a fish may also differ due to varying abundances of diurnal versus nocturnal predators (Katano et al. 2013). The surrounding fish composition changes from day to night, so

the aggression of the guarding males is likely to shift as well.

This study focused on potential explanatory factors for territorial nest guarding aggression in the Caribbean sergeant major damselfish, *Abudefduf saxatilis* (order: Perciformes, family: Pomacentridae). Sergeant major damselfish are omnivorous, abundant, widely distributed in tropical waters, and easily identifiable (Rodríguez-Fuentes et al. 2013; Soto and Rodríguez-Fuentes 2014). Predominantly planktivorous, sergeant major damselfish are commonly found foraging in the water column around shallow reefs less than 6 m deep (Foster 1987; Bessa and Sabino 2012). Typically ~15 cm, *A. saxatilis* reach sexual maturity after approximately one year or when they reach 10 cm (Robertson 1988). *Abudefduf saxatilis* are a yellowish hue above and white below, with five vertical black bars that taper in towards the belly (Emery 1978; Smith 1997).

When *A. saxatilis* males are ready to spawn, they adopt an alternative bluish body coloration (Breder and Rosen 1966; Bessa and Sabino 2012). The individual clears an area on dead coral and rock for a nest, then advertises their readiness to spawn with signal jumps, where the male undulates rapidly towards passing *A. saxatilis* females before turning and swimming slowly back to its territory (Myrberg et al. 1967; Fishelson 1970; Foster 1987). Over one to three days, typically in the morning, males externally fertilize a monolayer of adhesive eggs that females lay in their cleared territory, gathering up to 250,000 eggs (Foster 1987; Francini-Filho 2012). After spawning, the female leaves the clutch and the male assumes exclusive care of the eggs (Souza and Ilarri 2014). The eggs undergo a color shift from darker to lighter as they mature (Shaw 1955) and hatch 30-70 minutes after sunset 3.5 to 5.5 days after fertilization (Foster 1987; Robertson et al. 1993).

Guarding the nest is important to sergeant major damselfish eggs' survival; therefore, the guarding male is constantly vigilant, defending the nest as well as fanning and cleaning the eggs (Bessa and Sabino 2012). Common egg

predators that the *A. saxatilis* guard against include wrasses (Labridae such as bluehead wrasses, *Thalassoma bifasciatum*), other damselfishes (including non-parental *A. saxatilis*), butterflyfishes (Chaetodontidae), and parrotfishes (Scaridae) (Cummings 1968; Foster 1987). Guarding males appear to discriminate between harmful and harmless species, thus conserving energy by chasing more threatening passersby (Randall 1955).

The purpose of this study was to explore the relationship between the size of the egg patch, the number of eggs it contains, the age of the nest, the fork length of the guarding male, the time of day, and its aggressive behavior while guarding its eggs. The hypotheses were as follows:

- H₁: The number of eggs would increase with larger nest area
- H₂: The aggression of male guardians would correlate with nest area, increase with fish fork length, increase with the number of eggs, and decrease with nest age
- H₃: The aggression of male guardians would remain consistent throughout the day

Relatively little is understood about how size of the guarding fish, size of the territory, and aggressive behavior relate. This study aimed to further explore the factors contributing to territory size and aggression in guarding *A. saxatilis* males in order to better understand the high energetic investment in parental care for future fitness via higher offspring survival.

Materials and methods

Study location

The study was conducted at Yellow Submarine (12° 09.610' N and 68° 16.916' W) in Kralendijk, Bonaire, located in the Dutch Caribbean (Fig. 1). The site is centrally located on the leeward side of Bonaire, which has a

well-developed fringing reef (Perry et al. 2012). At Yellow Submarine, a shallow sand flat extends ~40 m from shore until the reef crest drops off at ~6 m depth. The study was centered around four sets of three concrete mooring blocks (~1 m³ each) on the sandy flat just shoreward of the reef crest and four individual mooring blocks in the middle of the sandy flat ~20 m from shore. At the mooring blocks, *Blennioidei* spp (blennies), Labridae (wrasses), Acanthuridae (surgeonfish and tangs), Pomacentridae (chromis and damselfish), and Scleractinia (hard corals) were the dominant fauna. The site was used for all behavioral observations to determine whether nest area, number of eggs, nest age, or fish size had an effect on aggressive behavior.

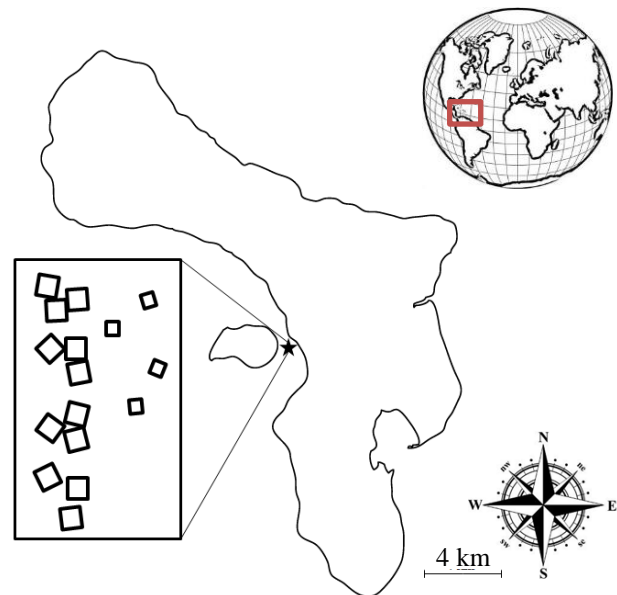


Fig. 1 Map of Bonaire with the study site, Yellow Sub and the layout of the mooring blocks (represented by squares) used for this study. The four sets of concrete mooring blocks furthest west sit along the reef crest ~5.5 m deep and the four mooring blocks more east sit in the more shallow sand flat ~3 m deep

Sampling method

Data collection via SCUBA diving occurred twice a week during daylight hours (09:00-16:00 hrs) for a five week data collection period from 7 March 2015 to 1 April 2015. For each nest, the following photos were taken with a Canon S110: (1) the guarding male from a lateral viewpoint, in line with a transect flush

against the mooring block (2) the entire nest including the transect tape as a scalar (3) close-up, macro photos of the nest with a 15 cm ruler and (4) the location of the nest on the mooring block.

Guarding fish size

The guarding male's fork length was determined using ImageJ software with the photograph of the side profile (lateral view) of the male, in line with a transect as a scalar.

Nest area

Using the photo of the entire nest with the transect tape flush against the mooring block as a scalar, an approximate nest area was found with ImageJ software. This was repeated three times on the same photo to account for user subjectivity. The three measurements were then averaged to give an approximate nest area.

Number of eggs

To calculate the number of eggs in each egg patch, close up, macro photos were taken of the nests with a 15 cm ruler as a scalar. In two close up photos for each nest, five 0.25 cm by 0.25 cm squares were drawn (n=10) in Microsoft Paint. Within each square, the number of eggs were counted and recorded. To avoid double counting the eggs, each egg was marked as it was counted. If any part of an egg fell within the square boundary, it was counted. Density of the ten 0.25 cm by 0.25 cm squares (0.0625 cm²) were then averaged, then multiplied by 16 to give the average density per square centimeter. The average density was multiplied by the average nest area from ImageJ to give an approximate egg count for that nest. The formula was:

$$N = \frac{d1 + d2 + d3 + d4 + d5 + d6 + d7 + d8 + d9 + d10}{10} \times 16 \times A$$

where N is the approximate number of eggs in a nest, d_n is the density in per 0.0625 cm² that was counted, which was multiplied by 16 to make it a density per 1 cm², and A is the

approximate nest area in cm² from ImageJ measurements.

Nest age survey

Regular 15-20 minute snorkels were made almost daily during the five week data collection period to four sets of mooring blocks along the reef crest and four individual mooring blocks in the sand flat where *A. saxatilis* nests could usually be found. Locations of nests, nest clearing behavior, number of individual *A. saxatilis* with typical coloration, and number of *A. saxatilis* individuals with dark coloration were all recorded. When nests were observed, the approximate age of the eggs in days was determined by looking back at the maps to see how many days previously the nest had first been recorded and the egg color observation. When *A. saxatilis* eggs are laid, they are bright orange or purple, then darken slightly in the next two days, and finally lose color and become grey-green. On the day the fry hatch, the egg membrane is transparent (Shaw 1955).

Aggression

Each guarding male (n=20) was observed for a 20 minute window for aggressive behavior. Aggression in this study was defined as any abnormal behavior, ostensibly because of a perceived threat, besides the regular aerating patterns. These aggressive behaviors included chasing and biting. When chasing an intruder, a guarding male would swim quickly after an intruder until the intruder retreated from its territory. Biting an intruder required contact by mouth with the perceived threat. A raster (five PVC poles marked every 10 cm, arranged like the spokes of a wheel) was laid down near the edge of the mooring block and a transect tape was hung vertically from the top to bottom of the mooring block to aid in distance estimation. A three minute acclimation period was used for the fish to become used to the object and observers in their environment. When an aggressive behavior was observed, two observers recorded whether it was a chasing or

Table 1 A compilation of linear regression results from possible explanatory variables on aggression. Also included are nest area versus number of eggs, fish size versus nest area, and fish size versus number of eggs

Explanatory Variable	Response Variable	F-Value	p-Value	R ² Value
Nest area	Number of eggs	25.57	0.000**	56.1%
Fish size	Nest area	1.10	0.308	5.2%
Fish size	Number of eggs	0.22	0.642	1.1%
Number of eggs	Attack rate	1.29	0.270	6.04%
Nest area	Attack rate	0.26	0.617	1.28%
Fish size	Attack rate	0.01	0.944	0.03%
Nest age	Attack rate	2.07	0.166	9.37%
Time of day	Attack rate	6.48	0.019*	24.5%
Number of eggs	Response distance	0.31	0.586	1.51%
Nest area	Response distance	0.48	0.497	2.34%
Fish size	Response distance	0.00	0.993	0.00%
Nest age	Response distance	0.00	0.998	0.00%
Time of day	Response distance	0.02	0.881	0.1%

* statistically significant at $p < 0.05$; ** statistically significant at $p < 0.005$; attack rate is aggressions per 20 minutes; response distance is average response distance (distance between the intruder and the egg patch at which the *Abudefduf saxatilis* first appears aggressive) in cm; nest area in cm^2 ; fish size is fork length in cm; approximate nest age in days

biting aggression, the species of the aggressor, and estimated the response and chase distances. The response distance was the distance between the intruder and the egg patch at which the *A. saxatilis* first appeared aggressive. The chase distance was the furthest distance to which the sergeant major would go from the center of the nest.

Data analysis

The data was analyzed using linear regressions in Minitab software to determine if relationships were significant. The independent variables were the fork length of the *A. saxatilis* (cm), the approximate nest area (cm^2), the approximate number of eggs in a nest, the age of the nest (d), and the time of day (hrs). The dependent variables were the attack rate (aggressions tallied/hr) and average response distance (cm). Linear regressions were also used to analyze associations between (1) nest area and number of eggs, (2) fish size and number of eggs, and (3) fish size and nest area. For this study, the assumption was made that an equal number of potential threats was surrounding each nest, such that the aggression of each fish was based on the variables used rather than the surrounding fauna. To be as consistent as possible with nest availability

during scheduled data collection dives, all but one nest observed were on the vertical faces of mooring blocks. If SCUBA divers approached the guarding males, they often swam away, and consequently predatory fish swarmed over the eggs, aggregating with increasing numbers the longer the guardian male was kept away (Cummings 1968; Cheney 2008). Observations were made ~2 m away from the mooring block in order to minimize interaction with the fish and researchers' effect was considered negligible for this study.

Results

The number of eggs in a nest was positively associated with the nest area ($R^2=0.561$, $p=0.000$; Fig. 2a, Table 1). There was no association between the guarding male's fork length and either number of eggs in the nest ($R^2=0.052$, $p=0.308$; Fig. 2b) or nest area ($R^2=0.011$, $p=0.642$; Fig. 2c, Table 1). No correlations were found between the attack rate and the number of eggs ($F=1.29$, $p=0.270$; Fig. 3a), nest area ($F=0.26$, $p=0.617$; Fig. 3b), size of fish ($F=0.01$, $p=0.944$; Fig. 3c), or nest age ($F=2.07$, $p=0.166$; Fig. 3d, Table 1). There was a positive association between attack rate and the time of day ($R^2=0.245$, $p=0.019$; Fig. 3e,

Table 1). There were no correlations found between average response distance and the number of eggs ($F=0.31$, $p=0.586$), nest area ($F=0.48$, $p=0.497$), size of fish ($F=0.00$, $p=0.993$), nest age ($F=0.00$, $p=0.998$), or time of day ($F=0.02$, $p=0.881$; Table 1).

Discussion

The hypothesized positive correlation between the nest area and number of eggs was supported. The other hypotheses were made based on findings in other fish species where mate choice and aggression were linked to nest area (Sikkel 1988), guarding male size (Knapp and Sargent 1989), number of eggs (Sargent 1988), and age of nest (DeMartini 1987). However, these hypotheses were not supported in this *A. saxatilis* study.

It was hypothesized that the aggression of male guardians would be related to nest area; however, no association between the two was found. These findings were consistent with Knapp and Warner (1991), who studied another pomacentrid, *Stegastes partitus*, whose males also provide exclusive parental care of eggs. They reported that nest area was not a relevant factor for behavioral differences. Nest area may not have had a clear effect on aggression across the *A. saxatilis* population as some fish may have favored smaller nests, concentrating their care on fewer eggs, thus lowering the cost of parental care, while other males may have preferred larger nests to maximize their reproductive output. Thus, the determinants for *A. saxatilis* aggression were not directly linked to nest area, but may have been more closely associated with the males' maximum potential reproductive output, which would determine the amount of aggression he would invest in parental care.

Another hypothesis was that the aggression of male guardians would increase with fish fork length, but no correlation was found. Jan et al. (2003) studied the Indo-Pacific dusky farmerfish, *Stegastes nigricans*, and also found that the maximum distance of attack, a metric of aggression, was not correlated to the owner's fork length. Additionally, in many other damselfish species—bicolor damselfish (*Stegastes partitus*), beaugregory damselfish (*S. leucostictus*), and garibaldi damselfish (*Hypsypops rubicundus*)—females have been reported not to mate preferentially with larger males (Itzkowitz and Makie 1986; Bisazza et al. 1989; Knapp and Warner 1991) because

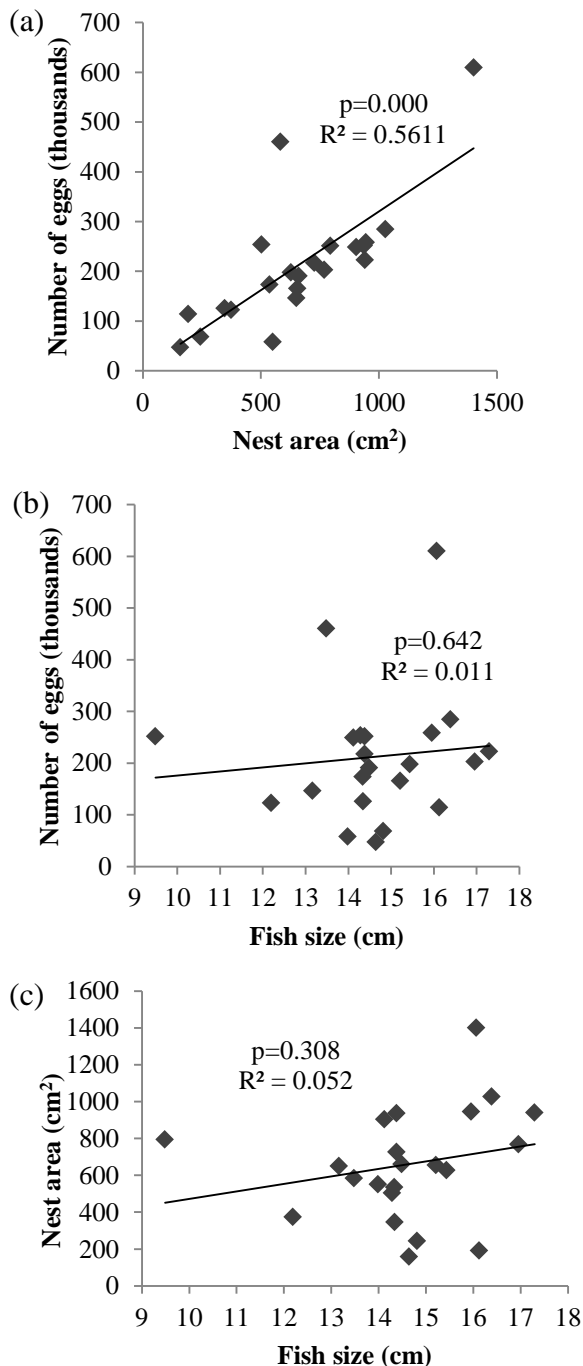


Fig. 2 Linear relationship between the number of eggs in a nest and (a) nest area in cm² and (b) the guarding male *Abudefduf saxatilis*'s fork length in cm. (c) The linear relationship between guarding male's fork length and the nest area in cm². The p-values listed are from linear regressions

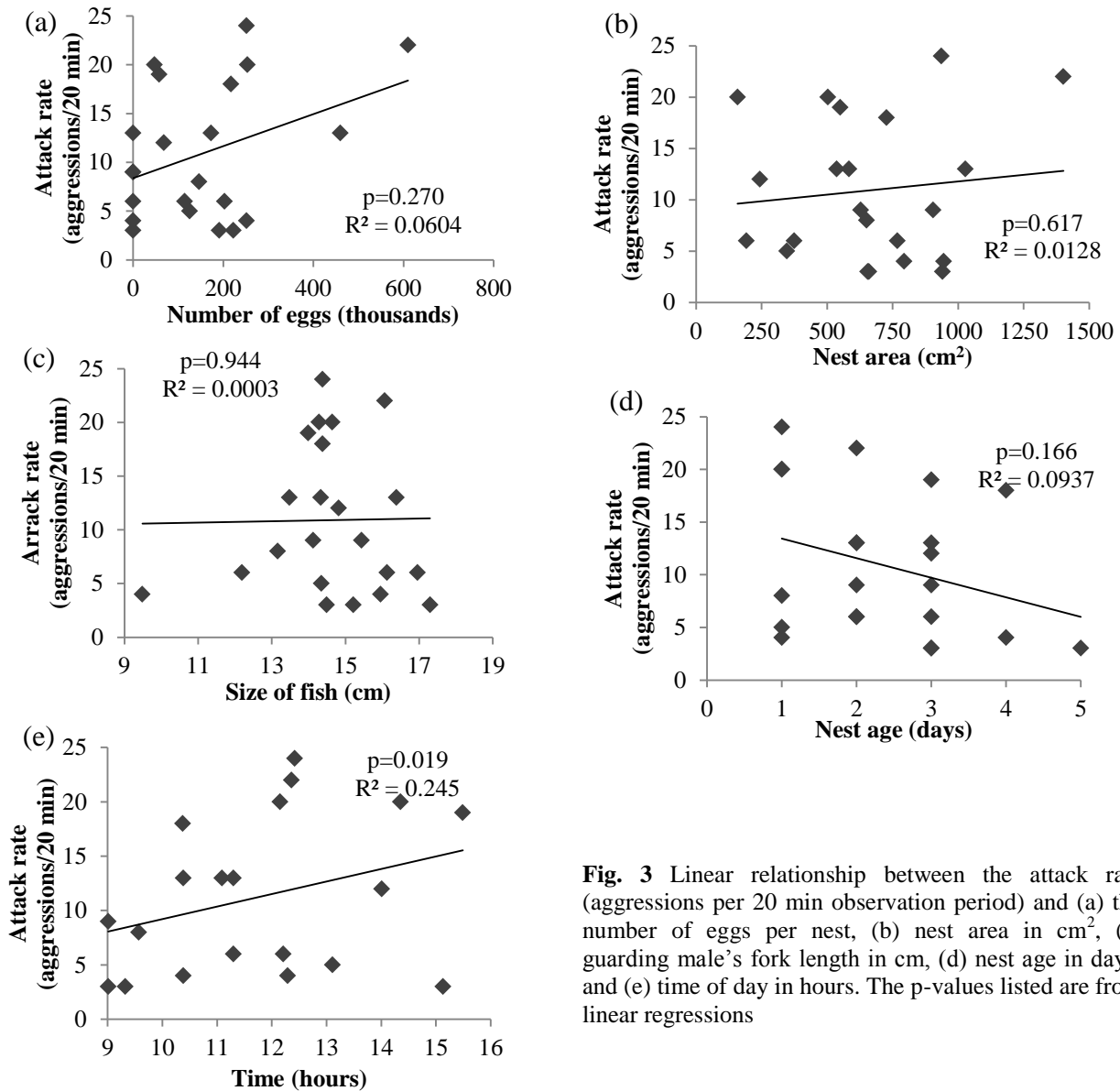


Fig. 3 Linear relationship between the attack rate (aggressions per 20 min observation period) and (a) the number of eggs per nest, (b) nest area in cm², (c) guarding male's fork length in cm, (d) nest age in days, and (e) time of day in hours. The p-values listed are from linear regressions

larger males were not correlated with an increasing percentage of egg-hatching success (Sikkel 1988). Thus, if larger males are not attracting more mates, the indicator of male parental care and nest guarding ability is not likely to be fish size, but another characteristic. Furthermore, the aggression of male guardians was expected to increase with the number of eggs, but was not observed. If females did not benefit from laying their eggs in nests with more eggs, males likely did not alter their behavior based on the number of eggs they were guarding. In another pomacentrid species, the number of eggs did not strongly influence the egg survival and hatching rate either (Knapp and Warner 1991). If the number of

eggs was not a driving factor for reproductive success, it would also likely not affect guarding male aggression.

It was also hypothesized that the aggression of male guardians would decrease with nest age, but no relationship was found. In his research, Cummings (1968) also found a poor correlation between aggression of *A. saxatilis* and developmental stage of the eggs. Since younger eggs contain higher nutritional value, they are preferentially eaten in filial cannibalistic species, such as the scissortail sergeant, *Abudefduf sexfasciatus* (Manica 2003), but *A. saxatilis* have not been observed to exhibit filial cannibalism, which could explain the different behavioral pattern.

Furthermore, since the eggs are laid on consecutive days, the age difference within the nests was negligible. Also, overall predation pressure on the eggs was low, so the difference between a new nest (0 days) and a nest about to hatch (3.5-5.5 days) was likely inconsequential to egg predators. If predation intensity was constant throughout egg development, the age of the nest would be an insignificant factor in aggression levels of guarding males.

The aggression of male guardians was hypothesized to remain consistent throughout the day, however an association was found between the time of day and attack rate. Many fish are either diurnal or nocturnal. Consequently, since all data was collected during sunlight hours, little variation was expected. However, the correlation could be explained by diurnal patterns in marine populations, which have been well documented (Walsh 1988; Engås and Soldal 1992; Michalsen et al. 1996; Korsbrekke and Nakken 1999). The aggression rate, or the number of threats the guarding male chases, could be dependent on the abundance of fish in the surrounding area, which varies throughout the day, rather than characteristics of the *A. saxatilis* or nest itself.

Aggression and territoriality are complex behaviors with many possible factors that could be responsible for variation between individuals and species. Other common variables studied are the surrounding environment, the males' performance in a courtship ritual, and intrinsic differences between individuals. The surrounding environment, such as the location on the single side of the mooring block or presence of other nests on the same side, could be a contributing factor to aggression variation between individual *A. saxatilis*. Kaya and Burgess (2007) studied people in classroom settings and found that spatially central individuals tend to be more aggressive than spatially peripheral individuals. Moreover, behavior can be density dependent, as seen in ayu fish, *Plecoglossus altivelis*, which become less territorial when densities increase (Tanaka et al. 2011). If other nests occurred on the same side of a mooring

block as the nest observed, the guarding *A. saxatilis* behavior could have been altered. In humans, responsibility diffusion is found in groups, where each individual has less responsibility in a group than on their own (Wallach et al. 1964). For the *A. saxatilis*, if other nests were present on the same side of the mooring block, one could have been a more active defender, leaving the other to not waste energy on defensive aggression.

Another factor that may have been more closely associated with the explanatory variables than aggression was how vigorously a male advertised himself when attracting mates with signal jumps, the undulating swimming pattern *A. saxatilis* utilize for mate attraction (Fishelson 1970). Females of some other damselfish species preferentially mate with more vigorously courting males because it advertises the male's parental ability (Gronell 1989; Knapp and Kovach 1991; Oliver and Lobel 2013). By attracting more mates with energetic courting, males may attract larger nests and more eggs, which this study hypothesized would correlate with higher aggression rates. However, a disconnect between courtship vigor and territorial defense vigor might exist if the courtship is not honest, that is, there are "cheater" males who falsely over-advertise their parental abilities (Knapp and Kovach 1991). Aggression was thought to be related to nest size and number of eggs because a more aggressive male is likely beneficial for a female's fitness as it would increase the survival rate of her offspring. However, if male aggression was evaluated by the rigor of the courtship ritual and the courtship does not correlate with parental ability, then no effect of nest size or number of eggs would be seen on aggression.

Individual fish may also have a specific temperament that they behave within; an individual may only be capable of a certain subset of behaviors. In this case, territoriality might vary with an individual's behavioral type, analogous to a person's personality (Kaya and Burgess 2007). If the behavioral plasticity among *A. saxatilis* is constrained by the limits of an individual's specific temperament, affects

of aggression may be masked by a “behavioral syndrome,” where the behaviors of individuals fluctuate consistently across various functional contexts (Snekser et al. 2008). Ideally, individuals change their behavior in accordance with external circumstances and environment; however, a behavioral syndrome would prevent individuals from adaptively altering their behavior, thus overriding external factors’ effects on nest guarding aggression.

Studying aggression and determining what traits contribute to an organism’s fitness is notoriously difficult *in situ* (Blais et al. 2004). Investigators’ expectations of dependence on secondary sexual traits and physical characteristics may lead to over-reporting correlations that may not actually have strong influences on behavior (Hall et al. 2000). Another complication when studying aggression and behavior is that the factors may be interacting. Blais et al. (2004) found that an individual male’s total attractiveness was not simply the sum of its traits, but more likely a context-dependent balance of the traits. For this study, the factors considered might not have correlated with aggression and territoriality or the factors might not have been independent of each other, instead affecting aggression in a certain combination.

Behavior, such as parental care, is quite nuanced as there is wide variation among and within species. Interspecific variation in parental care is often explained by the organism’s life history, where parenting improves offspring survival rate, but reduces parental fitness and residual reproductive value (Westneat et al. 2011). Studies across taxa have found that expected patterns, such as mate-choice based on mate condition when an individual would benefit from mating with a high-quality mate, are often not followed (Sundin et al. 2013). Parental care, although complex, is ultimately a balance between the highest reproductive output possible with the lowest cost to lifetime fitness.

In future studies, consistent sampling time and multiple observations of the same individual could be used to eliminate variables, data on surrounding fish could take into

account differences in aggression based on how many perceived threats were near, and the surrounding environment could be included to account for behavioral changes based on proximity to edges of the mooring block and dependence on other guarding *A. saxatilis* nearby. This study focused on nest guarding aggression in *A. saxatilis* as a way of studying investment in parental care. Nest guarding behavior is costly, so determining factors that lead to more aggressive defense of the territory could be used to further understand how individuals maximize their reproductive success.

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REPORT

Jillian R. Neault • University of Washington • jilliann@uw.edu

Determining how surroundings impact abundance and behavior of the yellow Atlantic trumpetfish *Aulostomus maculatus* phenotype

Abstract The use of color is seen throughout the animal kingdom. In coral reef ecosystems, organism colorations are suggested to assist in behaviors such as camouflaging and communication among schooling fish. The long and slender Atlantic trumpetfish, *Aulostomus maculatus*, is suggested to take advantage of both bright and neutral color schemes on the Caribbean reefs. This species has three known colorations, or phenotypes, that all exist on the reef of the island Bonaire, Dutch Caribbean: the yellow, blueheaded, and mottled phenotypes. The mottled camouflages easily into their surroundings, the blueheaded can change the color of its head in different surroundings, and the yellow is more brightly colored than most of the substrate on the reef. This study hypothesized that there would be a strong association between bright reef fish and the hunting behaviors of the bright yellow phenotype. To observe how coloration and surroundings may influence the abundance of the yellow *A. maculatus* in particular, a total of 1600 m² of abundance surveys for two phenotypes of trumpetfish and associating fish were conducted. Behavioral observations of six fish of each phenotype were conducted to examine links between coloration, surroundings, and behavior. While no links were found between coloration, surroundings and abundance, several significant links were found between coloration, surroundings, and behavior. There are a variety of factors that can affect the behavior of coral reef fishes; the data this study has collected suggests that the relationship between coloration of species and surroundings is one of these factors for the Atlantic trumpetfish.

Keywords Caribbean • Coloration • Behavioral ecology

Introduction

Throughout the animal kingdom, the use of color by organisms to increase survival rates is evident. By using colors that match an organism's environment, camouflage is used as a means to avoid predation and increase prey capture rates (Watson et al. 2014). Bright colors have suggested purposes; these include sexual selection to intra and interspecific communication to predator distraction (Peterman 1971; Flood 1989). In coral reef ecosystems in particular, bright colors are suggested to make subtle movements more dynamic, make schooling behavior more effective, and allow for minute color changes in a school to be noticeable to the schooling fish, allowing for early detection of predators (Peterman 1971; Kaufman 1976). In this same coral reef habitat, peacock flounders, *Bothus lunatus*, are known to avoid bright colors and camouflage with the neutral colored portions of the substrate using chromatophores in their skin as a means of lowering detection and recognition by predators (Tyrie et al. 2015).

A common coral reef fish that is suggested to take advantage of both bright and neutral color schemes is the trumpetfish (Kaufman 1976). The Atlantic trumpetfish *A. maculatus* is a narrow, tube snouted fish that averages 60 cm and is found in the coral reefs of the Western Atlantic at depths up to 25 m (Kaufman 1976; Bowen et al. 2001; Bickerstaff 2014). They come in three colorations: a bright to faded

yellow covering the whole body; vibrant to dull blue head and silvery body; and brown to orange to red with white markings; the yellow trumpetfish (YTF), blue headed trumpetfish (BHTF), and mottled trumpetfish (MTF), respectively. It has been suggested that these three colorations are genetically inherited and are fixed, meaning these trumpetfish cannot switch between colorations due to a lack of the necessary pigments and skin compounds that allow for certain colors to occur underwater (Lochmann 1989). Because they are genetically fixed, these different colorations can be considered different phenotypes. The BHTF phenotype is thought to be capable of changing the shade of blue of their heads (Kaufman 1976), possibly to help combat the potentially difficult time it has blending in to its surroundings. It might also help match the color of the blue fish they are hunting amongst, making it possible to hide within schools of fish such as blue chromis *Chromis cyanea* (Kaufman 1976). Considering many small reef fishes depend on communication within their large schools for a higher chance of survival (Peterman 1971), a predation method that takes advantage of these larger schools by blending in with their color scheme and disrupting this communication could potentially be highly effective. Bright colors are used by reef fishes to determine disturbances in their school; a break in the bright colors often indicates a predator in their midst. If these phenotypes of trumpetfish can remain undetected within schools of brightly colored fish, this camouflaging technique has the potential to assist trumpetfish with blending into their environment, regardless of the reef substrate below them. Considering that blue and yellow are the most frequently used colors in reef fishes (Marshall 2000a; 2000b) for these communicative purposes (Peterman 1971), it follows that the YTF and the BHTF would use this to their advantage.

The YTF phenotype, however, does not have the ability to change color; they are permanently a bright yellow color (Lochmann 1989). Experimental collections from South Florida (Lochmann 1989) and my own

personal observations from Bonaire suggest that the MTF phenotype is much more common than the YTF phenotype. There is a lack of research on why this actually is. It is possible that the limited stationary yellow color in the reef and therefore permanent places to hide for the yellow phenotype leave it at some sort of disadvantage. The presence, intensity, and effects of this disadvantage could be quantified in various ways if different phenotypes are found to behave or concentrate differently than others when comparing surroundings. For instance, we may find that a trumpetfish surrounded by objects that match its color may remain stationary while that same trumpetfish may remain mobile while surrounded by objects that make it stand out.

This study examined what components of trumpetfish behavior are influenced by their surroundings, phenotype, or perhaps a combination of both variables. Specifically, potential predictors were investigated for the abundance and behaviors of all three trumpetfish. Considering that trumpetfish are commonly observed hiding near or within corals and sponges, it could be the abundance of similarly colored coral cover that determines the abundance of different trumpetfish phenotypes in an area. The large number of personally observed yellow fish on Bonaire without a corresponding high number of YTF also supports this idea. On the other hand, personal observations on Bonaire have shown YTF to be found exhibiting the hunting behaviors outlined in Helfman (1989) among yellow fish more often than the other two phenotypes. This indicates that the abundance of yellow reef fishes that make up the trumpetfish prey (Kaufman 1976) could be what correlates more closely to the abundance of the YTF phenotype in an area. Previous studies have described BHTF following schools of bright blue fish (Kaufman 1976) while there is no data on that particular phenotype camouflaging into any substrate. Personal observations have shown MTF vertically camouflaging in rope sponges more often than other behaviors. Because of these

personal observations and previous studies, this study hypothesized:

- H₁: Each phenotype would spend a significant amount of time on different behaviors
- H_{1.1}: YTF would spend significantly less time camouflaging than hunting
- H_{1.2}: BHTF would spend significantly more time moving than camouflaging
- H_{1.3}: MTF would spend significantly more time vertically camouflaging than hunting
- H₂: There would be significant associations that exist between the behaviors of each trumpetfish phenotype and what their surroundings consist of
- H_{2.1}: There would be a significant difference between how often the MTF camouflage near coral and not near coral
- H_{2.2}: There would be a significant difference between how often the YTF and BHTF hunt near fish and not near fish
- H_{2.3}: There would be a significant difference between how often BHTF move with and without coral nearby

During personal observations, YTF appeared to spend more time hunting than camouflaging into the existing yellow cover, so it was proposed that:

- H₃: Out of all three phenotypes, the strongest relationship would exist between the abundance and behavior of yellow trumpetfish and the abundance of yellow reef fishes nearby.

Learning more about the relationships between the YTF habitat and their abundance and behavior begins to show the requirements that a habitat must meet in order for YTF to be expected in certain abundance. This study worked toward quantifying the effect that the color of surroundings has on the abundance

and behavior of a species understudied previously; preliminary research suggested that this is the first study to focus so intently on one particular phenotype of trumpetfish and its environment. Furthermore, this study provided new information on the animal behavior facets of ecology. Establishing links between YTF habitat components and behavior has showed how color of surroundings within an ecosystem can influence what a trumpetfish is doing depending on its phenotype. In efforts to suggest the presence or lack of relationship between YTF abundance and behavior and yellow fish abundance, this study has added information to the field of predator-prey interactions, which are a crucial structuring process in all communities, not simply coral reef communities (Loreau 2009). The links this study has suggested in trumpetfish could lead to similar studies in species more crucial to the health of the ecosystems of Bonaire and the entire Caribbean.

Materials and methods

Study site

Located on the west coast of the island of Bonaire just north of the major city Kralendijk, Yellow Sub (12°09'36.42" N, 68°16'54.84" W; Fig. 1) is a popular shore entry dive site where all three colorations of *A. maculatus* are present. Small fish such as blue chromis *C. cyanea* (Kaufman 1976) that comprise the trumpetfish diet are abundant on this reef, which begins at a depth of approximately seven to eight meters. Mooring blocks with an abundance of yellow brain corals and mooring ropes are also present in the back reef. Just south of Yellow Sub are multiple clusters of rope sponges where many vertically hovering mottled trumpetfish can consistently be seen. At 12 m, the percent cover of consistently yellow corals (*Meandrina meandrites*, *Porites astreoides*, *Eusmilia fastigiata*, *Diploria strigosa*) is approximately 7% as of fall of 2014 (Mailloux 2014).

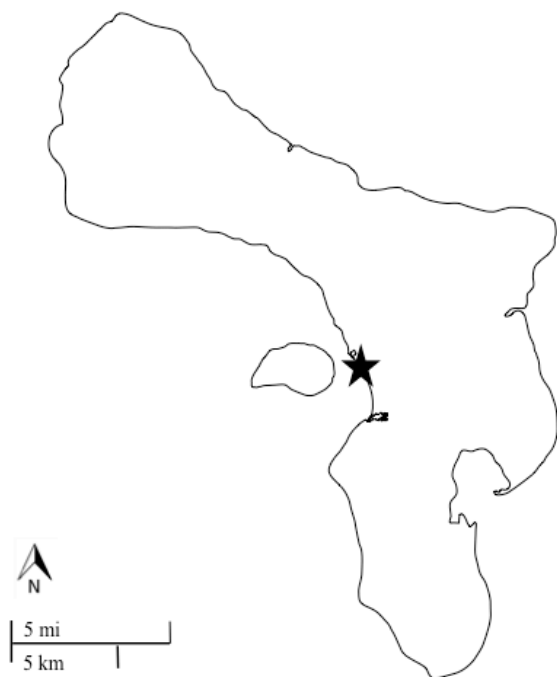


Fig. 1 Map of Bonaire, Dutch Caribbean showing the study site. Yellow Sub dive site is located on the western side of the island, shown with the black star

Behavioral observations

Start time of the dive was kept within 3-4 hours after sunrise and recorded to keep track of the timing of observations and keep them as consistent as possible. Before beginning each observation, start depth, color, and total length of the trumpetfish were recorded. As a reference, the length of the secondary surveyor (approximately 2 m) was used to gauge distances and lengths. Fin kicks were used to estimate distance traveled by each trumpetfish. For each behavior observed, the behavior, start and end time of the behavior, strike number during the behavior, number of colored fish within two meters, and percentage of colored coral within two meters were recorded. Observations lasted 10 minutes per fish unless the fish swam too deep to be followed, (approximately 20 m). Recorded behaviors and the behavioral categories used for analysis are outlined in Table 1.

Table 1 Behavioral categories for analyzing data, behaviors recorded, and descriptions of behaviors. Descriptions of behaviors are provided in the same order as listed in the behaviors column

Behavioral category	Behaviors	Descriptions
Horizontal camouflage (HC)	Horizontal camouflage (HC)	Horizontal, no movement, within 1 meter of an object of similar coloration or shape
Vertical camouflage (VC)	Vertical camouflage (VC)	Vertical, no movement, within 1 meter of an object of similar coloration of shape
Hunting	Shadow hunting; nuclear hunting; and bent tail	TF swims just above another fish to remain undetected by prey; TF displays hunting behaviors within 50 cm of another predatory fish, without swimming on top of it; suggested to happen just before a strike, where last 5-10 cm of TF bends to the side
Movement	Horizontal swimming; darting; and rapidly alternating between horizontal and vertical	Horizontal movement; very rapid swimming away from another aggressive fish; fluid movement between horizontal and vertical positions
Horizontal or vertical (H/V)	Horizontal or vertical	Horizontal, no movement; vertical, no movement

Abundance surveys

During observational dives, fish species seen to associate with each trumpetfish phenotype were recorded. Association was defined as an observed trumpetfish spending more than 60 seconds within 2 m of a species of fish at least once, regardless of the number of fish of that species observed. After all but four fish observations were completed, a list of associating fish for each trumpetfish phenotype was created. It was determined at this point that the BHTF had no species of fish that they associated with and that because of this, MTF and YTF would be the main comparison for the abundance surveys. The species that were determined to associate with the YTF were Smallmouth Grunts, *Haemulon chrysargyreum*, French Grunts, *Haemulon flavolineatum*, Yellowfin Goatfish, *Mulloidishthys vanicolensis*, Spanish hogfish, *Bodianus rufus*, Yellowtail snappers, *Ocyurus chrysurus*, and Schoolmaster snapper, *Lutjanus apodus*; for MTF, they were Blackbar soldierfish, *Myripristis jacobus*, Creolefish, *Paranthias furcifer*, and Mahogany snappers, *Lutjanus mahogoni*. Using random 10 m transects at a depth of 10 ± 3 m, abundance of all three trumpetfish phenotypes and the fish found previously to associate with YTF and MTF was recorded for approximately five meters on either side of the transect, making each transect area 100 m^2 . After laying the 10 m transect, a 60 second acclimation period was allowed for disturbance possibly caused by laying the transect, and then only stationary fish were counted during the pass. Over two dives, 16 transects were laid.

Data analysis

Average time that each phenotype spent performing each behavior in total, near associating fish, with coral and sponges, with both fish and coral/sponges, and without fish or coral/sponges was calculated and organized. Observed behaviors were divided into five different categories (Table 1). All behavior category data was organized into graphs, and

significance of the differences among different categories of data was calculated using multiple one-way analysis of variance tests (ANOVA $\alpha = 0.05$). Data compared in this way included time spent on different behaviors among phenotypes. Three one-way ANOVA tests were used to calculate whether the differences in time spent on each behavioral category were significant, one for each phenotype. No tests between individual behavioral categories were conducted, so a significant p-value suggests differences among the means but does not indicate which means are different from each other. Significance of the differences in time spent on each behavior in each possible surrounding (with fish, with coral/sponge, with both, or with neither) for each phenotype was also calculated using a series of one-way ANOVA tests. Each test calculated the significance of the differences in time spent displaying one behavior category in the four different surroundings for one phenotype. For example, one test would be used to calculate the significance of the difference among the mean times spent on movement near fish, near coral, or near both, or near neither for the blueheaded phenotype. These tests were repeated for behavior categories and phenotypes that had a sample size of more than two fish displaying that behavior category. Associated fish and trumpetfish abundances from the 100 m^2 surveys were organized and compared with a linear regression statistical analysis, one test for each phenotype.

Results

H₁: Each phenotype would spend a significant amount of time on different behaviors

For the yellow phenotype, there were statistically significant differences between percentages of time spent on different behaviors (one-way ANOVA, $df = 4$, $f = 4.77$, $p = 0.005$; Fig. 2). The behaviors that YTF spent the most time on were movement and

remaining stationary without camouflaging. No time was spent vertically camouflaging. For the blueheaded phenotype, there were statistically significant differences between percentages of time spent on different behaviors (one-way ANOVA, $df = 4$, $f = 28.62$, $p < 0.0001$; Fig. 2). The majority of time on average was spent moving. For the mottled phenotype, there were statistically significant differences between percentages of time spent on different behaviors (one-way ANOVA, $df = 4$, $f = 3.74$, $p = 0.016$; Fig. 2). The behavior that MTF spent the most time on was vertically camouflaging. No time was spent hunting. For all three phenotypes, differences between behaviors varied in magnitude.

H₂: There would be significant associations that exist between the behaviors of each trumpetfish phenotype and what their surroundings consist of

Horizontal camouflage

The difference in percentages of time spent horizontally camouflaging among different phenotypes was not statistically significant (one-way ANOVA, $df = 2$, $f = 0.57$, $p = 0.578$; Fig. 3). There were significant differences in time spent HC between surroundings for the blueheaded phenotype (one-way ANOVA, $df = 2$, $f = 5.33$, $p = 0.018$; Fig. 3) but not for the yellow (one-way ANOVA, $df = 2$, $f = 0.57$, $p = 0.578$; Fig. 3) or mottled (one-way ANOVA, $df = 2$, $f = 2.21$, $p = 0.144$; Fig. 3).

Vertical camouflage

The difference in percentages of time spent vertically camouflaging between phenotypes was statistically significant (one-way ANOVA, $df = 2$, $f = 9.53$, $p = 0.002$; Fig. 4). There were significant differences in time spent VC between surroundings for the mottled phenotype (one-way ANOVA, $df = 2$, $f = 3.87$, $p = 0.044$; Fig. 4). Due to small sample sizes, no tests were conducted for the yellow and blueheaded phenotypes.

Hunting

The difference in percentages of time spent hunting between different phenotypes was not statistically significant (one-way ANOVA, $df = 2$, $f = 1.47$, $p = 0.261$; Fig. 5).

Movement

The difference in percentages of time spent on movement between phenotypes was statistically significant (one-way ANOVA, $df = 2$, $f = 12.33$, $p = 0.001$; Fig. 6). There were significant differences in time spent on movement between surroundings for the blueheaded phenotype (one-way ANOVA, $df = 3$, $f = 18.14$, $p < 0.0001$; Fig. 6) but not for the yellow (one-way ANOVA, $df = 3$, $f = 0.70$, $p = 0.563$; Fig. 6). Due to a small sample size, no test was conducted for the mottled phenotype.

Horizontal or vertical

The difference in percentages of time spent horizontal or vertical between different phenotypes was not statistically significant (one-way ANOVA, $df = 2$, $f = 3.03$, $p = 0.078$; Fig. 7). There were significant differences in time spent horizontal or vertical between surroundings for the yellow (one-way ANOVA, $df = 3$, $f = 4.98$, $p = 0.010$; Fig. 7) and blueheaded (one-way ANOVA, $df = 3$, $f = 3.78$, $p = 0.027$; Fig. 7) phenotypes. Due to a small sample size, no test was conducted for the mottled phenotype.

H₃: Out of all three phenotypes, the strongest relationship would exist between the abundance and behavior of yellow trumpetfish and the abundance of yellow reef fishes nearby

Abundance surveys

Because no fish were found to associate with BHTF during observational dives, only MTF and YTF and their associating fish were counted in the sixteen 100 m² survey areas.

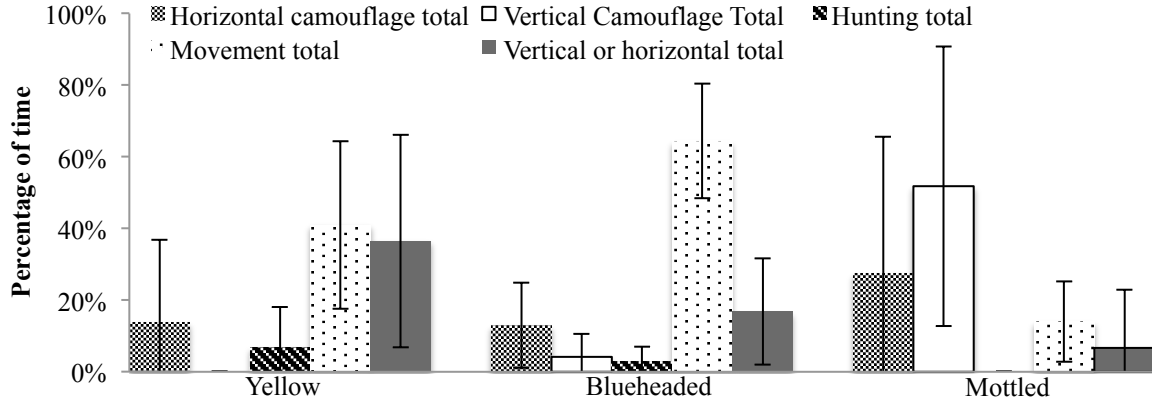


Fig. 2 Average percentage of 10 minutes observation spent in each of the five behavior categories for each phenotype (n=6). Error bars represent standard deviation. Note the scale, which ranges from 0-100%

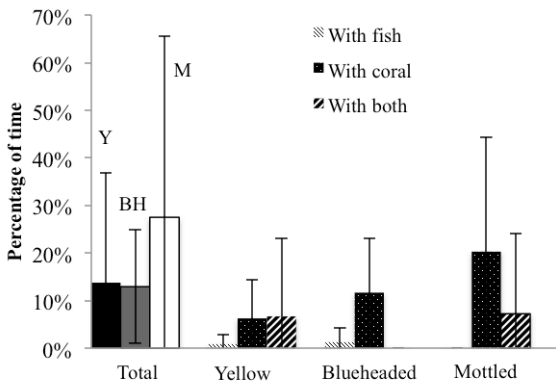


Fig. 3 Average percentage of 10-minute observation that each phenotype (yellow, Y; blueheaded, BH; and mottled, M; n=6) spent horizontally camouflaging in total and in different possible surroundings (with fish; with coral; with both; with neither). Error bars represent standard deviation. Note the scale, which ranges from 0-70%

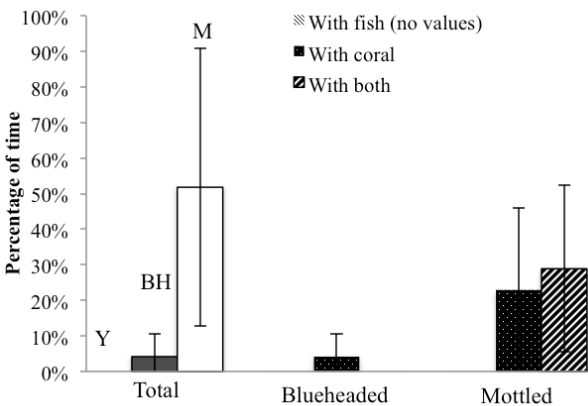


Fig. 4 Average percentage of 10-minute observation that each phenotype (yellow, Y; blueheaded, BH; and mottled, M; n=6) spent vertically camouflaging in total and in different possible surroundings (with fish; with coral; with both; with neither). Yellow was not included in the surroundings portion due to no observations of this behavior. Error bars represent standard deviation. Note the scale, which ranges from 0-100%

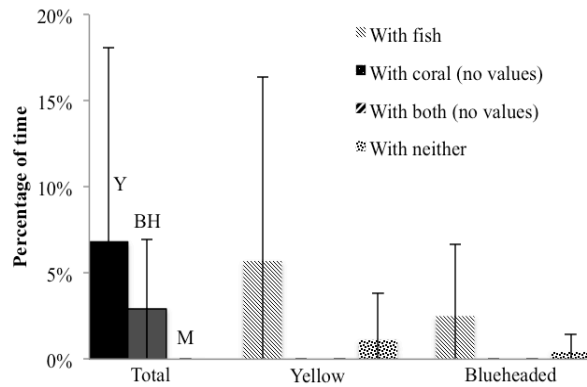


Fig. 5 Average percentage of 10-minute observation that each phenotype (yellow, Y; blueheaded, BH; and mottled, M; n=6) spent hunting in total and in different possible surroundings (with fish; with coral; with both; with neither). Mottled was not included in the surroundings portion due to no observations of this behavior. Note the scale, which ranges from 0-20%

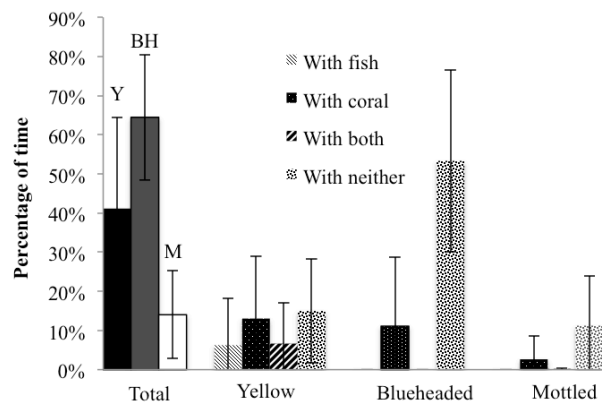


Fig. 6 Average percentage of 10-minute observation that each phenotype (yellow, Y; blueheaded, BH; and mottled, M; n=6) spent on movement in total and in different possible surroundings (with fish; with coral; with both; with neither). Error bars represent standard deviation. Note the scale, which ranges from 0-100%

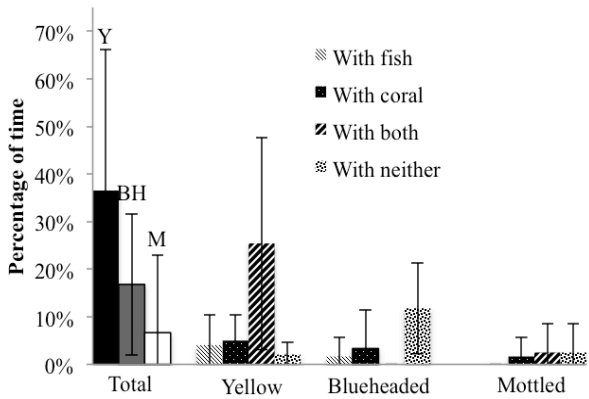


Fig. 7 Average percentage of 10-minute observation that each phenotype (yellow, Y; blueheaded, BH; and mottled, M; n=6) spent vertical or horizontal in total and in different possible surroundings (with fish; with coral; with both; with neither). Error bars represent standard deviation. Note the scale, which ranges from 0-75%

Table 2 Sixteen 100 m squared areas were surveyed for mottled trumpetfish (MTF), yellow trumpetfish (YTF), and the fish observed to associate with them. Blueheaded trumpetfish were not recorded because no fish were observed associating with them during observational dives

Transect	MTF		YTF	
	MTF	MTF assoc. total	YTF	YTF assoc. total
1	0	2	1	71
2	1	20	0	45
3	5	6	0	6
4	2	8	0	65
5	1	30	0	17
6	1	11	0	5
7	2	44	0	20
8	4	10	1	12
9	1	9	0	35
10	1	9	0	15
11	2	11	1	43
12	1	6	0	17
13	1	7	0	0
14	2	20	1	22
15	1	8	0	3
16	0	0	0	7

Linear regressions between the MTF and its associating fish ($df = 1$, $f = 0.14$, $p = 0.713$; Table 2) and the YTF and its associating fish ($df = 1$, $f = 2.04$, $p = 0.175$; Table 2) showed no significant relationship between the abundances surveyed.

Discussion

H₁: Each phenotype would spend a significant amount of time on different behaviors

While there were statistically significant differences in the amount of time spent on different behaviors for the yellow phenotype, there was no difference in the time spent camouflaging and the time spent hunting. While no statistical tests were performed to determine this for sure, the entire span of the hunting data values are encompassed by the camouflage data values, so this study assumed that there are no differences between them. In addition to statistically significant differences in the amount of time spent on different behaviors for the blueheaded phenotype, the behavior observed for a majority of each observation on average was movement. While no statistical tests were performed to determine this for sure, the visual representation of the data supports the hypothesis that BHTF spend more time moving than camouflaging. In addition to statistically significant differences in the amount of time spent on different behaviors for the mottled phenotype, no hunting behaviors at all were observed for the mottled phenotype while the highest percentage of time on average was spent vertically camouflaging. Because every observed mottled trumpetfish spent at least some time vertically camouflaging and no time hunting, the data supports this hypothesis in that MTF spend more time vertically camouflaging than hunting.

H₂: There would be significant associations that exist between the behaviors of each trumpetfish phenotype and what their surroundings consist of

For the yellow phenotype, the behavior category suggested to be influenced by surroundings was time spent horizontal or vertical. For the blueheaded phenotype, behavior categories suggested to be influenced by surroundings include horizontal

camouflaging, movement, and being horizontal or vertical. For the mottled phenotype, the behavior category suggested to be influenced by their surroundings was vertical camouflage.

While no significant differences in time MTF spent horizontally camouflaging between surroundings were found, significant differences in time spent vertically camouflaging between surroundings were found. Because all time spent vertically camouflaging was near coral, my hypothesis regarding MTF camouflaging near coral is supported for vertically camouflaging, but not horizontal camouflaging. No statistical differences were found in the percentage of time spent hunting between different phenotypes or different surroundings, so my hypothesis regarding hunting was not supported by the data. There were significant differences in time spent on movement between surroundings for the blueheaded phenotype, but because no statistical analysis was done to determine if there was a statistical difference between the means for movement with coral and movement without coral, it is not completely clear whether my hypothesis regarding movement with and without coral nearby is supported or not. The data does suggest that there is a significant difference between the means for movement with fish and without fish, which was unexpected. Previous studies suggested that BHTF follow schools of blue fish, and the lack of evidence found to support this was surprising (Kaufman 1976). The lack of relationship between BHTF movement and fish presence is likely due to the lack of any data for BHTF moving with schools of blue fish. Due to this and the large standard deviations for the movement behavioral data across phenotypes and surroundings, more data is necessary to support this hypothesis. The significance in the differences in time spent horizontal/vertical for BHTF among surroundings was likely because the phenotype lingered far enough above corals/sponges that these behaviors didn't meet the camouflage criteria. Additionally, this study did not consider that this phenotype might be using the dark blue water column as a means of

camouflage, as this was difficult to distinguish from hovering.

H₃: Out of all three phenotypes, the strongest relationship would exist between the abundance and behavior of yellow trumpetfish and the abundance of yellow reef fishes nearby

After surveying sixteen 100 m² areas and calculating linear regressions for MTF and their associating fish and YTF and their associating fish, no significant associations were found for either phenotype. This means that the abundance portions of this hypothesis were weakly supported. This could be because there is a greater link to the abundance of certain types of corals or sponges in the area, particularly for the mottled trumpetfish. The abundances of either phenotype of trumpetfish may have been too low for the size of survey areas used to collect data to propose associations. While the behavioral observations focused on individuals, the abundance surveys were focusing on whole populations of trumpetfish. There is no way to know just how big the trumpetfish or associating fish populations are in whole, making it difficult to say whether these associations would be representative of the whole population. This is a problem that extends far beyond colorful reef fish; marine ecologists working to determine accurate population sizes for restoration and conservation projects worldwide require multiple facets of a species' ecology to create an accurate population estimate, and for many species, this information can be very difficult to come by (Sands et al. 2009).

The behavioral results for the yellow phenotype indicate that what we recreationally observe YTF to be doing may not necessarily be what they spend most of their time doing and that it may be more difficult to interpret what different behaviors are for this phenotype compared to the two others. While BHTF and MTF appear to have one predictable behavior that dominates their time (moving and vertically camouflaging, respectively), YTF spend similar amounts of time moving and

remaining horizontal or vertical, neither of which this study predicted to be greater than hunting. A potential reason for this could be the use of a different camouflaging mechanism that does not match the aforementioned camouflaging criteria, which would increase the amount of time in the “horizontal or vertical” category. If yellow trumpetfish are only camouflaging to prevent being noticed from above, it would not matter how far they are from a substrate, meaning whenever they were above a yellow coral or completely under any structure, they could have been camouflaging. This idea is supported by one of my YTF observations where the fish spent over half of the observation lingering under a large overhang. It is also possible that much of the time spent horizontal or vertical was actually spent hunting with less outwardly aggressive behaviors. One study showed that lizardfish, also present on Bonaire and found to blend into their surroundings, can have higher prey capture rates when they struck at prey less often (Sweatman 1984). This could explain why we did not observe hunting behavior from the mottled trumpetfish as well, which was suggested by the present study to spend a majority of their time vertically camouflaging in coral or sponges that match their color.

A lack of support for H_1 and a lack of significant data from the abundance surveys means H_3 is not supported by this data. It is possible that the link between YTF behavior and surroundings is less predictable than BHTF or MTF behavior because of the multitude of ways that YTF can potentially use their surroundings to their advantage. Because YTF were seen doing such a large spread of behaviors in comparable amounts, this could also indicate that they use a variety of behaviors to achieve what they need to and depend less on their surroundings to assist them because of their bright color. Cues besides environmental ones could have a bigger effect on YTF behavior; animal behavior is a complex combination of both internal and external cues (Colgan 1993). Presence of olfactory cues from small prey fish or predatory fish (Loreau 2009) or even the

authors’ observations as a diver could have changed their behavior in various ways as well, regardless of what colors and objects their surroundings were made up of. Six replicates of each phenotype were observed and the length of time each behavior was displayed, the color of nearby objects, and what each object was were all recorded. Recordings and methods were kept consistent across phenotypes as well. Because significant differences were found despite these considerations, my data does suggest that these colored objects influence behavior for certain phenotypes of trumpetfish.

This is the first study of its kind to attempt to quantify the behavioral differences found between trumpetfish phenotypes when compared with differences in their surroundings. While the focus of this study was on how the behavior and abundance of YTF may be influenced by color in their environment in ways different that the other trumpetfish phenotypes, studies on other aspects of the environment and behavior of any marine species could use similar methods to add even more information to what is known about marine animal behavior. Supporting to some degree that different phenotypes quantitatively act differently in different surroundings may encourage further behavioral research of other species with a variety of different colorations.

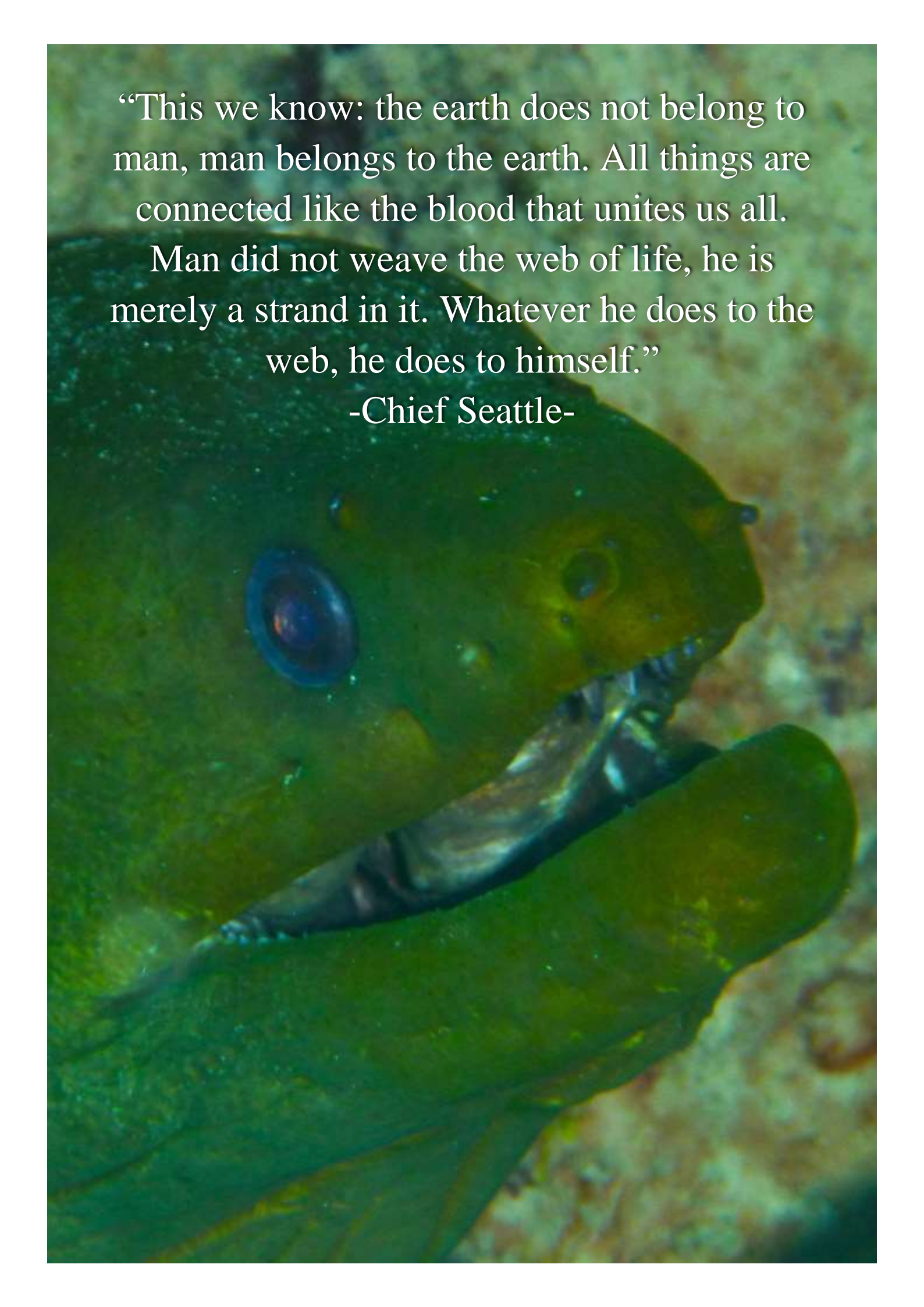
For future studies regarding the impact of surroundings on trumpetfish abundance and behavior, more data could be collected on how other factors such as diver impacts (Lindfield et al. 2014) or surroundings such as rugosity (Schrandt et al. 2012) and predator and prey interactions (Loreau 2009) change the time spent on different behaviors. If all other factors that make up the surroundings could be controlled for and this experiment repeated with a higher sample size, there could be more certainty on whether or not the results found were actually due to changes in color and objects in the surroundings of these fish. This is true for all studies of this nature; if work is done to eliminate the confusion caused by extra variables and calculating population sizes, comparative behavioral and abundance studies

such as this one can become more effective at determining links between surroundings, behaviors, and abundances.

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“This we know: the earth does not belong to man, man belongs to the earth. All things are connected like the blood that unites us all.

Man did not weave the web of life, he is merely a strand in it. Whatever he does to the web, he does to himself.”

-Chief Seattle-

