

PHYSIS

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CIEE Research Station Bonaire

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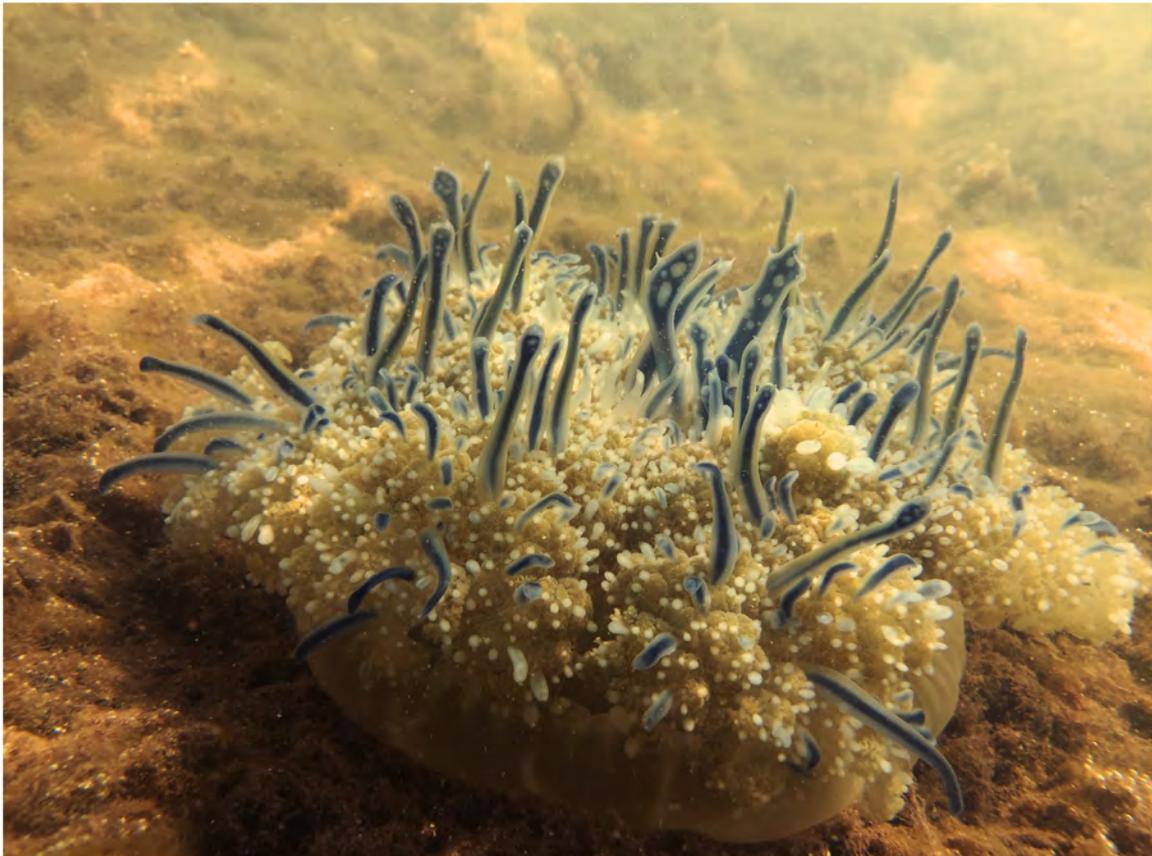
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Physis

Journal of Marine Science



CIEE Research Station Bonaire
Tropical Marine Ecology and Conservation
Volume XXI • Spring 2017

FOREWORD

Publication of Volume XXI of the student journal *Physis: Journal of Marine Science* was the ultimate goal of the Independent Research in Marine Ecology/Biology course. The course is part of the semester program in Tropical Marine Ecology and Conservation at the CIEE Research Station Bonaire. Franziska Elmer, PhD, and Zac Kohl, PhD Candidate, co-taught the course. Additionally, student projects were supported by an intern, Madeleine Emms, MS, Sarah Jean Byce, MS, and Courtney Klatt, BA (CIEE Alumnus). The academic advisors guided the projects through course content delivery and weekly meetings with each student. Astrid de Jager Verstappen directed the Dive Safety Program for the semester.

Research was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment and Nature. Projects were conducted near the research station, which is located on the leeward side of Bonaire to the north of the town of Kralendijk. The students presented the findings of their research projects in a public forum on the 3rd of May, 2017 at the CIEE Research Station lecture room.

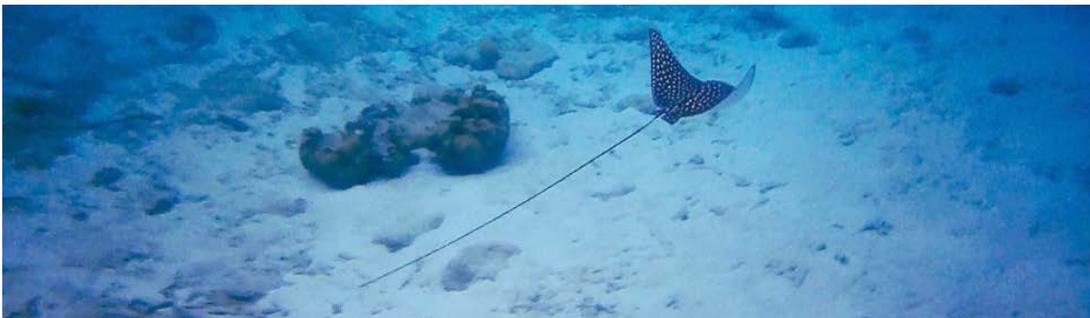
The Tropical Marine Ecology and Conservation program in Bonaire is designed for upper level undergraduates majoring in Biology or Ecology. There is a field-based orientation to the program with a strong focus on research-skills acquisition. In addition to the Independent Research course, students enroll in five courses: Coral Reef Ecology, Marine Ecology Field Research Methods, Advanced Scuba, Tropical Marine Conservation Biology, and Cultural & Environmental History of Bonaire. A noteworthy accomplishment is that students earned a Scientific Dive certification with the American Academy of Underwater Sciences during the program.

Part of the mission of the CIEE Foundation, which is a Bonairean not-for-profit organization, is:

“to provide outstanding educational opportunities to students in Tropical Marine Ecology and Conservation. We strive to provide interdisciplinary marine research opportunities for CIEE students as well as visiting scientists and their students from around the world.”

Thank you to the students and staff that participated in the program this semester. A final word to the students: **Congratulations on publishing this volume of *Physis* and best of luck as you embark on your future careers!**

Dr. Rita BJ Peachey





“After the final no there comes a yes, and on that yes the future world depends.”
-Wallace Stevens

Humans have always strived to understand what we do not know. This thirst for knowledge has manifested into a need to conquer the natural world that surrounds us. We have been successful, but at what cost? Man has finally reached the point where our combined effects as a species on this planet are palpable: our fisheries have been exhausted, our atmosphere altered beyond natural bounds, our wetlands have been permanently lost, and our polar icecaps have begun to see their final days. When does the world finally say yes to a sustainable future?

The word *physis* is derived from Greek, meaning the phenomenon and processes that aid in nature’s ability to heal itself. Throughout history, humankind as a species has believed that the resources provided by the surrounding landscape were inexhaustible. Presently, we have begun to realize that we have built an insidious house of cards below us – one more move and it crumbles. We can no longer assume that nature’s ability to heal itself overcomes our destructive way of life. Our focus as a species must shift from one of selfish exploitation to one geared towards reparations. The question that always seems to impede our progress towards a more sustainable future remains: where do we even begin? We believe that through science, research, and advancements in technology, humans can begin to better understand the effect our species has on the planet. Our understanding can not be a superficial one, rather it must be profound. With this wisdom, we might be able to penetrate and change the ambivalence our society has towards maintaining and returning to the natural state of the world’s ecosystems, to ones that are healthy and able to heal themselves.

Much like Sisyphus, a man condemned to a life of pushing a large boulder to the top of a mountain - just to watch it fall hopelessly back to the bottom - our scientific efforts seem futile at times. Progress is met with backlash, and ignorance seems to drive the path our society follows. Presently, under the current political climate, faith in a brighter tomorrow seems to fade dimmer. Still, hope remains and our community has shown resilience through these trying times. The March for Science showcased our resistance to being silenced. The job we have ahead of us, though daunting, is one we must continue towards and must be sustained by our generation.

The participants in this publication firmly believe that we all have individual roles to play to ensure our planet not only survives human impacts, but rather thrives as a result. By continuing to expand our knowledge of the natural world, we hope to instill deeper appreciation for conservation and sustainability in current and future generations. As society begins to place greater value on sustainability, mankind’s insatiable quest for knowledge will continue to drive us, but in a way that will benefit not only our species, but all those that share this world.

And with these ideals, we bring into fruition Volume XXI of *Physis: Journal of Marine Science*.

Evan Claggett and Hannah Yi

FACULTY AND STAFF



Dr. Rita Peachy has been the Director of CIEE Research Station since 2006. She has a BS and an MS from the University of South Florida and a PhD in Marine Sciences from the University of South Alabama. Dr. Peachy is also the Executive Director of the Association of Marine Laboratories of the research grant from the National Science Organization of the Netherlands to study algae production for food, feed or energy.



Dr. Franziska Elmer is the Coral Reef Ecology Faculty for CIEE and co-teachers Marine Ecology Field Research Methods and Independent Research. Franziska is from Switzerland but has been working on coral reefs in both the Caribbean and Pacific Ocean. She has been researching how biological and physical factors affect coral recruitment and calcium carbonate accretion by CCA.



Zachary Kohl is the Tropical Marine Conservation Biology Faculty from CIEE and co-teachers Marine Ecology and Field Research Methods and Independent Research. He is from Denton, Texas but spent most of his life in Oregon and was one of the first students at CIEE Bonaire in 2006. His research focused largely on cardiovascular and development physiology in fish, amphibians, reptiles, birds, and mammals.



Astrid de Jager Verstappen is CIEE's Dive Safety Officer and instructor for the Advanced Scuba class and Cultural and Environmental History of Bonaire course. She studied in the Netherlands, but has been and DAN. Since 2013 she's been part of CIEE Research Station Bonaire.

FACULTY AND STAFF



Sara Buckley is the Office and Laboratory Manager. She received her BS in Oceanography from the University of North Carolina at Wilmington. She is a PADI/SDI SCUBA instructor. Sara started at CIEE as an intern and teaching assistant, where she studied the effects of UV on zooplankton communities and diversity. After a year-long internship, she was hired on as a full-time staff member to be the Laboratory and Office Manager alongside finishing her zooplankton study.



Mary Dianza works in accounting at CIEE. She was born and raised in Colorado. Bonaire's early commitment to protecting the environment was what first drew her to the island, where she worked as a dive instructor, boat captain, and retail manager for a local dive shop before branching out to the resort/management side of the business.



Marc Tsargaris is the facilities manager at CIEE and an instructor for the Advanced Scuba course. He is interested in diver impacts on coral reefs.

INTERNS



Sarah Jean Byce is a teaching assistant for Coral Reef Ecology and Independent Research; she co-manages the residence hall. She holds a BS in Biochemistry and Molecular Biology from the University of Richmond and a MS from the College of Charleston in Environmental Studies, focusing on evaluating the effectiveness of community-based management of the Crown-of-Thorns sea star in Romboln, Philippines.



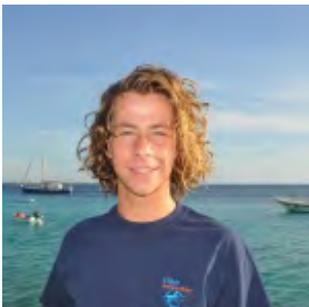
Maddie Emms is a teaching assistant for Tropical Marine Conservation Biology and Independent Research. She is from England and studied for her BS in Marine Biology at the University of St. Andrews, Scotland. She was based at KAUST, by the Red Sea, for her MS in Marine Science focusing on the population genetics of 'host' sea anemones. Before arriving at CIEE, she completed her PADI Divemaster and worked as a Caribbean Reef Ecology lecturer for Operation Wallacea in Mexico.



Jamie Emm is volunteer research intern working with zooplankton. He holds a BS degree in Ecology/Environmental Science from the University of North Texas. Jamie will continue his education in January 2018 to earn a MS in Marine Biology and Ecology in his continued pursuit of PhD. Jamie is a former intern and student at CIEE.



Courtney Klatt is a research intern. She was a student at CIEE Bonaire during Fall 2015 and January 2017. studied biology and Spanish at Indiana University in Bloomington.



Martijn Koot is a lab intern, with a background in nutrients and chemicals. He is still studying for his BS as a chemical/physical analyst in the Netherlands and is also the youngest person that is working at CIEE Bonaire. After CIEE, he is going to work in the Netherlands and is going to study as a lab technician, gaining with DNA, nutrients, and blood.

STUDENTS



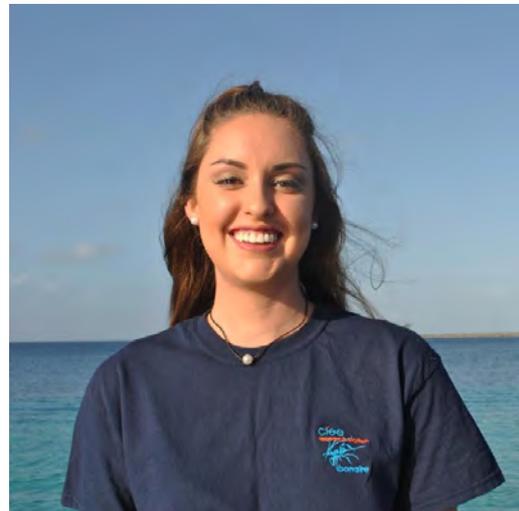
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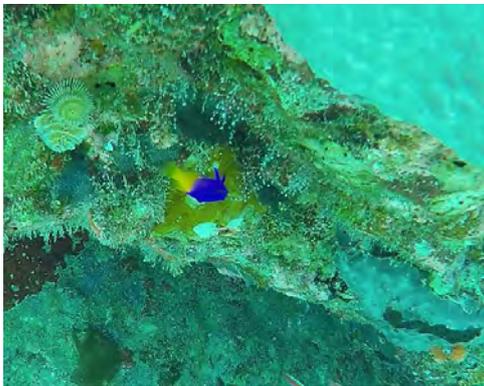
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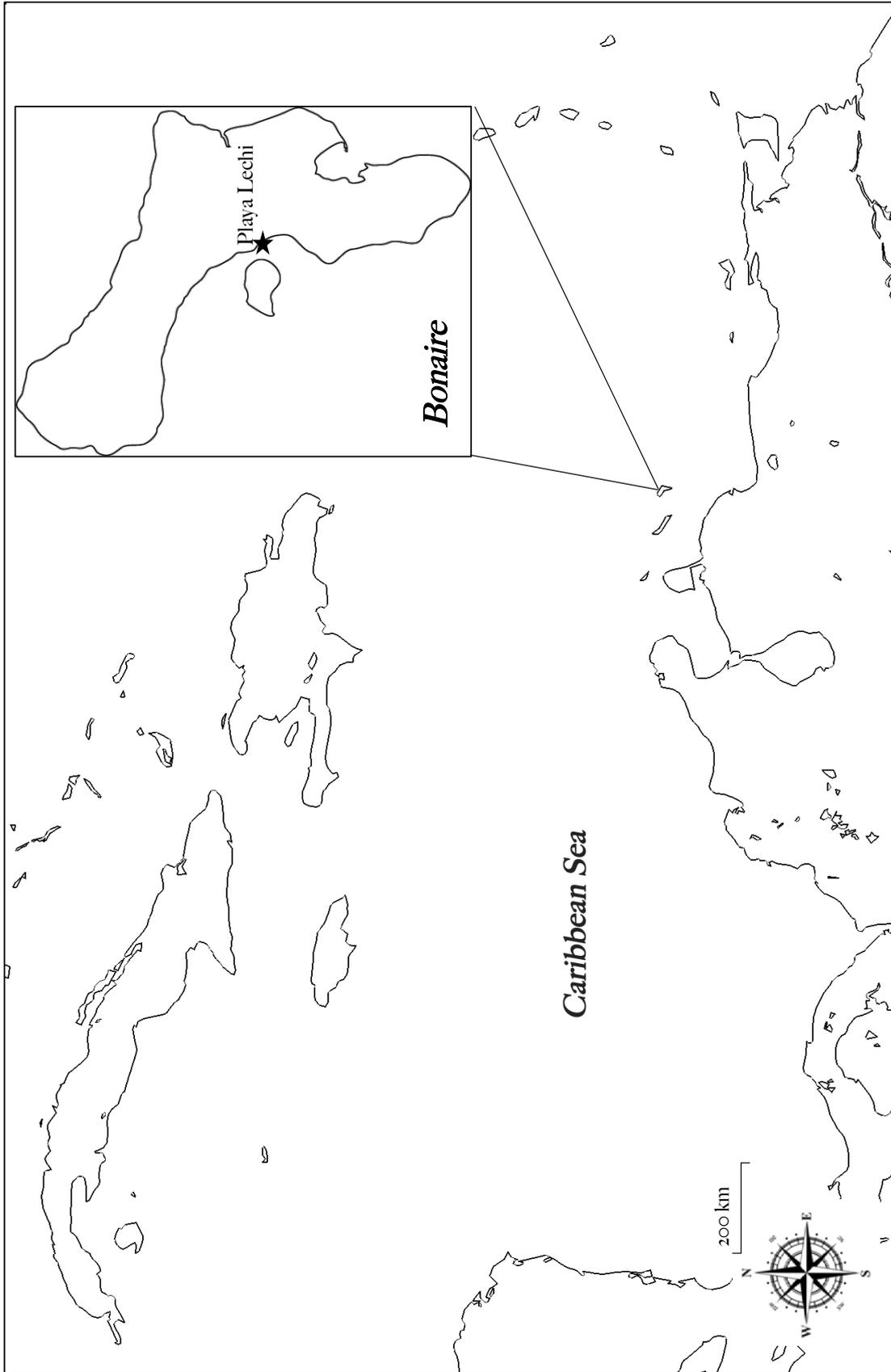
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REPORT

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Monitoring the effect of sedimentation on *Cassiopea* spp. bell pulsation dynamics

Abstract Mangrove forests are productive, carbon-rich ecosystems that help to entrap sediment and prevent it from entering coral reefs. *Cassiopea* spp. (upside-down jellies) are typically found in mangroves and may assist with nutrient regulation as well as serve as a bioindicator species for anthropogenic disturbances, such as nutrient-loading and water-metal exposure. *Cassiopea* spp. use their bell pulsations to perform functions such as oxygen exchange, gamete distribution, and prey capture. Currently there is little known about how sedimentation impacts the bell pulsation dynamics of *Cassiopea* spp. This study investigated the effect of increasing sedimentation levels on *Cassiopea* spp. on the bell pulsation rate (BPR) and bell pause variability (BPV) over 115 min trial periods in a laboratory setting (n = 144). The average BPR increased alongside increasing sediment treatment, from low to high, while there was no significant difference between the control and low sediment treatment. Further, the low sediment treatment had a significantly greater average BPV than the medium sediment treatment. Neither the BPR nor the BPV significantly changed over the 115 min observation period. These results indicate that sedimentation impacts bell pulsation dynamics. This could be due to *Cassiopea* spp. needing to alter the frequency of their bell pulsations to clear sediment from the bell. These results have important implications, as *Cassiopea* spp. may need to adapt to meet their increased metabolic demands under increased exposure to sedimentation. Furthermore, it could have an overall impact on nutrient cycling within mangrove ecosystems.

Keywords Mangroves • Upside-down jellies • Bell pause variability

Introduction

Mangrove forests are productive, carbon-rich ecosystems that provide many ecosystem services (Donato et al. 2011). They help protect sensitive seagrass habitats and coral reefs by serving as barriers against land-derived nitrogen loads and pollutants (Valiela and Cole 2002) and are essential nurseries for reef fish communities (Mumby et al. 2004). Until 2001, at least 35% of worldwide mangrove forest area had been lost (Valiela et al. 2001). Mangroves aid in entrapping sediments, but heavy sedimentation associated with the degradation of these important ecosystems has led to reefs with fewer coral species, reduced coral recruitment, and slower rates of reef accretion (Rodgers 1990; Humanes et al. 2017). Thus, mangrove conservation helps to protect coral reefs, which are one of the most biodiverse ecosystems in the world.

Cassiopea spp. (upside-down jellies) are typically found along mangroves and may assist in regulating the nitrogen and carbon that these ecosystems receive (Welsh et al. 2009; Jantzen et al. 2010; Freeman et al. 2016). *Cassiopea* spp. have a symbiotic relationship with dinoflagellates in the genus *Symbiodinium*, commonly known as zooxanthellae, which photosynthesize and transfer carbon to the host as a source of energy (Verde and McCloskey 1998). In human-impacted areas, *Cassiopea* spp. have been found to have greater average zooxanthellae densities than in low-impacted

areas, which suggests that nutrient loading from anthropogenic disturbances may lead to *Cassiopea* spp. blooms (Stoner et al. 2011). Additionally, it has been found that *Cassiopea marametensis* is capable of accumulating copper and zinc in their tissues (Templeman and Kingsford 2015). These studies suggest that *Cassiopea* spp. may be a good bioindicator species for both nutrient loading and heavy metal pollution.

Along with the symbiotic relationship that *Cassiopea* spp. have with zooxanthellae, they also rely on filter-feeding to fulfill functions such as capturing prey, oxygen exchange, and gamete distribution (Arai 1997). A bell pulsation can be defined as each expansion and contraction of the bell. Hamlet et al. (2011) found that as *Cassiopea* spp. pulsate their bell, fluid from the substrate is passed over the bell and through the oral arms. Further, the pauses between bell pulsations may lead to an increase in the flux of fluid from the substrate to the bell (Hamlet et al. 2011). This suggests that *Cassiopea* spp. may impact the nutrient fluxes of their environment (Jantzen et al. 2010).

As *Cassiopea* spp. may play an important role in marine ecosystems, it is essential to understand how environmental factors, such as sedimentation, impact *Cassiopea* spp. behavior. It has been theorized that sedimentation intensity can increase significantly before filter-feeders show large changes in resistance to clogging (Rubenstein and Koehl 1977). This suggests that when the sedimentation intensity exceeds the capacity of filter-feeding mechanisms, resistance to clogging drops. Sedimentation has been shown to reduce the feeding efficiency of large filter-feeding benthic epifauna, such as sponges and pinnid bivalves, by blocking their filtration systems (Lohrer et al. 2006). Other filter-feeders, such as corals, work to actively remove sediments through a variety of methods, such as ciliary action and mucus production (Hubbard and Pocock 1972). By looking at the responses and mechanisms used to clear sediment in filter-feeding organisms, it can be hypothesized that increased levels of sedimentation may lead to a decline in the feeding efficiency of *Cassiopea* spp. unless they

have a built-in response to clear sediment. This study aimed to understand how sedimentation affects *Cassiopea* spp. bell pulsation rate (BPR).

- H₁: When exposed to higher sedimentation levels, *Cassiopea* spp. specimens will have a lower average BPR than when exposed to lower sedimentation levels
- H₂: When exposed to higher sedimentation levels, *Cassiopea* spp. specimens will show a decrease in their average BPR over time.

By understanding how *Cassiopea* spp. respond to increased sedimentation levels, better predictions can be made for how *Cassiopea* spp. may alter their behaviors linked with bell pulsations, such as filter-feeding, within mangrove ecosystems. Further, this study aimed to expand the current methodologies for analyzing the biomechanics of *Cassiopea* spp. bell pulsations in relation to changes in environmental conditions, which could ultimately alter ecosystem processes such as nutrient cycling.

Materials and methods

Site description

Bonaire is located in the southern Caribbean Sea approximately 80 km off the northern coast of Venezuela. All *Cassiopea* spp. specimens were collected in February of 2017 from the mangroves at Lac Bay (12°05'33.9"N, 68°14'22.1"W) within the Bonaire National Marine Park (Fig. 1). The approximately 700 ha lagoon of Lac Bay is located on the eastern, windward side of Bonaire and hosts a mangrove forest comprised of three mangrove species—Red Mangrove (*Rhizophora mangle*), Black Mangrove (*Avicennia germinans*), and White Mangrove (*Languncularia racemosa*). This area was selected for its abundance of *Cassiopea* spp. along the mangroves in the bay. All *Cassiopea* spp. were released at Lac Bay after completion of experimental trials.

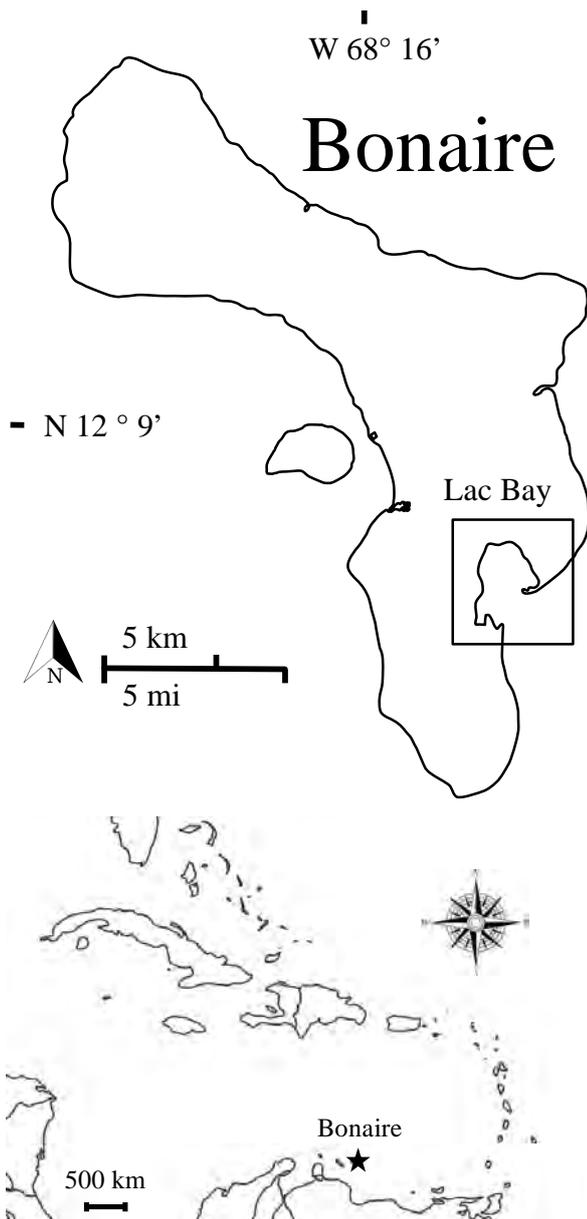


Fig. 1 Bonaire (star) is a 29,400 ha island in the Caribbean. Lac Bay ($12^{\circ}05'33.9''\text{N}$, $68^{\circ}14'22.1''\text{W}$; square) is a 700 ha lagoon on the eastern side of Bonaire and consists of a rich mangrove ecosystem which hosts an abundance of *Cassiopea* spp.

Collection procedure

Cassiopea spp. specimens ($n = 12$) of 4.3-8.7 cm in bell diameter were collected at a depth of 2-3 m. Each *Cassiopea* spp. specimen had a differing combination of blue and green oral arms of various lengths and shades, and a white or brown bell band pattern. Three surface-sediment cores were collected from the specimen collection area for manipulation of sediment levels in the experimental procedures

and for further nutrient and particle size analysis. A water sample was collected to perform nutrient analyses. All specimens were transported approximately 20 min to the CIEE Research Station, Kralendijk, Bonaire, in a cooler with sufficient saltwater.

Husbandry of *Cassiopea* spp. specimens

Cassiopea spp. specimens were kept in two 30 l containers of unfiltered seawater pumped from, and overflowing into, a single aerated 30 l container with multiple bubbler-systems (Fig. 2). *Cassiopea* spp. specimens were fed brine shrimp (*Artemia* spp.) 1-3 times weekly (0.5 g of eggs were hatched in 1000 ml of seawater, approx. 80 ml of solution was given per *Cassiopea* spp. specimen). Half of the seawater in the aerated container was changed 24 h after each feed, and containers were shaded from direct sunlight. Each specimen's bell diameter (cm) was measured, the morphology described, and an identification number assigned.

Experimental acclimation period

Cassiopea spp. specimens ($n = 4$) were monitored prior to removal from the 30 l container for their resting bell pulsation rate (RBPR; beats min^{-1}) by counting the number of bell contractions and expansions per minute. After transfer from the main container to a 10 l aerated experimental tank, specimen RBPR was monitored for 1 min every 2 min until reaching

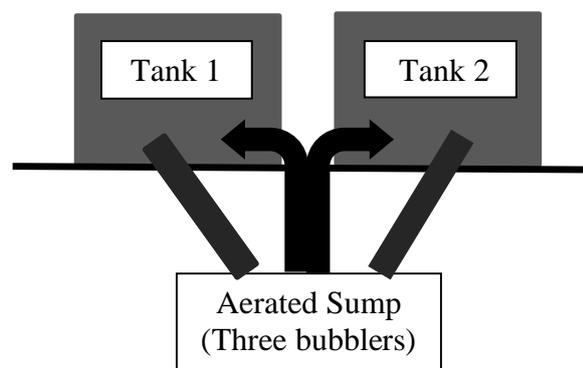


Fig. 2 *Cassiopea* spp. specimens were kept in unfiltered seawater in two elevated 30 l containers (Tank 1 and Tank 2: six *Cassiopea* spp. specimens per tank) overflowing into (grey rectangles = PVC pipes), a shared 30 l sump containing three bubbler-systems

a consistent RBPR. The time of the total experiment was recorded and the procedure repeated for each of the four *Cassiopea* spp. specimen to determine an adequate acclimation period prior to any experimental observations.

Sedimentation manipulation

Four 10 l experimental tanks were used for the following conditions: control, low, medium, and high sedimentation. Six *Cassiopea* spp. specimens were randomly divided into two groups of three specimens. Each of these six specimens were randomly exposed to the control, low, and high sedimentation treatments once over the course of four weeks (with a resting period between each trial of 3-7 d). Four different *Cassiopea* spp. specimens were exposed only once to the medium sedimentation treatment during this trial period. During a single trial, one of the three *Cassiopea* spp. specimens from one of the two random groups was selected based on a pre-determined alternation schedule and one *Cassiopea* spp. specimen in the medium sedimentation treatment group was individually placed in an aerated 10 l tank. Prior to placing the *Cassiopea* spp. specimen in the aerated 10 l tank, the bottom of each of the four experimental tanks were coated with 600 g of sand and filled with filtered seawater. Each *Cassiopea* spp. was placed in the appropriate tank and given an acquisition period of 45 min based on initial results. At the end of the acquisition period, all visible nematocysts ejected from the *Cassiopea* spp. specimen were removed from the water using a pipette. Once the trial began, the *Cassiopea* spp. specimens were monitored at 1 min intervals at the start of the trial, and then every 5 min over the course of two hours. At every 5 min mark, the following sediment was added for each sedimentation level: control = 0 g; low = 0.1 g; medium = 0.3 g; high = 0.5 g. After each sediment load input, the time in seconds since the sediment was added was recorded for every bell pulsation (one contraction and expansion) so that the average BPR could be determined. After the completion of each trial, specimens were returned to their

original holding container.

Sediment grain size and water quality analyses

The Lac Bay mangrove sediment sample was placed in a weigh boat and dried at 38°C for 48 hrs in an oven. The dried sample was weighed (263.6 g) and large clumps of sediment were broken apart. The sample was loaded into sieves, arranged from <63 µm up to 2 mm mesh size, starting in the 2 mm sieve and placed onto the Vibratory Sieve Shaker (©RETSCH) at 30% amplitude for 30 min. After the allocated time, the sieves were removed from the Vibratory Sieve Shaker and the mangrove sediment particles in each sieve were collected using wire brushes to remove any fine particles that remained on the face of the sieve. The mass (g) of each grain size was determined by weighing the sediment particles collected from each sieve. The percentage of the total sample for each grain size was calculated by dividing the mass (g) of the grain size by the total mass of the mangrove sediment sample. The Lac Bay water samples were tested for ammonia (ppm), nitrate (ppm), nitrite (ppm), and pH using the Salt Water Aquaculture Test Kit (©LaMotte).

Statistical analyses

Following preliminary trials, the bell pause variability (BPV) was analyzed because the BPR did not appear to capture the full change in behavior for each sediment treatment. The BPV was determined by subtracting the shortest bell pause from the longest bell pause for each minute-long observation, which shows the bell pause variation within one minute. The individual sample unit was a single minute-long measurement of bell pulsations (n = 24 per trial). BPR was determined by counting the number of bell pulsations within each 1 min observation period over the 115 min trial. One-way ANOVAs were performed to determine: 1) the effect of sedimentation treatment on BPR and BPV and; 2) the effect of time on BPR and BPV. A Tukey post-hoc analysis was conducted if the p-value was less than 0.05 and the mean and standard deviation were reported.

Results

Effect of sedimentation on BPR and BPV

Over six days of data collection, the BPR and the BPV for twelve *Cassiopea* spp. specimens were recorded over 1 min long observations for various sedimentation treatments (n = 144; Table 1). Based on observations, one *Cassiopea* spp. specimen (ID #4) had abnormally high activity during the control treatment (Fig. 3). After eliminating this specimen from the dataset, all statistical analyses were performed. Sedimentation treatment had a significant effect on the BPR, (ANOVA: $F = 43.66$, $df = 3$, $p < 0.001$; Fig. 4). A Tukey post-hoc analysis showed that the average BPR (\pm SD) increased from the low (13.58 ± 5.43), to medium (19.11 ± 8.75), and high (21.78 ± 8.72) sedimentation treatments. There was no significant difference between the control and low sedimentation treatments. Treatment did have a significant effect on the average BPV (\pm SD) (ANOVA: $F = 3.27$, $df = 3$, $p = 0.021$). The low sediment treatment (9.44 ± 0.41 s) had a significantly greater BPV than the medium sediment

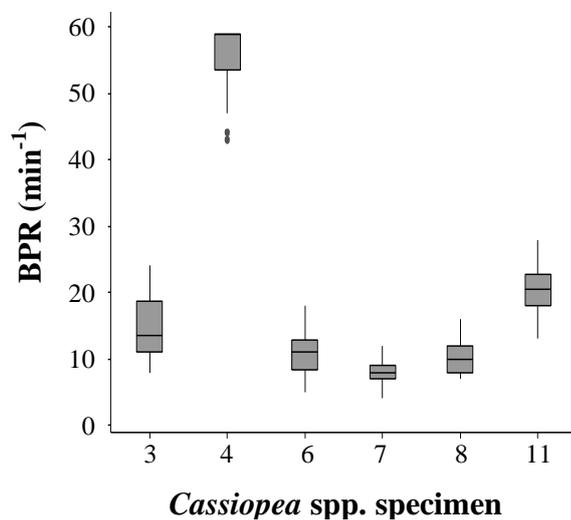


Fig. 3 The interquartile range (boxes), median (line in center of boxes), and total range (maximum and minimum; lines) for the BPR during control treatments for each *Cassiopea* spp. specimen exposed to the control treatment (n = 72)

treatment (7.72 ± 0.47 s; Fig. 5). Neither the BPR (ANOVA: $F = 0.37$, $df = 23$, $p = 0.996$) nor the BPV (ANOVA: $F = 0.43$, $df = 23$, $p = 0.998$) significantly changed over the 115 min trial period for the control treatment. The same was true for the sediment treatments (low, medium and high combined) for the BPR (ANOVA: $F = 0.64$, $df = 23$, $p = 0.903$) and the BPV (ANOVA: $F = 1.05$, $df = 23$, $p = 0.402$).

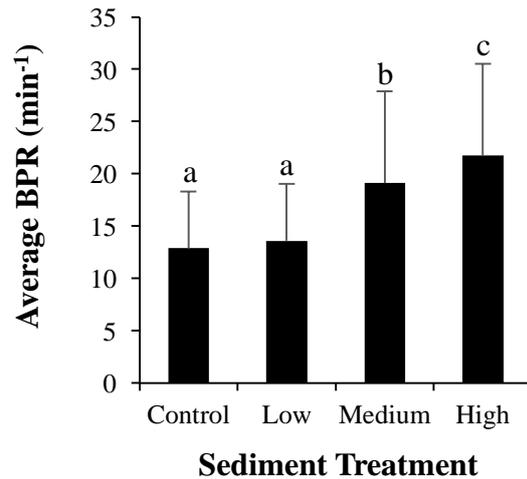


Fig. 4 The average *Cassiopea* spp. BPR by sediment treatment (control, low, high: n = 120; medium: n = 144). Averages reported are the mean; error bars show the standard deviation. Unique letters indicate significant difference (Tukey post-hoc analysis)

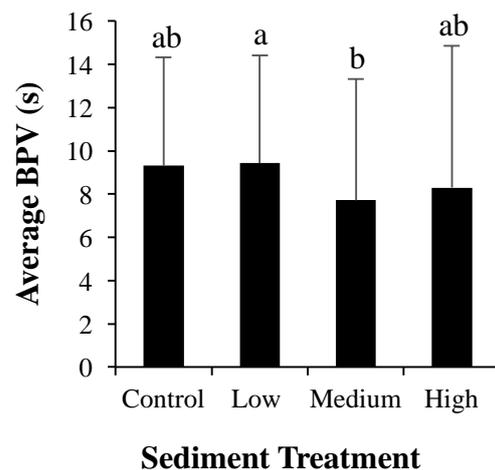


Fig. 5 The average BPV of *Cassiopea* spp. within one minute of recording by sediment treatment (control, low, high: n = 120; medium: n = 144). Averages reported are the mean and the error bars show the standard deviation. Unique letters indicate significant difference (Tukey post-hoc analysis)

Table 1 *Cassiopea* spp. specimen bell diameters, average resting bell pulsation rates min⁻¹, standard deviation and sediment treatments

<i>Cassiopea</i> spp. ID #	Bell diameter (cm)	Average resting bell pulsation rate/min	Standard deviation	Sediment treatments
1	8.5	38	--	Medium
2	6.1	21	--	Medium
3	4.3	17	5.5	Control Low High
4	5.6	35	6.8	Control Low High
5	7.9	15	--	Medium
6	8.3	24	12.0	Control Low High
7	8.7	12	13.7	Control Low High
8	6.9	22	15.9	Control Low High
9	6.9	25	--	Medium
10	5.3	28	--	Medium
11	8.2	27	2.5	Control Low High
12	7.6	6	--	Medium

Sediment grain size and water quality results

The Lac Bay mangrove sediment sample grain size analysis revealed that the sediment is mainly comprised of 2 mm (40.6% of total mass) and 1 mm (25.5 % of total mass) grain sizes (Table 2). The water quality analysis showed the following: ammonia = 0.05 ppm; nitrate = 0.25 ppm; nitrite = 0.05 ppm; and pH = 6.5.

Table 2 Grain size analysis for Lac Bay mangrove sediment sample

Grain size	Mass (g)	Total mass (%)
2 mm	106.9	40.6
1 mm	67.2	25.5
500 µm	41.1	15.6
250 µm	24.8	9.4
125 µm	12.3	4.7
63 µm	7.7	2.9
< 63 µm	3.6	1.3

Discussion

This study aimed to determine how sedimentation effects *Cassiopea* spp. BPR and BPV over a 115 min observation period. The BPR increased under high sedimentation, thus rejecting the first hypothesis that *Cassiopea* spp. specimens will have a lower average BPR when exposed to higher sedimentation levels. The effect of sedimentation on the BPR and BPV did not appear to change over a 115 min trial period. Thus, the second hypothesis was also rejected that when exposed to higher sedimentation levels, *Cassiopea* spp. will show a decrease in their average BPR over time. Sedimentation does seem to have some effect on bell pulsation dynamics.

Effect of sedimentation on BPR

The average BPR was highest during the high sedimentation treatments (0.5 g min⁻¹). This may be due to *Cassiopea* spp. needing to clear their bell more frequently so that they have direct access to the water column for functions such as filter-feeding and oxygen exchange (Arai 1997). By altering the bell expansions and

contractions, *Cassiopea* spp. could adjust the flow of fluid from the water column through the oral arms and bell, thus allowing them to clear the bell of sediment more effectively (Hamlet et al. 2011). It is not yet known if *Cassiopea* spp. have natural response mechanisms for clearing sediment or if a stress response was observed. Future studies could investigate whether *Cassiopea* spp. can sustain these BPRs over extended periods of time as a way to clear sediment. There was no significant difference between the control (0.0 g min^{-1}) and low (0.01 g min^{-1}) sedimentation treatments, suggesting that *Cassiopea* spp. are tolerant to low levels of sedimentation. Another possibility for sediment clearing could include nematocyst ejection, which was occasionally observed as a cloud of a sticky mucus when sediment would contact the bell (Blondheim, pers. obs.) This could be similar to other cnidarians, such as corals, which use a mucus to clear sediment from their substrate (Hubbard and Pocock 1972).

Effect of sedimentation on BPV

The low sedimentation treatment had a significantly greater BPV than the medium sedimentation treatment. Considering there were no other significant differences between the other sedimentation treatments (including the control), this result may have been due to differences in *Cassiopea* spp. specimens selected. As the medium sedimentation treatment included four separate *Cassiopea* spp. specimens that were not used for the replications of the other sedimentation treatments (control, low, high: $n = 120$; medium: $n = 144$) there may have been differences between individuals in the medium treatment *Cassiopea* spp. specimen group when compared to the other treatment groups.

Effect of sedimentation over time on BPR and BPV

There was no change in the effect of sedimentation on the BPR and BPV over a 115 min trial period. Overall sedimentation seemed to have more of a direct effect than the accumulation of sediment over the observation

period. This result may indicate that *Cassiopea* spp. are able to effectively clear the bell after immediate contact with sediment, and thus no changes are observed over time. One method that could be used to test this hypothesis is the use of videography and digital particle image velocimetry, which can be used to see if the vortices that *Cassiopea* spp. create with their bell pulsations are used to actively clear sediment from the bell (Santhanakrishnan et al. 2012). Effective and immediate clearing may be a way for *Cassiopea* spp. to restore natural filtering flows through the oral arms and bell, preventing the need to change their behavior over time. Future studies could use longer monitored trial periods as previously suggested.

Conclusions

The results from this study suggest that sedimentation has an effect on the bell pulsation dynamics of *Cassiopea* spp. As mangrove ecosystems decline globally, *Cassiopea* spp. may need to use more energy to meet the increased metabolic demands that are associated with increased bell pulsations and decrease the energy used for growth and reproduction. Further, as these species may be important in nutrient cycling, the increased energy usage of *Cassiopea* spp. may have longer lasting impacts on the overall health of mangrove ecosystems by increasing nutrient cycling with more bell pulsations.

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REPORT

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Time budgeting and community structure of the fairy basslet, *Gramma loreto*

Abstract The coral reef fish *Gramma loreto* is an abundant planktivore found living in small aggregations of conspecifics throughout the Caribbean. Outside of reproduction and territoriality, little information is known about *G. loreto*; therefore, this study examines the community structure and the behavioral time allocation of *G. loreto*. Time budgeting studies are used to quantify how organisms allocate their energy and make behavioral trade-offs. To investigate the time budgeting of *G. loreto*, various populations (n = 8) were videotaped for 15 min and footage was analyzed in the laboratory. *Gramma loreto* are found significantly more often near the coral species *Undaria agaricites*, *Orbicella annularis*, and *Orbicella faveolata*. Most of the fish species found in proximity to the populations of *G. loreto* are invertivores ($80.3 \pm 8.1\%$). On average, *G. loreto* spent more time feeding compared to other observable behaviors (e.g. hiding, swimming, chasing, and floating stationary). The percentage of time spent on a specific behavior is not significantly influenced by the population size, percentage of piscivores within a 2 x 2 x 1 m frame, or fish size. Planktivores induced most of the chase behavior of *G. loreto*, whereas *G. loreto* hide in response to all other functional groups. Further studies are required to determine the interaction of various factors and the role they play to influence the behavior of *G. loreto* and other important coral reef fish.

Keywords Behavior • Coral preference • Planktivore

Introduction

Fairy basslets (*Gramma loreto*) are common, planktivorous coral reef fish (10-70 individuals per population) found throughout the Caribbean and Bahamas (Webster 2004). Ranging from 1-8 cm in total length (TL), these bicolored yellow and purple *G. loreto* appear to exhibit no sexual dimorphism; however, a previous study found that males on average tend to be larger (Asoh and Shapiro 1997; Kindinger 2016). Populations of *G. loreto* exhibit no coral preference on coral reefs, but they do favor high vertical relief due to the protection provided by ledges and cave corners (Freeman and Alevizon 1983; Kindinger 2016). While it is currently unknown whether coral assemblage is associated with community settlement, *Undaria* (often known as *Agaricia*) *agaricites* is the most likely candidate of the corals present at their depth range. Alvarez-Filip et al. (2011) found that when *Orbicella* spp. (massive corals) and *Agaricia* spp. (leafy corals) dominate the reef as coral cover increases, a higher rugosity is observed, which provides essential habitats for fish. In addition to contributing to a high rugosity, *U. agaricites*, at shallow depths (7-12 m) is an encrusting or unifacial plate coral that creates small ledges on the reef (Helmuth and Sebens 1993). As diurnal feeders, *G. loreto* potentially move from the protected alcoves of *U. agaricites* and feed on zooplankton (Webster 2004). Territory size of an entire population ranges from 0.9 to 10.0 m² (Kindinger 2016).

Gramma loreto are highly social animals whose populations are organized by a dominance hierarchy (Webster and Hixon 2000). Larger *G. loreto* aggregate at the front of ledges where they have better access to the plankton-filled water column, while smaller

individuals retreat to the back of the ledge (Freeman and Alevizon 1983; Webster and Hixon 2000). Individuals forced to the back of the ledge have reduced feeding rates and higher mortality due to their territory overlap with predators (Freeman and Alevizon 1983; Webster and Hixon 2000). Predators of *G. loreto* include *Cephalopholis fulva*, *Cephalopholis cruentata*, *Aulostomus maculatus*, *Caranx ruber*, *Mycteroperca tigris*, *Serranus tigrinus*, and *Ocyurus chrysurus*. Even though smaller individuals appear to be in a disadvantageous position, little emigration between populations is observed (Webster 2003, 2004).

In both marine and terrestrial environments, organisms are often required to make trade-offs and allocate more time to certain behaviors due to limited energy availability. Their allocation of time is often dependent on various abiotic or biotic factors (Matsumoto and Kohda 2000). For example, in *G. loreto*, the energy demand required to chase another individual may result in reducing the amount of time an individual can allocate towards feeding or reproduction (Kindinger 2016). Throughout the literature, time allocation studies have been completed to assess how the time spent performing a behavior changes due to outside influences (Talbot 1979).

A time budgeting study can be used to examine the behavior of prevalent coral reef fish such as *G. loreto*. The purpose of the current study is to examine the community structure and behavioral time budgeting of the fairy basslet, *G. loreto*.

H₁: *Gramma loreto* will demonstrate a coral preference for *U. agaricites*

H₂: Larger *G. loreto* (> 4 cm) will budget more time to the chasing and feeding behaviors

H₃: Smaller *G. loreto* (< 4 cm) will allocate more time to swimming and hiding behaviors

H₄: *Gramma loreto* will allocate more time to feeding when there are fewer piscivores

H₅: *Gramma loreto* will chase more invertebrates, compared to other functional groups

Little is known about the behavior of *G. loreto* outside of reproduction and territoriality, therefore, examining time budgeting of the species is important. In addition, by providing a more detailed behavioral background for *G. loreto*, researchers can observe the effect of a changing environment on the behavior of a population. For example, the presence of the invasive lionfish, *Pterois volitans*, who feed on *G. loreto*, may alter the time budgeting behavior of *G. loreto* on the coral reef (Albins and Hixon 2008). While multiple behavioral studies exist for freshwater systems, few exist for marine systems (Lima and Dill 1990). The proposed methodology of the current study could therefore be applied to similar small, planktivorous coral reef fish to understand their time allocation trends.

Materials and methods

Study site

The behavioral time budgeting of *G. loreto* was studied at the Playa Lechi dive site (12°9'36.2"N, 68°16'55.8"W) in Kralendijk, Bonaire located in the Dutch Caribbean (Fig. 1). The sloped, fore-reef dive site is located on the island's surrounding fringe reef. Between February and April 2017, all data were collected during mid-day dives (11:30 to 14:30) between 9-15 m. Captured footage of *G. loreto* and nearby coral and fish communities was analyzed at the CIEE Research Station in Kralendijk, Bonaire.

Data collection

To investigate the time budgeting of *G. loreto*, several populations (n = 8) were randomly selected for examination. A population was defined as a group of *G. loreto* who interact or swim within 1 m of each other regularly. To randomize population selection, all dives began

at a small mooring block with a white buoy located ~30 m southwest of the entry point at the edge of the reef crest. Before data collection began, a random number generator was used to calculate randomized depths between 9-15 m and randomized distances between 0-40 m for each individual population. The direction of travel from the white buoy was also randomized by using a random number generator to determine whether to swim with or against the current for each individual population. During each dive, the researcher began at the mooring block, traveled to the randomly calculated depth, and then swam the pre-established distance in the direction previously determined based on the current. Fin kicks estimated the distance traveled. Once kick cycles were completed, the closest *G. loreto* population was selected for video capture. In the case of no visible populations, the diver traveled further, rather than changing depths.

When a population was identified, the depth and number of individuals within a population was recorded. At each population, a GoPro Hero 4 was used to film the behavior of each population for 15 min. *Gramma loreto* acclimated to the diver for 30 s before footage capture began. To reduce disturbance of the population and to capture the full territory within the frame, the footage was captured from a 1 m distance at a 45° angle. At the end of 15 min, a fixed, 50 cm T-bar was placed within their territory and included in the video for body length determination of each *G. loreto*. For data analysis of coral cover and fish community, the T-bar was referenced to approximate a 2 x 2 x 1 m (L, H, D) frame with the observed *G. loreto* at the epicenter. Even if the diver changed position during filming, the frame position remained constant.

Data processing

Analysis of coral community

In the laboratory, based on the Atlantic and Gulf Rapid Reef Assessment (AGRR) protocol v5.4, the type of species and the number of coral colonies was determined by pausing the video

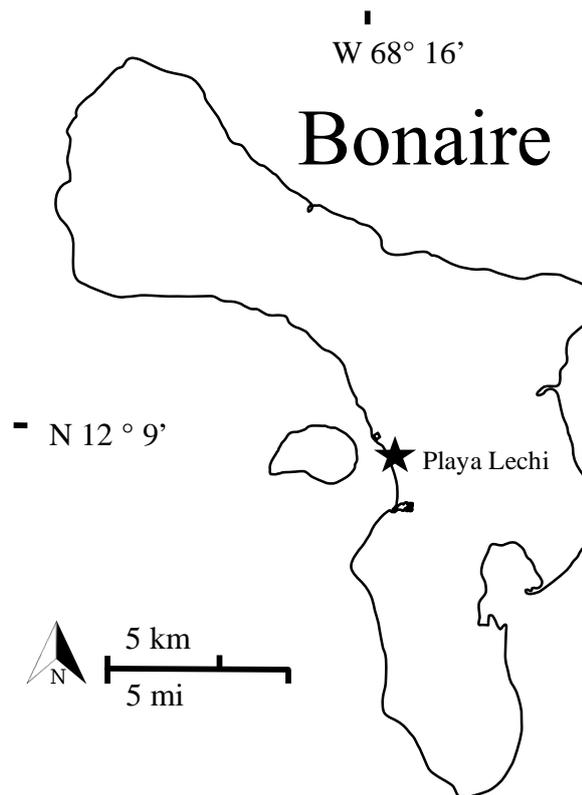


Fig. 1 Map of Bonaire showing the location of Playa Lechi, where the study took place

captured and identifying the colonies in an approximately 2 x 2 x 1 m (L, H, D) frame. Coral community analysis was performed on each population of *G. loreto* recorded. If no visible separation existed between the same species of coral, a connection was assumed and only one colony was recorded. Type of coral species was only recorded if a positive identification was made.

Analysis of the fish community

In the laboratory, the type of species and number of individuals per species that swam within an approximately 2 x 2 x 1 m (L, H, D) frame were recorded. Video analysis was performed by watching each recorded video of a population of *G. loreto* at half-speed. Due to distance and visibility, the type of species and total number were estimated for gobies. Individuals who were territorial (e.g. *Abudefduf saxatilis*, *Stegastes partitus*, *Stegastes planifrons*) were carefully observed to prevent double counting. Some

Table 1 The various behaviors demonstrated by fairy basslets, *Gramma loreto*

Observable behaviors of <i>G. loreto</i>		
Behaviors	Descriptions	Source
Feeding	Identified as bites into the plankton filled water column	(Webster and Hixon 2000)
Chasing	Characterized by approaching target fish with mouth open wide or curving body in a sigmoidal position. Aggressive defense sometimes results in biting.	(Asoh and Yoshikawa 1969)
Hiding	Quickly move behind crevice or into hole	Personal Observation
Stationary	Floating within the water column while performing no other noticeable behaviors	Personal Observation
Swimming	Characterized by swimming around territory with no clear demonstration of other behaviors	Personal Observation

species (e.g. *Myripristis jacobus*, *Haemulon chrysargyreum*) remained in the frame for multiple minutes but were only recorded once. If one fish remained hidden for most filming, after the 15 min recording period, the diver swam closer to the unidentified individual to ensure identification during data analysis.

Behavioral analysis of G. loreto

Before video analysis, each behavior was classified based on previous literature and field observations (Table 1). In the laboratory, video footage was analyzed to determine how much time each *G. loreto* in a population spent on each specified behavior. One stopwatch was assigned to each behavior and used to record the exact time an organism spent on each behavior. Fifteen minutes (total survey time) minus the sum of the time spent on each behavior equals the amount of time unaccounted for due to *G. loreto* visibility. The type of species that caused the *G. loreto* to hide or chase during the observation period was also noted.

Data analysis

In the laboratory, the length of each *G. loreto* was calculated by taking a screenshot from the captured video and using ImageJ to estimate the length based on the fixed T-bar placed within their territory. All means calculated were listed with standard deviation. All coral species

observed were given codes and categorized into types based on AGRRA protocol. All fish species observed in each video were categorized based on functional groups assigned via AGRRA protocol v5.4. Some species (e.g. *Lutjanus mahogoni*) were recorded as having two functional groups based on their diets. All quantitative data was organized and graphs were produced in Microsoft Excel 2013. Model I linear regression analysis was performed with R-Studio Version 0.99.467 to examine how size, population, and percentage of piscivores influenced the time budgeting of various *G. loreto* behaviors. Bar graphs were created to investigate coral colonies in proximity to aggregations of *G. loreto* and the overall time budgeting pattern of *G. loreto*.

Results

Coral community and fish community

The number of coral colonies located in proximity of each population of *G. loreto* ranged from 9 to 37 colonies. On average, massive and leaf/plate corals dominated these coral communities (Fig. 2A). Across all populations, ten species of coral were identified. More colonies of *U. agaricites*, *Porites porites*, and *O. annularis*, and *O. faveolata* on average were observed near the populations of *G. loreto* (Fig. 2B). Not all populations of *G. loreto* were

surrounded by the same species and types of coral.

The average number of fish counted in proximity of each population of *G. loreto* during a 15 min period was 62.4 ± 32.7 . Within a $2 \times 2 \times 1$ m frame of all populations of *G. loreto* survey, $17.6 \pm 5.8\%$ of the fish were herbivores, $80.3 \pm 8.1\%$ invertivores, and $7.6 \pm 6.1\%$ piscivores. Gobies, which are categorized as invertivores, were excluded from the percentage calculations due to sheer number. Six of the observed populations of *G. loreto* had between 20-70+ gobies of an unidentifiable species.

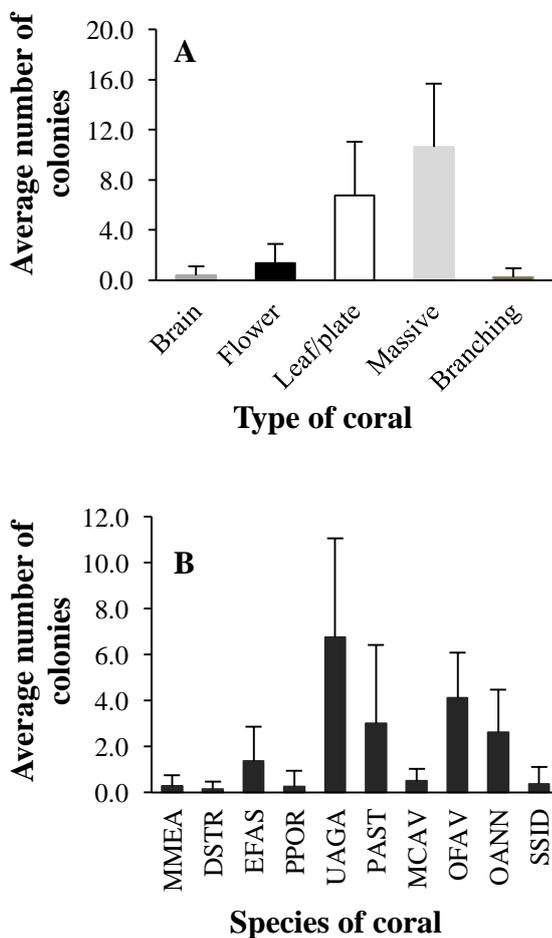


Fig. 2 Average number of coral colonies counted within a $2 \times 2 \times 1$ m frame of all observed populations of *Gramma loreto* ($N = 8$). (A) Number of colonies per coral type. (B) Number of colonies per species of coral. Abbreviations for species of coral are AGRRA coral codes. Error bars represent standard deviation. MMEA: *Meandrina meandrites*; DSTR: *Diploria strigosa*; EFAS: *Eusmilia fastigata*; PPOR: *Porites porites*; UAGA: *Undaria agaricites*; PAST: *Porites astreoides*; MCAV: *Montrastaea cavernosa*; OFAV: *Orbicella faveolata*; OANN: *Orbicella annularis*; SSID: *Siderastrea siderea*

Behavior of *G. loreto*

Over the course of experimentation, twenty-nine *G. loreto* ($N = 8$) were examined. On average, *G. loreto* spend more time feeding compared to all other observable behaviors (Fig. 3). All relationships concerning behavior were tested with a Model I linear regression. Population size, which ranged from 1 to 8 individuals, did not significantly influence the percentage of time each *G. loreto* spent feeding (Fig. 4). In addition, the percentage of time a *G. loreto* hid or fed was not influenced significantly by the percentage of piscivores in the nearby community of fish (Fig. 5). The average size of all observed *G. loreto* was 4.1 ± 1.3 cm. The size of an individual did not significantly influence the amount of time spent performing various behaviors (Fig. 6). Some individuals committed no time to hiding or chasing behaviors. During observation, several species caused *G. loreto* to hide including *Clepticus parrae*, *C. ruber*, *Sparisoma aurofrenatum*, *S. planifrons*, *Chromis multilineata*, *Lutjanus apodus*, *Stegastes diencaeus*, *H. chrysargyreum*, *Scarus taeniopterus*, *Bodianus rufus*, *Haemulon sciurus*, and *L. mahogoni*. In addition to hiding, various fish species were chased by *G. loreto* from their habitat including *Coryphopterus personatus*, *Canthigaster rostrate*, *S. taeniopterus* and multiple smaller *G. loreto*.

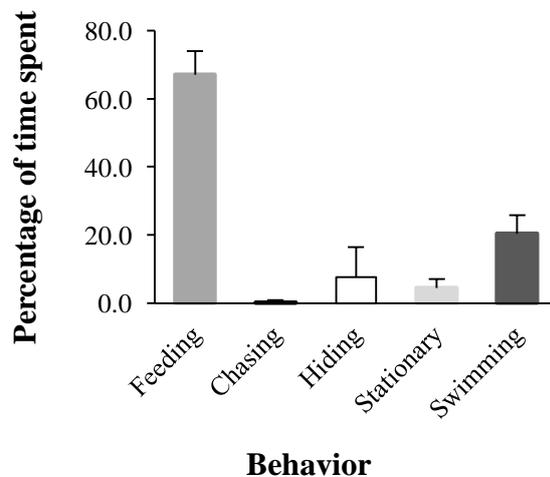


Fig. 3 The average percentage of time that a *Gramma loreto* ($N = 29$) performs five distinguished behaviors (Table 1). All error bars represent standard deviation

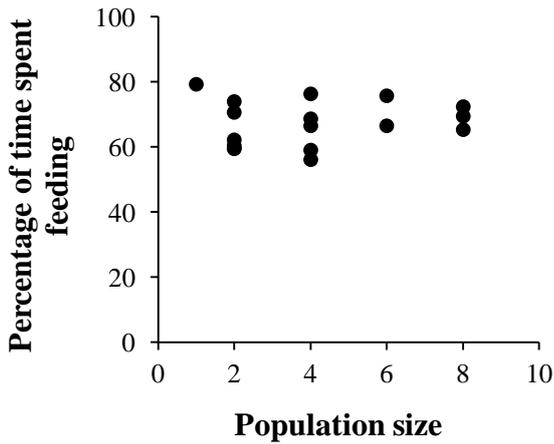


Fig. 4 The relationship between the number of *Gramma loreto* in a population and the percentage of time individuals ($n = 29$) spent feeding. The slope of the regression was not different from zero

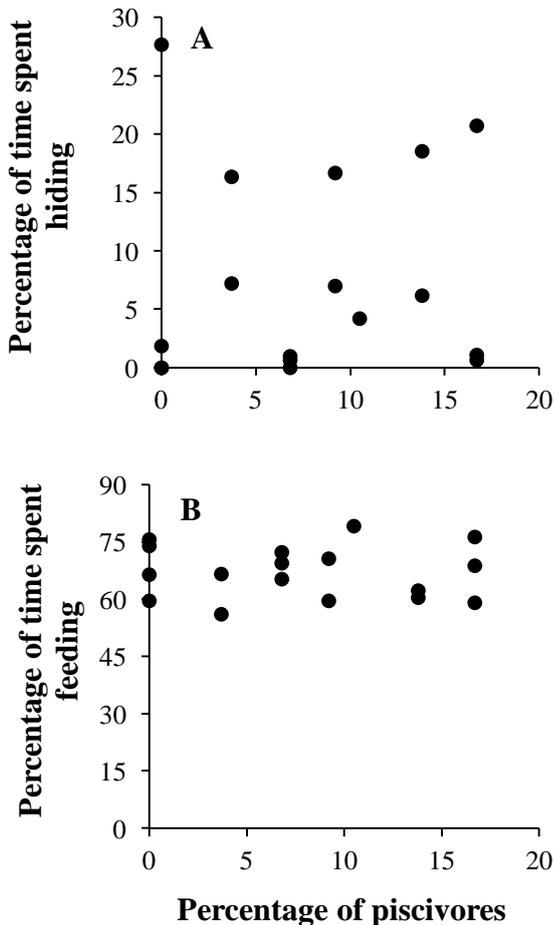


Fig. 5 The relationship between the percentage of piscivores within a $2 \times 2 \times 1$ m frame of a *Gramma loreto* population ($N = 8$) and the percentage of time spent hiding (A) and feeding (B). The slopes of the regressions in A and B were not different from zero

Discussion

Within aggregations, the common, planktivorous *G. loreto* display a multitude of behaviors including hiding, feeding, swimming, chasing, and floating stationary (Webster 2004; Kindinger 2016). The current study examines the community structure and behavioral time budgeting of the fairy basslet, *G. loreto*. Populations of *G. loreto* are found near massive and leafy corals because of the rugose environment they create. Invertebrates dominate the region around *G. loreto* populations at Playa Lechi, potentially due to the favorable access to the water column. *Gramma loreto* spend more time feeding than other behaviors; however, population size, fish size, and percentage of piscivores does not influence the percentage of time spent on each behavior. Similar to previous data, the study found that *G. loreto* more frequently chase other planktivores.

Coral community and fish community

Gramma loreto favors reefs with high rugosity because they provide protection from predators and access to the water column for feeding (Asoh and Shapiro 1997). Due to its encrusting and unifacial structure, it was hypothesized that *G. loreto* would demonstrate a coral preference for *U. agaricites*. At Playa Lechi, populations of *G. loreto* prefer massive and leaf corals (Fig. 2A). This preference aligns with previous research that states with increased coral cover, these two types of coral result in reefs with higher rugosity which *G. loreto* favor (Alvarez-Filip et al. 2011). More specifically, the populations of *G. loreto* live in proximity of *U. agaricites*, *O. faveolata*, and *O. annularis* significantly more than other AGRRA coral species (Fig. 2B). In Bonaire, these species of coral account for the highest percent cover in areas of low fishing pressure, such as Playa Lechi (Steneck et al. 2015). On the other hand, in the Grand Bahamas, Freeman and Alevizon (1983) noted that populations of *G. loreto* associated with *O. annularis*, *Acropora palmata*, and *Diploria labyrinthiformis*. Due to varying coral health and reef diversity in the

Caribbean, the coral preference of *G. loreto* may differ locally. In addition to examining the coral preference of *G. loreto*, measuring vertical relief is another method to characterize their habitat. In previous studies, a population's habitat occurred in significantly greater mean vertical relief areas when compared to the surrounding reef (Freeman and Alevizon 1983). Future experimentation could investigate whether this trend persists throughout the rest of the Caribbean.

The community of fish on the reef, based on AGRRA protocol, is composed of multiple functional groups (e.g. invertivores, herbivores, and piscivores). Fish communities in proximity to the populations of *G. loreto* are dominated by invertivores. Based on an AGRRA fish survey at Playa Lechi in 2017, herbivores and invertivores are found in equal abundance on the overall reef (unpublished data). The trend potentially differs from the observed communities around *G. loreto* because small,

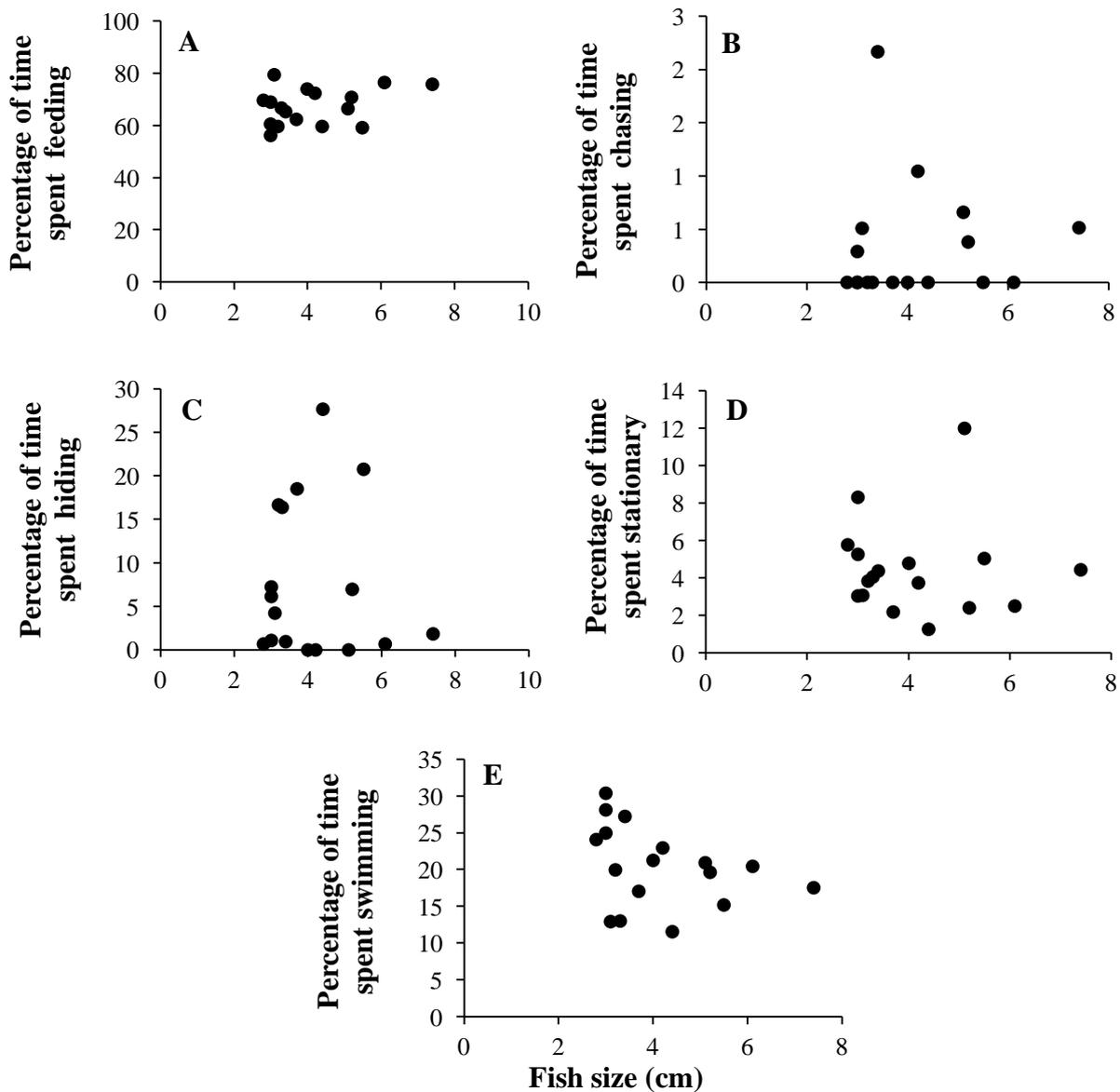


Fig. 6 The relationship between *Gramma loreto* size (cm) and the percentage of time spent performing various behaviors. Behaviors listed include feeding (A), chasing (B), hiding (C), stationary (D), and swimming (E). The slopes of the regressions in A-E were not different from zero

planktivorous fish favor protective habitats (i.e. alcoves and ledges). Some invertivores, like *G. loreto*, feed on zooplankton in the water column; therefore, the high abundance of invertivores may be due to *G. loreto* holding a prime feeding location on the reef. In addition, the surrounding community structure may have resulted from territorial invertivores (e.g. *A. saxatilis* and *S. partitus*) who favor similar habitats to *G. loreto* (Humann and Deloach 2002). In the Grand Bahamas, researchers recorded the number of interspecific encounters with *G. loreto* (n = 23) within 10 cm of the population. Including both non-chase and chase encounters, 56.6% of the fish community was invertivores and 51.5% herbivores, likely due to location availability and habitat type (Freeman and Alevizon 1983).

Behavior of *G. loreto*

Gramma loreto are found to spend most of their time feeding compared to other observed behaviors. Since *G. loreto* are planktivores, a single bite of the water column will not provide them with ample energy for the day. Medium-sized piscivores, such as adult *P. volitans*, can consume around 8.5 g per day (i.e. ~80 adult fish) (Albins and Hixon 2008). When piscivores feed, often consuming a single fish, can supply a significant proportion of their needed energy for the day. Therefore, the size of *G. loreto* zooplankton prey may cause them to allocate more time to feeding. Lima and Dill (1990) discuss how predation risk influences the rate of feeding per species. The habitat of *G. loreto* aggregations could reduce the species' overall threat of predation and allow them to allocate more energy to feeding.

Influence of population size and fish size

At the Playa Lechi dive site in Bonaire, the population size of *G. loreto* ranged from 1-8 individuals, which is smaller than aggregations in the Bahamas. For instance, in an environment with both *G. loreto* and *Gramma melacara*, the populations of *G. loreto* ranged from 11-19 individuals (Kindinger 2016). Another study in the Bahamas found aggregations of 10 to 70

individuals at 5-7 m (Webster 2004). The difference in population size between Bonaire and other Caribbean islands may be a result of habitat availability and predator abundance (e.g. *P. volitans* and *C. fulva*). On average, a higher abundance of *G. loreto* is observed in shallower waters (1-30 m); however, during the current repetitive study, the population number is relatively low (Kindinger 2016). The dive site did not allow observation below 30 m; therefore, it is unknown whether in Bonaire populations of *G. loreto* decrease dramatically at depth. During analysis, the data showed that population size did not influence feeding rate (Fig. 4). Such relationship may exist because the high abundance of zooplankton prevents a single *G. loreto* from reducing the stock enough to cause another *G. loreto* to feed less frequently, however, such a trend may change when populations reach 70 individuals.

In the current study, one hypothesis predicted that larger *G. loreto* would budget more time to consuming more plankton and to chasing away invaders of their territories. Another suggested that smaller individuals would allocate more time to swimming and to hiding due to their inferior location on ledges. Both hypotheses are rejected because none of the five recorded behaviors performed by *G. loreto* are influenced by their size. Freeman and Alevizon (1983) also found that when defending a territory from predators, the chasing of another species is independent of the defending fish size. In both the current study and previous research, in the case of chasing conspecifics, larger *G. loreto* were observed chasing more than smaller *G. loreto*. Such trend is expected because the dominance hierarchy displayed by the species results in larger individuals occupying higher territories on their ledge habitat (Freeman and Alevizon 1983). Smaller individuals found at the back of ledges experience more interaction with predators and less access to the water column (Webster and Hixon 2000). In terms of feeding, previous examination has found that feeding rates of *G. loreto* are lower for those individuals who are smaller (Kindinger 2016). A non-significant relationship between fish size and feeding in the

current study could have resulted from the small sample size, average population size, or even the reef complexity (i.e. access of each *G. loreto* to the water column). Smaller individuals are also expected to allocate more time to hiding due to their lower location, nevertheless, the lack of a relationship between the time allocated towards hiding and size is potentially observed for similar reasons explained for feeding.

Influence of other fish species

The relationship between the percentage of piscivores present on the reef had no influence on the amount of time *G. loreto* allocate to hiding. To determine how various factors (e.g. population size, fish community, and fish size) interact and affect the energy allocation of *G. loreto*, a principle component analysis (PCA) can be run taking new factors into account such as depth, territory size, and dive site (Marchand et al. 2003).

Throughout experimentation, only a small list of species induced the chasing or hiding of *G. loreto*. The current study hypothesized that *G. loreto* would spend more time chasing invertivores than fish categorized in other functional groups. All recorded aggression, except for the single attack on an herbivorous *S. taeniopterus*, was directed at other planktivorous species. Freeman and Alevizon (1983) observed the same trend when recording chase and non-chase behaviors. Compared to other reef species, such as *S. planifrons*, in the current study, *G. loreto* exhibit aggression less often. Previous research also found a similar trend with only 10% of the 490 interspecific encounters resulting in a chase by the *G. loreto* (Freeman and Alevizon 1983). Unlike *S. planifrons*, who are protecting their garden, *G. loreto* chase other planktivores due to competition for space and available zooplankton; therefore, the frequency of attack is low because these are not often limiting resources. In comparison to chasing, a wider range of fish species induced the hiding of *G. loreto*. Fish from all three functional groups caused *G. loreto* to hide. From observations, it is apparent that size and speed of the fish species

often contributes to whether the *G. loreto* retreated to the protection of surrounding coral. For example, only one *G. loreto* hid from a swiftly moving *S. aurofrenatum* which posed no threat to them, while several hid from a large swarm of *C. multilineata* that quickly overcame their habitat. In future studies, a more in-depth analysis can be used to determine what factors significantly contribute to the chasing and hiding performed by aggregations of *G. loreto*.

Conclusion

Overall, the current study adds more knowledge to a limited field. In addition, it provides time budgeting methods that can be applied to a multitude of other coral reef fish species. In future research, additional sample sites and more aggregations of *G. loreto* at various depths can be utilized to add more detailed information regarding behavioral energy allocation and the community structure of *G. loreto*.

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REPORT

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Monitoring factors that affect coral bleaching recovery in *Undaria agaricites*

Abstract *Undaria agaricites* is a Caribbean coral species that has in recent years been affected by coral bleaching. Coral bleaching poses an important issue for the overall health of coral species on the reef. The causes and effects of coral bleaching have been extensively studied, but how corals show recovery from bleaching events is not as well known. To investigate possible means of recovery from bleaching in *U. agaricites*, the roles of depth and angle were investigated at Playa Lechi, Bonaire. Coral pigmentation was used as an indication of possible recovery from bleaching. No significant relationship was found to show that depth or angle is influential in recovery from coral bleaching. However, there was some indication of recovery as indicated by pigmentation restoration. This study demonstrates that *U. agaricites* do show some minor recovery from bleaching. Further studies of bleaching in *U. agaricites* is required to understand the full effects of bleaching and the mechanisms corals use to recover.

Keywords Depth • Pigmentation restoration • Angle

Introduction

The Earth's equatorial region is home to many coral reefs, which are productive, vibrant marine communities. These coral communities have a crucial role in overall ocean health, however, coral reefs appear to be one of the most vulnerable marine ecosystems to environmental change (Hoegh-Guldberg 1999). Coral bleaching has become a major concern for coral reefs in recent decades. (Donner et al. 2007). Anomalously warm ocean

temperatures, climate variability, and anthropogenically induced thermal stress have all been attributed to mass coral bleaching events (Donner et al. 2007). Coral bleaching occurs from the expulsion of photosynthetic algae, known as, zooxanthellae, which results when a coral is exposed to environmental stresses. Bleaching can be linked to ultraviolet radiation or increased ocean acidification (Gleason and Wellington 1993). The combination of harmful events can lead to mass coral mortality and have severe negative impacts on environmental connectivity and economic sustainability through tourism and the dive industry.

Although the effects of coral bleaching can be detrimental, the resilience of coral reefs can make recovery possible. Recovery from coral bleaching can be observed by recolonization of healthy zooxanthellae that can produce photosynthetic pigments. Unfortunately, long-term studies suggest that coral reefs affected by global ocean temperature spikes may take many years to recover from bleaching induced mortality (Glynn 1993). Coral cover recovery can also be hindered by swift rates of macroalgal growth and recruitment caused by increased water temperatures (Roff and Mumby 2012). The implementation of marine reserves can assist in recovery from bleaching induced mortality by protecting important macroalgal grazers, like herbivorous fish. This helps balance the ecosystem, preventing a possible phase shift to macroalage-dominated state. By reducing competing flora around coral colonies, corals are also less likely to experience mortality due to bleaching (Mumby and Harborne 2010).

The southern Caribbean coral reefs are a prominent habitat for different coral species.

Undaria agaricites is a common coral in the southern Caribbean and has experienced coral bleaching. It grows as thin leafy plates or shelves and can be found throughout the Caribbean, Gulf of Mexico, and Bahamas (Huston 1985). It is located in a number of reef environments including lagoons, back reefs, fore reefs, and sea grass beds. Scleractinian corals such as *U. agaricites* serve a critical role as framework building of reefs in the Caribbean and globally (Rhodes 2010). One way that scleractinian corals like *U. agaricites* show the effects of coral bleaching is by reducing color pigmentation (Kleppel et al. 1989). A study done by Kobluk and Lysenko (1994) on the island of Bonaire, highlights the contributing factors of coral bleaching, such as rapid temperature change, on a southern Caribbean reef with a particular focus on *U. agaricites*. Although, no relationship between the bleached *U. agaricites* peripheral tissue and their growth orientation, depth, or locations of colonies could be determined. (Kobluk and Lysenko 1994). In Bonaire, coral bleaching of *U. agaricites* is still prevalent. This study aims to provide evidence of coral bleaching recovery of *U. agaricites* through pigmentation restoration and whether growth, orientation, and depth affect this recovery. Kobluk and Lysenko (1994), study was done from May 1992 to July 1992, however this study will be conducted from February 2017 to March 2017. This study aims to provide evidence that coral-bleaching recovery of *U. agaricites* is occurring at a colony level. It is also important to understand how coral-bleaching recovery varies at different depths and positions in the water column.

H₁: *Undaria agaricites* that are bleached at greater depths will show evidence of a faster recovery than *U. agaricites* in shallow areas

H₂: *Undaria agaricites* that are less vertically orientated will show evidence of a faster recovery

Materials and methods

Study site

Playa Lechi (12°09'36.2"N, 69°16'55.8"W) is located on the western side of the island of Bonaire in the Southern Caribbean (Fig. 1). Playa Lechi, Bonaire consists of a rocky beach, sand flats, and a fringing coral reef. It is critical to note that although Playa Lechi is a marine protected area, it is still vulnerable to and impacted by humans, which may inhibit possible bleaching recovery due to additional stress.

Data collection

A GoPro HERO4 Silver camera with a red filter was used to take multiple photographs of bleached *U. agaricites* specimens over the course of three weeks in February and March 2017. The photos captured each bleached coral in its entirety as well as a Coral Health Chart (Siebeck et al. 2006) beside it, not overlapping the coral. The coral health chart is provided by CoralWatch.org and indicates the health of a coral by referencing color to the concentration of symbionts on the coral. D1 and D2 are two reference points from the coral health chart that resembled the color of *U. agaricites*. D1 represented white (severely bleached) tissue while D2 represented brown pale tissue (mildly bleached) tissue on the coral. In total, 14 corals (*U. agaricites*) showing loss of pigment (white or pale tissue) were selected by the diver based on respective depth range. The corals chosen were in close proximity to each other and within the boundaries of Playa Lechi to increase accessibility during data collection. Once a coral was located, it was marked with flagging tape for future relocation. Four corals were at 5 - 10 m, five corals were at 10 - 15 m, and five corals were at 15 - 20 m depth. Coral colonies were monitored over a period of 3 weeks. Eight of these 14 coral colonies were photographed after one week and the remaining six were photographed after two weeks. All 14 corals were photographed during the third and final week. Monitoring the corals consisted of

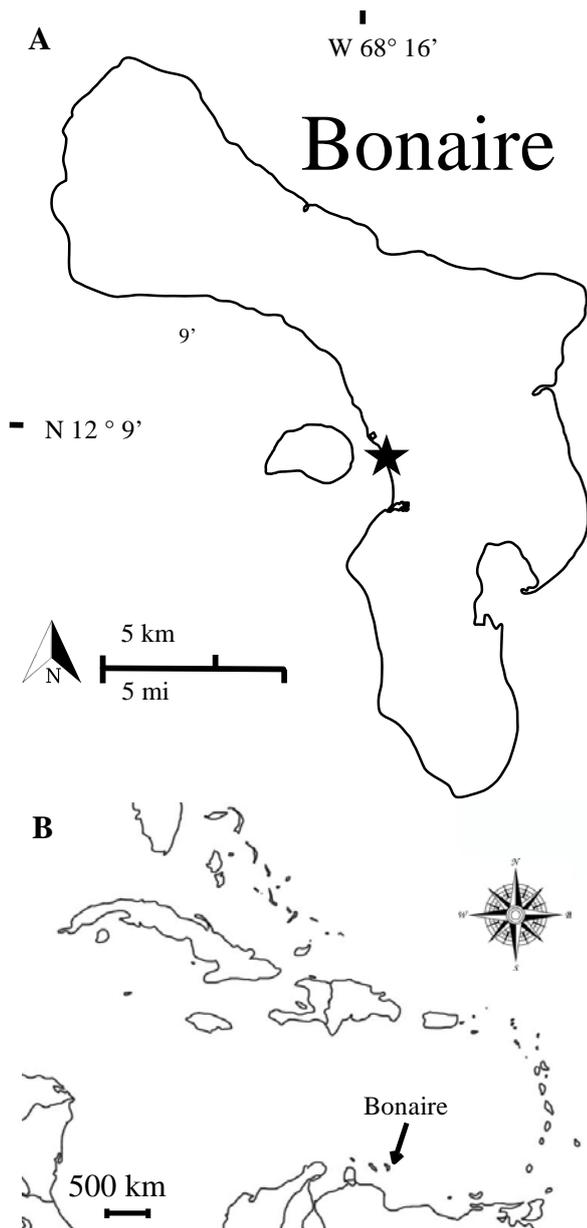


Fig. 1 (A) Map of Bonaire, a small island in the Dutch Caribbean. Marked point denotes the study site, Playa Lechi. (B) Map showing the location of Bonaire within the Caribbean Sea

recording depth, temperature, and angular orientation measurements with a protractor. All 14 coral angle measurements were recorded when the coral was initially located. If a coral was positioned completely vertical, the angle measurement was 0° . Each time a coral colony was photographed, several photographs were taken to ensure the best picture quality for computer analysis. Multiple photos also ensured that the coral photos resembled the

same frame positioning as in the initial photograph at week 0.

Photoshop editing

The photographs of the corals were transferred to a computer for analysis. In Adobe Photoshop CC, the outline of individual corals was traced. The background was blackened, except for the traced coral and D1 and D6 from the coral health chart. This was to ensure only the coral and respective coral health chart references could be seen for data analysis.

Pixel analysis

ImageJ was utilized to look at possible progression in pigmentation restoration based on angle position and depth. In ImageJ, a color threshold analysis was performed on the coral photos edited in Photoshop using the brightness tab. This step was used to clearly separate the color shades of D1 and D2 from each other as well as other color shades on the D color scale. The D color scale also was used to select what part of the coral tissue resembled D1 respectively D2 in color. Once the D1 color spectrum was selected, the number of pixels that matched that color shade were measured. The same procedure was repeated for the D2 color shade. This step showed the number of pixels that were present in each coral colony photograph within the scope of the D1 and D2 color shades. The number of pixels that appeared on the coral were then counted and entered into a data spreadsheet. Finally, the color threshold was adjusted to encompass the entire coral. The pixel count of the entire coral was recorded and divided by the total pixel count that represented the D1 and D2 color shade from the coral. That value was multiplied by one hundred to get a percent value. The percent values were then entered into an Excel data spreadsheet with corresponding angle measurements and depth values.

Results

Fourteen corals were photographed, but only pictures of eight corals were analyzed due to issues in photo quality. Two corals were measured at a depth range of 5 – 10 m, three corals were measured from 10 – 15 m, and three corals were measured from 15 – 20 m. A one-way ANOVA revealed that depth did not influence the D1 percent change ($F = 1.54$, $df = 8$, $p = 0.289$), D2 percent change ($F = 0.75$, $df = 8$, $p = 0.512$) and the D1 and D2 total percent change ($F = 1.00$, $df = 8$, $p = 0.422$) were not significant. The mean and standard deviation percent change in coral tissue with D1 color scale was $-0.01\% \pm 0.02$ at the deep depth (15 – 20 m), $-11.00\% \pm 19.00$, at the middle depth (10 – 15 m), and $17.60\% \pm 29.20$ at the shallow depth (5 – 10 m). The mean and standard deviation percent change in coral tissue with D2 color scale was $2.36\% \pm 5.04$ at the deep depth, $-15.00\% \pm 20.70$ at the middle depth, and $-0.10\% \pm 24.50$ at the shallow depth.

In the first week, the deepest corals showed a mean increase in bleaching while the other showed a mean decrease in bleaching (Fig. 2A). After 2 weeks there was a mean positive percent change in the deep depth and shallow depths indicating an increase in white or pale tissue (Fig. 2B). There is no available data for the middle depth range (Fig. 2B). Varied responses (increasing and decreasing trends) across depth ranges were seen after 3 weeks (Fig. 2C). D1 percent change shows negative relationships with depth at the deep and middle depth but a positive relationship at the shallow depth (Fig. 2C). D2 and D1 and D2 total percent change show a positive relationship at the deep and shallow depth but a negative relationship at the middle depth (Fig. 2C).

D1 percent change after 3 weeks showed a non-significant negative relationship with angle (Fig. 3A, $R^2 = 0.02$, $p = 0.743$). D2 percent change after 3 weeks shows a non-significant positive correlation with angle measurement (Fig. 3B, $R^2 = 0.01$, $p = 0.866$). D1 and D2 percent change after 3 weeks showed no apparent relationship relative to angle (Fig. 3C, $R^2 = 0.0$, $p = 0.961$).

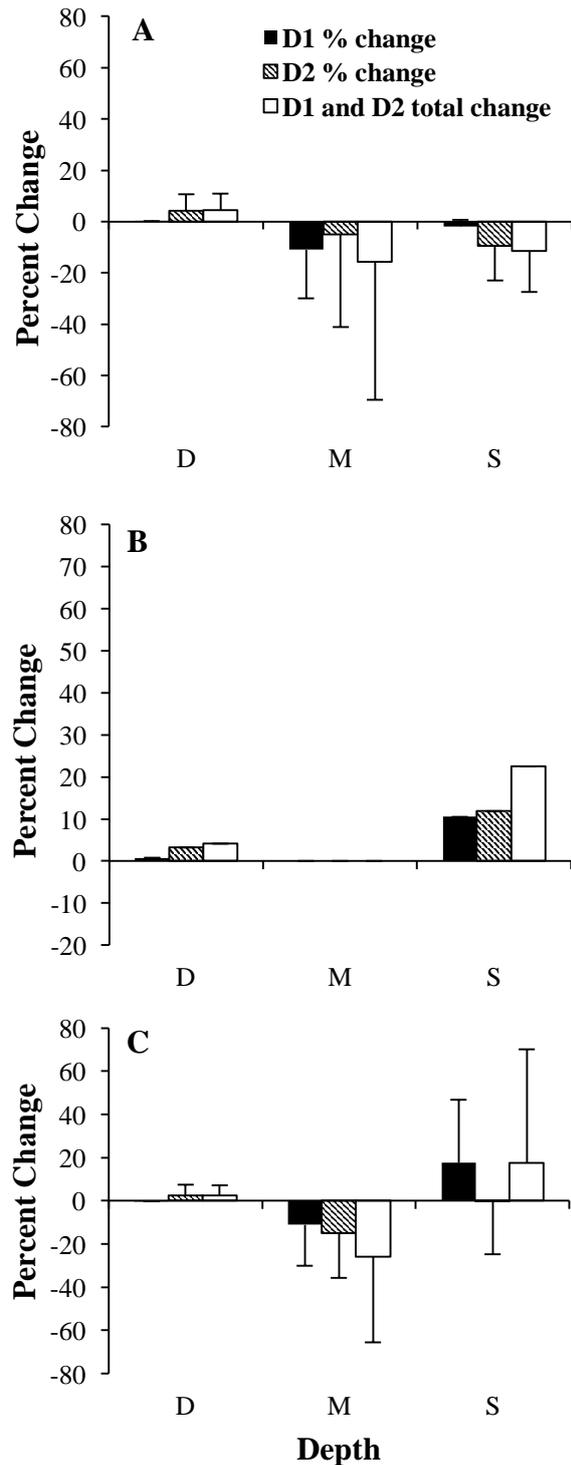


Fig. 2 D1, D2 and D1 and D2 percent area changes in *U. agaricites* ($n = 8$). CoralWatch health card was used to reference D1, white (bleached tissue) and D2, pale (mildly bleached tissue). Percent changes after 1 week (A), 2 weeks (B) and 3 weeks (C) shown for 3 depth ranges deep (D), middle (M) and shallow (S). “X” indicates no available data for middle depth. Standard deviation is represented by error bars

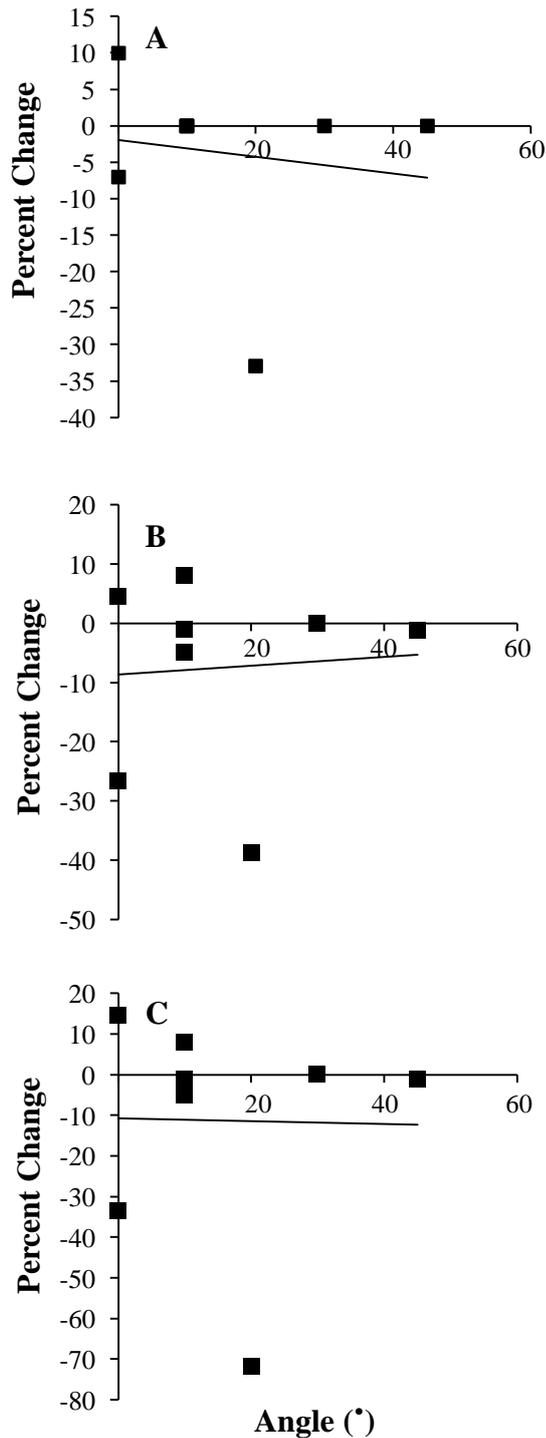


Fig. 3 Percent area change of D1 (A), D2 (B), and D1 and D2 combined (C) color shade from CoralWatch health card, indicating D1, white (bleached) and D2, pale (mildly bleached) section of *Undaria agaricites* (n = 8) over a 3 week period at Playa Lechi, Bonaire. Angle represents the angle of the coral in relation to vertical (0°)

Discussion

By monitoring pigmentation restoration rates, the process of bleaching recovery in affected coral colonies can be better understood. Recording the depth and angle measurements of *U. agaricites* corals over a 3-week period was a relative method of monitoring recovery in relation to abiotic factors. This study did not find correlations between coral pigmentation restoration and depth or, respectively angle, therefore both hypotheses were rejected.

Effect of depth on recovery

The comparison between recovery and depth showed no significant relationship, ultimately rejecting the original hypothesis, which, stated that deeper *U. agaricites* would show a faster recovery rate than the *U. agaricites* that were recorded at shallower depths. Although a slight increase in bleached tissue can be seen at the deep depth (15 - 20 m) after the 2nd week there is a lesser increase between D1 and D2 percent change seen after 1 week and 3 weeks. Even though these corals did not show signs of recovery, the progression of the bleaching appeared to slow, as the increase after 3 weeks was less than the increase after 1 and 2 weeks. Shallow corals showed a decrease in bleaching during the first week but an increase thereafter. At both shallow and deep depths, corals had increased bleaching between the initial week and week two and three. The difference observed in percentage change could indicate that *U. agaricites* both bleach and recover from the effects of bleaching at similar rates, regardless of depth ranges. However, *U. agaricites* found at the middle depths showed recovery. Optimum conditions and position of the coral measured at the middle depth range likely provide reason as to why there is improvement seen. At the same time the absence of data for percent change after 2 weeks does limit the credibility. While depth did not have an effect on bleaching recovery, other factors that affect coral health such as sedimentation and turbidity levels limiting sunlight and acquisition of necessary nutrients

could limit bleaching recovery (Lucas 2015). It is likely that biological factors such as pigmentation restoration are not affected by depth as depth does not affect growth rate of *U. agaricites* (Huston 1985).

Effect of angle on recovery

The second hypothesis was not supported by the data. Some corals did show recovery, however the results did not support that more horizontal angled corals positioned showed signs of faster recovery. The coral measured at 20 degrees and one of the corals measured at zero degrees had the highest bleaching recovery rate of all the corals, while the coral measured at 90 degrees had the highest progression of bleaching. The remaining *U. agaricites* did show very minor decreases in percent change from D1 to D2. As zero degrees is a vertical angle, this information indicates that direct sunlight may not be a critical factor in supporting the restoration process in *U. agaricites*. This data could indicate that coral angles, and therefore direct sunlight exposure, may not be very important in the overall restoration process. Angular positioning relative to light is likely to be irrelevant for bleaching recovery because light is used for nutrient acquisition (Kühl et al. 1995). Light intensity and warm temperature change throughout the day indicating that corals with different angles are still exposed to various effects of the same conditions.

Limitations and further study

The study was restricted by some limitations. The time of day of the in-situ data collection was not kept consistent over the course of three weeks. Light intensity could have slightly altered the coloration of the coral at the time the pictures were taken. Also, differences in photographs taken each week may have affected pixel count during Photoshop and Image-J analysis.

Subsequent research to further this work could possibly include repeating this experiment with a longer duration of study. A

longer duration of study can track bleached corals over a longer time period. It would be important to look at additional factors such as human-induced eutrophication or comparing bleached corals both in and out of marine protected areas. Other studies have shown evidence that coral growth rates can increase as a direct cause of increased eutrophication (Tomascik and Sander 1985). This study could also be developed by focusing on algal coverage on bleached corals as well as surrounding coral species in regards to competition with corals that are bleached. These factors could limit coral regenerative health, which may slow the bleaching restoration process. Finally, using more than one study site could provide more conclusive data about the recovery from coral bleaching in Bonaire and the southern Caribbean. Implications to this study included further research on the affects of coral bleaching recovery.

Conclusion

It can be determined that while coral bleaching is occurring on Bonaire, there is not a direct relationship between recovery of bleached corals and specific depths. Also, angular position of corals likely does not contribute to overall pigmentation restoration in *U. agaricites* corals. However, it is critical to continue to implement conservation strategies to stimulate healthy coral reef processes such as recovery from bleaching events. If not, there may be dire consequences to such a vulnerable and critically biodiverse marine ecosystem.

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REPORT

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Prevalence and progression of the black-spot causing parasite in *Acanthurus bahianus*, *Acanthurus coeruleus*, and *Caranx ruber*

Abstract Marine diseases are difficult to track because of the sheer size and connectivity of the world's oceans. Since 1985, a parasite that causes black spots has been observed on multiple coral reef fish in the Caribbean. Little is known about the spread and effects of this parasitic infection. Multiple surveys were conducted at Playa Lechi dive site in Kralendijk, Bonaire to determine the percentage of the populations of *Acanthurus bahianus*, *Caranx ruber*, and *Acanthurus coeruleus* with the black-spot parasite at 2, 5, 11, and 18 m depths. Water samples and invertebrates were collected from the feeding grounds of all three species for later testing to detect parasite presence. In *A. bahianus*, there was a higher percentage of fish with the parasite at shallower depths. When the results at 5, 11, and 18 m were compared to surveys in 2012 and 2015, there was a significant decrease in parasitised fish between 2012 and 2017 ($p = 0.027$). When comparing all three species of fish surveyed in 2017, there was a significant difference between the percentage of fish infected and the species type ($p < 0.001$). It is possible that there could be a decline in the prevalence of the understudied black-spot disease from 2012 to 2017; however, the parasites have been observed in many other coral reef fish species since 2015. More research is needed to examine the effects of the parasite on its hosts as well as an identification of the parasite to determine the lifecycle.

Keywords Disease monitoring • Host cycle • Parasite infection

Introduction

The Caribbean is a hot spot for many marine life diseases. However, many of these diseases are difficult to track or recognize due to the size and connectivity of the world's oceans. The effects of marine diseases may be amplified by environmental stressors, such as ocean acidification and ocean temperature rise (Hoag 2012). These diseases can originate from many sources, including bacteria and parasites. The black-spot parasitic infection has been observed in Bonaire, Curaçao, Belize, and the Turks and Caicos Islands since 1985 (Hoag 2012; Bernal et al. 2016; Peachey et al. unpublished data). This black-spot disease was first studied in detail in 2012 on *Acanthurus bahianus*, a Caribbean reef fish (Penn 2012). The black spots were found on 89% of *A. bahianus* around the island of Bonaire, Dutch Caribbean (Penn 2012). A similar presentation of black spots appears to occur in *Acanthurus coeruleus* and *Caranx ruber* on Bonaire (Jarnagin 2015).

It was found that the black-spot disease is caused by a parasite, and that the diseased fish are usually located at shallower depths between 2-5 m, which have higher water temperatures (Hoag 2012; Penn 2012; Rodriguez 2013). These black spots have been associated with infections by trematodes, turbellarians, and protozoans (Bernal et al. 2016). Each parasite in the fish is an isolated individual within a cyst, causing the visible black spot on the body of *A. bahianus* (Rodriguez 2013). There is no current evidence to suggest that the parasite has any negative effects on the organs or bodily functions of the host fish, besides causing

physical damage to the fin rays when the cysts are located there (Rodriguez 2013; Bernal et al. 2016). However, there are instances of wounds from the dermal parasites causing *Vibrio* infections in *Zebrasoma flavescens* (Kent and Olson 1985). Additionally, there has been no reported correlation between infection percentage and abundance, diet, mobility, schooling behavior, or position in water column (Bernal et al. 2016). *Acanthurus bahianus* and *Acanthurus coeruleus* play a vital role in coral reef ecosystems as herbivores that prevent the algal biomass from taking over the reefs (Lewis 1986). For this reason, conservationists must ensure the stability of these species to maintain a coral-dominated reef. *Acanthurus coeruleus* and *A. bahianus* are in the same family, and as a result share similar genetics and traits, increasing the likelihood that this parasite could be infecting both species of surgeonfish.

In 1986, *C. ruber* was found to be the host to *Opecoeloides brachyteleus*, a trematode parasite off the coast of Puerto Rico (Dyer et al. 1986). However, there is no further research on the effects this parasite has on the fish itself. In 2013, the unknown parasite in *A. bahianus* was identified as a type of digenetic trematode (Rodriguez 2013; Jarnagin 2015). If the identification is correct, the type of parasite that infects *A. bahianus* could be the same parasite that infects *C. ruber*.

Trematodes have a three-stage life cycle, and *A. bahianus* is most likely the secondary intermediate host. There are unknown hosts for the primary intermediate stage and the definitive or final stage of the life cycle. In most trematodes, the primary intermediate host is a species of snail or small invertebrate, and the definitive host is a type of predator or larger animal (Routtu et al. 2014). The diet of *A. bahianus* consists of green or brown algae and small invertebrates and is similar to the diet of *A. coeruleus*, which consists mainly of algae (Humann and Deloach 2002). *Acanthurus bahianus* eat sand to aid in the digestion of algae (Deloach 1999). This behavior makes it possible for *A. bahianus* to consume a parasite-infected invertebrate either in the sand or in the algae. *Caranx ruber* eat small invertebrates as well,

which suggests that the parasitic vector is an invertebrate that *A. bahianus* and *A. coeruleus* also consume. Due to the unknown severity and number of fish infected by this disease, along with the recent discovery of *A. coeruleus*, *C. ruber*, and other coral reef fish displaying black spots, the identification of the parasite and its life cycle would give insight into the population health of *A. bahianus*, *A. coeruleus*, and *C. ruber* and an idea of overall reef health (Hayes and Goreau 1998; Marcogliese 2005).

This study evaluated the prevalence of *A. bahianus*, *A. coeruleus*, and *C. ruber* with black spots. In addition, the study aimed to establish a tissue bank to allow identification of the black spots in *A. bahianus*, *A. coeruleus*, and *C. ruber*.

- H₁: The prevalence of the black-spot disease in *A. bahianus*, *A. coeruleus*, and *C. ruber* is higher at shallower depths
- H₂: The prevalence of the black-spot disease in *A. bahianus* has increased when compared to 2012 and 2015 surveys
- H₃: There is a higher percentage of *A. bahianus* infected with the parasite currently when compared to all other fish species studied

Materials and methods

Study sites

The laboratory portion of this study was conducted at the CIEE Research Station in Kralendijk, Bonaire. The island of Bonaire is located about 80 km north of Venezuela and 79 km east of Curaçao (Fig. 1). Dives were conducted at the Playa Lechi dive site (12°9'36.2" N, 68°16'55.8" W), which is a popular dive site on the leeward (western) side of the island (Fig. 2). Observations of *A. bahianus* feeding behavior were recorded to determine a possible means of parasite transmission. After a popular *A. bahianus* feeding area was recognized, turf algae and invertebrate samples in the sand flats were collected to preserve for later parasitic DNA presence testing. The prevalence of the black

spot disease in *A. bahianus*, *A. coeruleus*, and *C. ruber* was surveyed at the Playa Lechi dive site to calculate the current population of parasitized individuals. Intertidal snails were collected on the leeward (western) shore of Kralendijk at the Playa Lechi dive site from the dock to 100 m north of the dock.

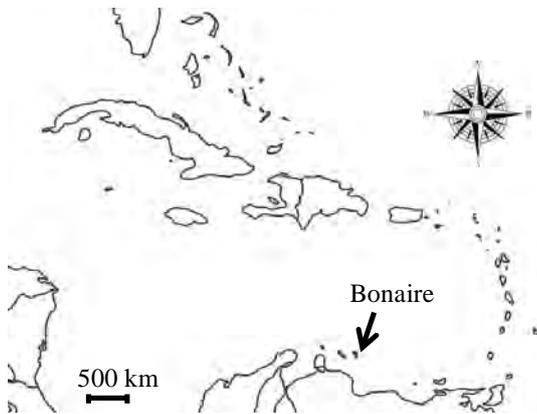


Fig. 1 A map of the Caribbean. The island of Bonaire is indicated by the arrow in the map

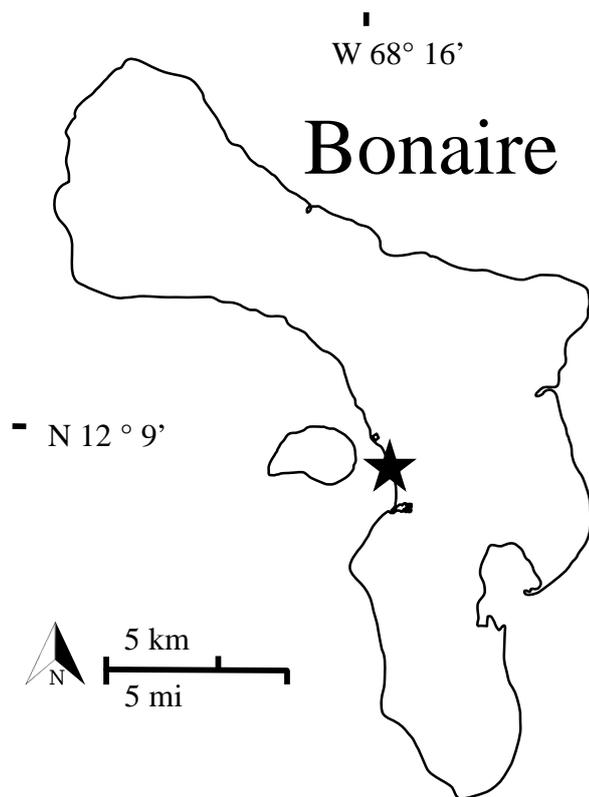


Fig. 2 A map of the island of Bonaire. Playa Lechi dive site is indicated by the star on the leeward side of the island

Study organisms

Acanthurus bahianus lives mostly in shallow, sandy areas in the Western Atlantic between 4-24 m in depth in schools that normally include *A. coeruleus* (Humann and Deloach 2002). Their total body length ranges from 15-31 cm (Humann and Deloach 2002). *Acanthurus coeruleus* have a total body length of 13-38 cm and usually reside at approximately the same depths as *A. bahianus* (3-18 m). They can be found within a similar geographical range in the Western Atlantic and in the same areas of the coral reef (Humann and Deloach 2002). *Caranx ruber* can also be found in the same geographical regions as *A. bahianus* and *A. coeruleus*; however, they usually swim in open water over the reefs (Humann and Deloach 2002). *Caranx ruber* are opportunistic fish that often feed with goatfishes and stingrays (Humann and Deloach 2002). *Caranx ruber* usually grow to a total length of 20-61 cm (Humann and Deloach 2002).

Prevalence of the disease

Roving diver surveys were conducted from March to April of 2017 at Playa Lechi dive site at four different depth intervals (2, 5, 11, and 18 m). All surveys began between 15:00-16:30 for consistency. A random number generator was used to determine whether the survey would be performed north or south of the Playa Lechi swimming area on each given survey date (1 = north; 2 = south). Fifteen min surveys at each depth were conducted four times to collect data on disease prevalence. The total number of *A. bahianus*, *A. coeruleus*, and *C. ruber* observed during each survey were tallied and the presence or absence of black spots on each specimen was recorded. If a fish of interest was seen, but too far away to determine the presence of spots, the fish was not counted in the tally.

Parasite vector

Four species of marine intertidal snails were collected from the intertidal zone at the shore of the Playa Lechi dive site to determine if a

parasite could be encouraged out of a snail's body by shining a bright light on the snails for several hours (Routtu et al. 2014; Table 1). Five individuals of each snail species collected were separated into containers with 50 mL of sea water filtered with 0.7 μm pores to remove DNA and RNA fragments. These containers were then placed under four different photosynthetically active radiation (PAR) intensities of 18.2, 21.0, 60.5, and 73.9 watts m^{-2} . The snails were kept in the lit containers for 24 hrs. Each sample was studied for parasite ejection every hour for the first 4 hrs, then once at 12 hrs and once at 24 hrs. After 24 hrs, the water from each container was filtered and the filter paper was stored at $-40\text{ }^{\circ}\text{C}$ for future environmental DNA (eDNA) analysis.

Two dives were conducted to observe means of disease transmission by watching *A. bahianus* behavior and collecting invertebrate samples in the sand flats of the Playa Lechi dive site. Small invertebrates found in the sand flats (2 – 5 m depth) were collected, homogenized, and preserved to be analyzed as a possible alternate vector alongside the turf algae samples. One water sample of 500 mL was taken above the sand flats, which is an area that *A. bahianus*, *A. coeruleus*, and *C. ruber* feed (Easley, pers. obs.). The water samples collected were filtered and the filter paper was stored at $-40\text{ }^{\circ}\text{C}$ for future eDNA analysis to determine if parasitic DNA can be detected in the water column. *Acanthurus bahianus* were observed to determine the type of algae they commonly feed on. These species of algae were inspected for parasitic cysts. Tissues were sampled and preserved for future polymerase chain reaction

(PCR), gel electrophoresis, and enzyme digestion analysis to determine if the algae could be the parasitic vector, rather than an invertebrate.

Data analysis

After data collection was complete, the disease prevalence data for 2017 were organized by species and by depth of survey to calculate percentage of infected fish species. The mean percentage and standard deviation were also calculated for each depth group per species. After calculations, all percentages were graphed to compare results between species and depths. A two-way ANOVA and a Tukey HSD test were used to analyze any differences in percentage of infected fish between species and depths.

Data from Penn (2012) and Jarnagin (2015) were included in this analysis because they too surveyed the percentage of infected *A. bahianus*. Their results were graphed alongside 2017 data in order to visualize changes in the infection percentage between years. A two-way ANOVA of percentage of infected *A. bahianus* between year(s) (2012, 2015, and 2017) and depth(s) (5, 11, and 18 m) was conducted. Since the 2015 study did not survey at 2 m depth, this was not included in the comparison. Penn (2012) conducted surveys at 11 m, while Jarnagin (2015) performed surveys at 12 m of depth. A comparison was performed including 11 m and 12 m measurements from all three studies due to their minimal difference. A Tukey HSD test was used to determine which individual year(s) or depth(s) had significant differences in infection percentage.

Table 1 Common and scientific names of all intertidal snail species collected at Playa Lechi for the parasite vector portion of the study

Scientific Name	Common Name
<i>Nerita fulgurans</i>	Antillean Nerite
<i>Nerita tessellata</i>	Checkered Nerite
<i>Nerita versicolor</i>	Four-tooth Nerite
<i>Nerita peloronta</i>	Bleeding Tooth Nerite

Results

Disease prevalence analysis

After a total of 120 min of observation, a total of 544 fish were observed. The number of spots on each fish ranged from one or two faint spots, to numerous spots that almost covered the body of the fish. For *A. bahianus*, $81 \pm 13\%$ of the fish observed at 2 m, $75 \pm 9\%$ at 5 m, $50 \pm 14\%$ at

11 m, and $44 \pm 18\%$ at 18 m were found to be infected with the parasite (Fig. 3). There was a significant difference in the frequency of infection of *A. bahianus* between the four depths surveyed ($F = 6.98$, $df = 3$, $p = 0.005$). There was no significant difference between the infection frequency and depth of both *A. coeruleus* and *C. ruber* (Fig. 3).

In the 2017 survey, there was no significant difference between the percentage of infected fish of all species at each depth, but there was a significant difference in percentage of infected fish between species ($F = 12$, $df = 2$, $p < 0.001$) (Fig. 3). A Tukey HSD test determined that the overall percentage of infected *A. bahianus* was significantly greater than *A. coeruleus* ($p = 0.0016$). Additionally, *C. ruber* also had a significantly higher percentage of infection when compared to *A. coeruleus* ($p = 0.0002$).

The analysis of variance of the disease prevalence in *A. bahianus* from 2012, 2015, and 2017 revealed that there was a significant difference between all years and depths when compared collectively ($F = 5.44$, $df = 2$, $p = 0.011$; $F = 4.03$, $df = 2$, $p = 0.029$, respectively) (Fig. 4). The results of the Tukey HSD test showed that there was a significant decrease in the percent of

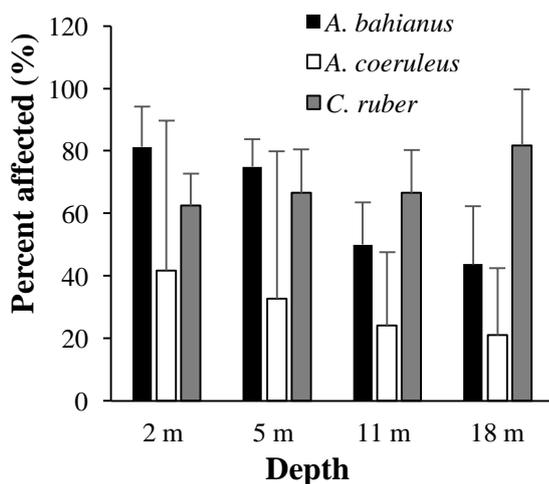


Fig. 3 Average percentage of parasite-infected *Acanthurus bahianus*, *Caranx ruber*, and *Acanthurus coeruleus* observed at 2, 5, 11, and 18 m. There is a significant difference between all species ($p < 0.001$). All error bars are standard deviation

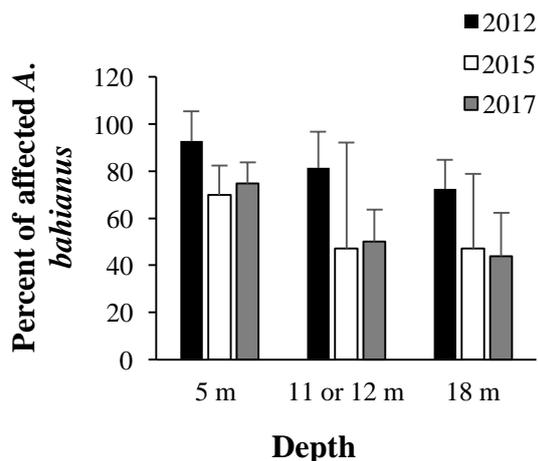


Fig. 4 Comparison of percentages of infected *Acanthurus bahianus* between surveys in 2012, 2015, and 2017. There is a significant difference between all years and depths ($p = 0.0104$; $p = 0.0294$, respectively). All error bars are standard deviation

infected *A. bahianus* between 2012 and 2015 ($p = 0.017$) and between 2012 and 2017 for all depths ($p = 0.027$). However, there was no significant difference between 2015 and 2017. For all years, there was a significant difference between the percentage of infection between 5 m and 18 m ($p = 0.031$) (Fig. 4).

Parasite light-extraction test

After 24 h of observation, there were no visible parasites in each of the four samples of varying light intensities.

Discussion

Disease prevalence study

This study showed that infected *A. bahianus* is found to be more prevalent at shallower depths (2 m and 5 m) (Fig. 3). On the other hand, *A. coeruleus* and *C. ruber* exhibit no significant difference of infection percentages between depths.

The null hypothesis of H_1 states there is no significant difference of infection percentage between all species at all depths surveyed. This null hypothesis is rejected for *A. bahianus* and accepted for *A. coeruleus* and *C. ruber*.

Therefore, these results lend support to H₁. These results support a 2012 study that observed a significant percentage of the parasite-infected fish at shallower depths (Penn 2012). It is likely that the infection starts in shallower, warmer waters, but the parasite can survive in deeper, cooler depths if its host travels there.

The comparison section of the current study is promising for reef fish species in Bonaire. There seems to be a decline, or at least a stasis, of infection frequency of *A. bahianus* when compared to Penn (2012) and Jarnagin's findings (2015). This trend could mean that the parasite's host cycle in *A. bahianus* is not as successful as before. The parasite population may have adapted to using other fish species as their secondary hosts, causing the frequency of infected *A. bahianus* to stay the same while it spread to other species. Jarnagin (2015) observed the black spots in 11 other marine fish species. Alternatively, the small sample size in the Penn study (2012) could have caused the infection percentage in 2012 to appear higher than it was in actuality. If this is the case, the percentage of infected *A. bahianus* could potentially be constant since 2012. Regardless, these results reject the null hypothesis that there is no significant difference between the infection frequency from 2012-2017.

Since surveys in 2012 and 2015 were conducted by different researchers, there is a chance that there were some deviations and differences between how the protocol was followed in all three studies. This could have led to deviations in results. Two different surveyors conducted research on different species at the same time, which gives room for error. It is possible that one of the researcher's positioning while conducting the survey gave them an advantage or disadvantage of viewing spots on surveyed fish. Overall, possible sources of error were minimized as the survey protocol was followed closely by the surveyors who worked together to correctly classify each observed fish.

Parasite vector

Future analysis should identify the primary intermediate host to the secondary intermediate

host of the black-spot parasite life cycle. More reliable techniques are needed to determine the life cycle of the parasite. The light extraction method conducted in this study needs to be thoroughly developed with a detailed methodology to identify the primary host (Routtu et al. 2014). There is a chance the parasites do not respond to light exposure or the parasites were extracted through the light exposure, but were not visible to the researcher. In case the parasites were ejected into the water, samples from each container were filtered and preserved. In the future, these water samples, along with parasitic and invertebrate tissue and turf algae samples, will be analyzed using restriction enzyme and eDNA analysis. This will indicate if the parasitic DNA is present in the collected samples. Confirmation of parasitic DNA in eDNA and/or tissue samples could be obtained by using restriction enzymes and trematode-specific primers and subsequently comparing them using PCR analysis to determine if the same DNA was found in both samples. Trematode-specific sequences in the Routtu et al. (2014) protocol would be a promising starting point in finding the correct primers to test for the black-spot parasite's DNA in all water and tissue samples collected. DNA sequencing of positive samples using a database, such as GenBank, would allow for a better understanding of the life cycle and behavior of the parasite. Genetic identification would allow for verification of the taxonomic identification of the parasite given by Rodriguez (2013) and Jarnagin (2015). The hypothesis that black spots are caused by the same species of parasite in all three species of fish studied can neither be accepted nor rejected at this time. Subsequently, future research could investigate whether the disease has the same source for all fish species with the spots.

It may be worthwhile to conduct a behavioral study to determine if *A. bahianus*, *A. coeruleus*, *C. ruber* change their behavior when infected with the parasite. Observing behavioral changes could give insight into how the parasite affects its host. Additionally, a researcher might consider another behavioral study to determine whether or not unaffected fish change their

behavior when they are near other parasite-infected fish. A greater understanding of infection-associated behavior changes would allow insight into intraspecific and interspecific relationships between fish on the reef (Barber et al. 2000).

Before examining the parasite across several species, researchers should examine whether the parasite threatens the overall health of the fish before examining the parasite across several species. Since black spots have also been observed on other islands around the Caribbean, it would be beneficial to track the spread of the parasite, especially if it is found to have negative effects. Additionally, a necessary component of future studies is an analysis of the physiological impacts of parasitic infection, which are currently unknown.

Overall, the study has assisted in the continuous monitoring of the spread of black spot disease in *A. bahianus*, *A. coeruleus*, and *C. ruber*. In addition, the study has provided baseline data and tissue samples that can be used in future studies to identify the parasite and determine its life history. There is an immediate need for more research to establish the host cycle of this parasite as well as the effects, long-term and short-term, on its hosts. With answers to these pressing questions discussed above, future conservationists and wildlife managers can whether or not they must take action against the parasite, or allow it to naturally take care of itself.

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REPORT

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Tracking dark spot syndrome on the coral reefs of Bonaire

Abstract Coral diseases have increased in their pervasiveness since the mid-1990s and are currently threatening some of the most biodiverse ecosystems in the world — coral reefs. Dark spot syndrome (DSS) is the most prevalent coral pathogen in the Caribbean, affecting scleractinian corals throughout the region. DSS is characterized by irregularly-shaped, dark discolorations of maroon, brown, and purple hues on normal coral tissue. This may make corals more susceptible to infection by other diseases. This study investigated DSS by analyzing lesion activity (progression or recovery) through afflicted polyp counts as well as analyzing the transmission of DSS *in situ*, using video analysis of the following scleractinian corals: *Siderastrea siderea*, *Stephanocoenia intersepta*, *Orbicella annularis*, *Orbicella faveolata*, *Montastraea cavernosa*, *Orbicella franksi*, and *Undaria agaricites*. Over a minimum of 15 and a maximum of 19 d, DSS cover increased at an average rate of $18.4 \pm 0.4\%$ of afflicted polyp counts per colony. Although DSS-afflicted coral counts had decreased over the span of 26 d, mean DSS progression per coral occurred at a greater rate than initially predicted. This can be detrimental to the reef as a larger afflicted area leads to greater vulnerability to infection. Ecosystem-wide consequences, including loss of fish biodiversity, arise when considering the vulnerability of major reef-building corals such as *O. annularis* and *M. cavernosa*. Severe reef degradation due to loss of structural complexity and habitat loss would result in a catastrophic loss of fish diversity and abundance, ultimately leading to a potential phase shift to an algae-dominated reef.

Keywords *Stephanocoenia intersepta* • Caribbean • Coral disease

Introduction

Coral reefs, some of the most diverse ecosystems in the world, are currently being threatened by an increase in the pervasiveness of coral diseases, first observed in the mid-1990s (Sutherland et al. 2004). Coral diseases, defined as any impairment of vital body functions, systems, or organs, are causing significant losses in hard coral cover in the Caribbean (Weil 2004). The Caribbean has been known as a coral disease “hot spot” due to the high prevalence, high virulence, wide geographic distribution, and fast emergence of these diseases (Weil 2004). Dark spot syndrome (DSS) is one of these afflictions and has been identified as the most prevalent coral pathogen in the region (Meyer et al. 2016). DSS was first discovered in 1990 on the reefs of Islas del Rosario in the Colombian Caribbean and was mistaken for a type of bleaching (Solano et al. 1993). Further examination of DSS uncovered which coral species are most affected by the ailment: *Siderastrea siderea*, *Stephanocoenia intersepta*, and *Orbicella annularis*. The associated characteristics were also described, including round, small, dark spots growing larger over time. However, the causative agent is still unknown (Gil-Agudelo and Garzón-Ferreira 2001). DSS is characterized by irregular-shaped, dark discolorations of maroon, brown, and purple hues on healthy coral tissue. DSS begins as dark spots among normal tissue, however, DSS can include a dark band along the edge of tissues which slowly dies, leaving a ring surrounding dead coral in the later stages

(Goreau et al. 1998). The spot variant, DSS-II, being mostly observed in the southern and western Caribbean (Weil 2004). For this reason, this experiment will solely be looking at DSS-II syndrome.

The average rate of DSS progression is 0.12 mm d⁻¹ (Borger 2005). Borger (2005) photographed six colonies over the span of two years with no observed tissue mortality, as opposed to some tissue mortality reported by Goreau et al. (1998). The overall impact of DSS directly causing death of coral tissue was quite low; however the presence of DSS is thought to expose tissue to potential infection by other coral diseases. Therefore, DSS could be utilized as an indicator to predict future disease spread (Borger 2005).

The number of affected massive coral species has increased since the initial assessment of DSS in the Colombian Caribbean (Gil-Agudelo and Garzón-Ferreira 2001). DSS is currently found to afflict *S. siderea*, *S. intersepta*, *O. annularis*, *Orbicella faveolata*, and *Montastraea cavernosa* in Bonaire (Mathe 2015). In addition, 56% of *S. siderea* were affected by DSS in Bonaire (Goreau et al. 1998). The distribution of DSS was not found to be depth-dependent, however, DSS tends to be associated with specific corals and thus its distribution can be correlated with the distribution of these corals (Gil-Agudelo and Garzón-Ferreira 2001). It is not clear if DSS negatively affects corals directly, since afflicted polyps can still function, or rather DSS is a consequence of affected zooxanthellae, indicating environmental stress. It has been proposed that DSS lesions are likely a general stress response with respect to rising water temperatures (Borger 2005). Similar lesions were observed with field equipment irritation on *S. siderea*, possibly implying that the observed effect is rather an overall stress response (Borger 2005). Further study of DSS is a necessary step in fully understanding the mechanisms behind this complex condition.

While eastern Caribbean DSS has been measured (Borger 2005) no such effort has been undertaken in the southern Caribbean. Although studies attempting to quantify the rate of DSS

progression among colonies have been executed, there has been little research conducted regarding the activity of DSS lesions within individual coral colonies. Studying lesion activity not only provides insight into DSS progression or recovery but also how DSS lesions morph and interact with one another. This aids in fully understanding how DSS is affecting individual corals and, by extension, ecosystems as a whole. It is predicted that there will not be any observable changes in DSS progression as the study was conducted over a relatively short time period.

An *ex situ* disease transmission experiment concluded that direct contact and waterborne transmission showed no signs of infection and that artificially-created lesions completely healed over the course of 28 d, without signs of infection (Randall et al. 2016). There has never been an *in situ* DSS transmission study conducted. Therefore, this study will be analyzing transmission *in situ* to test the observational findings in a natural environment.

- H1: There will be no observable progression of DSS on individual coral colonies
- H2: There will be no transmission of DSS from affected colonies to healthy colonies

Materials and methods

Site selection

The dive site was located at Playa Lechi, Kralendijk, Bonaire (12°9'36.2"N, 69°16'55.8"W), a semi-arid island in the southern Caribbean (Fig. 1). This site was chosen due to its easy accessibility and its close proximity to the CIEE Research Station, Bonaire. The fringing reef at Playa Lechi has a relatively dense coral cover with patches of sand and rubble. Playa Lechi is a popular SCUBA diving site which experiences high diver traffic. During preliminary data collection, the greatest DSS occurrence was observed at approximately 15 m and 20 m; however, due to time and air constraints this study will be analyzing the

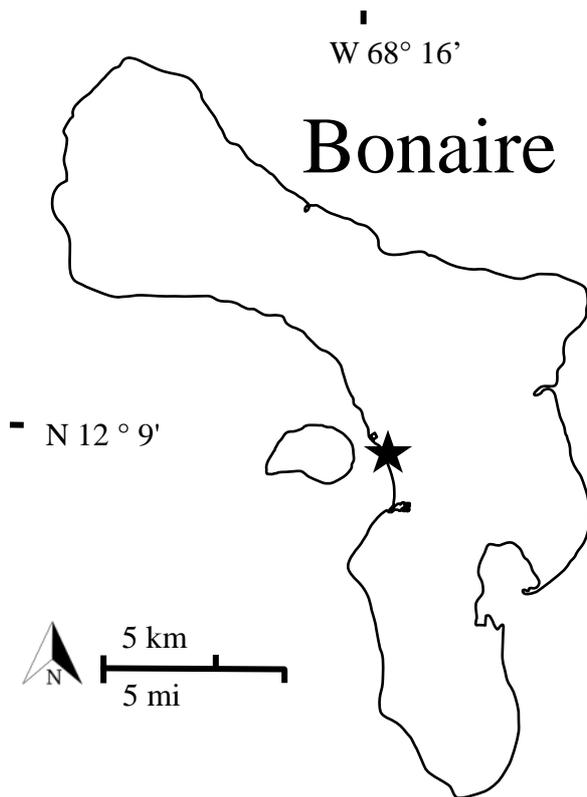


Fig. 1 Map of Bonaire, Dutch Caribbean. Filled star indicates location of study site, Playa Lechi (12°9'36.2"N, 69°16'55.8"W)

prevalence of DSS at approximately 15 m.

Lesion progression

To analyze the progression of DSS on individual corals, photos were taken semi-weekly with an underwater camera (Canon PowerShot S110 in Canon WP-DC47 housing) to determine change in the number of afflicted polyps per coral colony over time. To facilitate coral specimen identification, an identification tag bound to colored flagging tape was fixed to nearby dead substrate using a masonry nail. A floating cork was attached to the tape so afflicted colonies could be easily recognized. Colonies were discovered and marked by swimming approximately 120 m northward from Playa Lechi at approximately 15 m in depth. Nineteen DSS-afflicted corals were monitored over a minimum of 15 and a maximum of 19 d (15-19 d) from March to April 2017.

Ailment transmission

A transect line was laid at 15 m at the first located presence of DSS, running parallel to the shore. The nail and cork technique was utilized to mark the start and end points of the transect line for population sampling consistency. Counts of DSS-afflicted coral colonies were taken over time. A T-bar was utilized to conduct a 2 m wide belt transect to count DSS-afflicted colonies over time. This belt transect was conducted twice over a 26 d interval. This transect was also used to count the number of corals greater than 4 cm that were within the boundaries of the belt. This study recorded DSS occurrences on the following scleractinian corals: *S. siderea*, *S. intersepta*, *O. annularis*, *O. faveolata*, *M. cavernosa*, *Orbicella franksi*, and *Undaria agaricites* (formerly known as *Agaricia agaricites*). These corals were chosen because they are primarily impacted by DSS (Gil-Agudelo et al. 2004).

Data analysis

After data collection, Microsoft Paint was utilized to mark and count afflicted polyps. Polyps that were $\geq 50\%$ afflicted were counted as afflicted. Lesion progression data illustrated the average percent change of total afflicted polyps per coral colony over time. In cases of lesion fusion, polyp counts of separate lesions were added together prior to fusion for neutral comparison. The lesion progression data was tested using two one-sample t-tests as well as a one-way ANOVA with colony number. Ailment transmission data were used to compare the number of afflicted colonies over a 26 d interval, depicting whether healthy colonies are showing signs of DSS acquisition or whether afflicted corals recovered from DSS affliction. Due to a low number of afflicted corals, coral colonies of different species were combined to get an overall number of afflicted corals.

Results

Overview

A total of 19 corals were photographed, however, five corals were omitted from data analysis due to poor image quality ($n = 14$). DSS was found to increase by an average rate of $18.4 \pm 0.4\%$ per colony over a span of 15-19 d, with corals showing a maximum decrease of 19% and a maximum increase of 64% (Fig. 2). All corals analyzed in the sample study were *S. intersepta* and found in the 10.5-16.5 m depth range. In addition, DSS-afflicted coral colony counts had decreased over the span of 26 d in the population study (Fig. 3). All coral colonies that exhibited DSS lesions on both day 0 and day 26 of the population study were *S.*

intersepta, however, one coral, an *O. faveolata*, exhibited DSS lesions on day 0 and not on day 26.

Test results

A one-sample t-test showed that the mean percent change in size of each lesion in the total population was higher than the hypothesized mean of zero ($df = 112$, $t = 4.53$, $p < 0.001$). Another one-sample t-test showed that the mean average percent change in lesion size of all lesions per colony was greater than the hypothesized mean of zero ($df = 13$, $t = 2.68$, $p = 0.019$). ANOVA results showed no significant effect of colony on mean percent change of DSS lesions ($df = 13$, $F = 1.58$, $p = 0.104$).

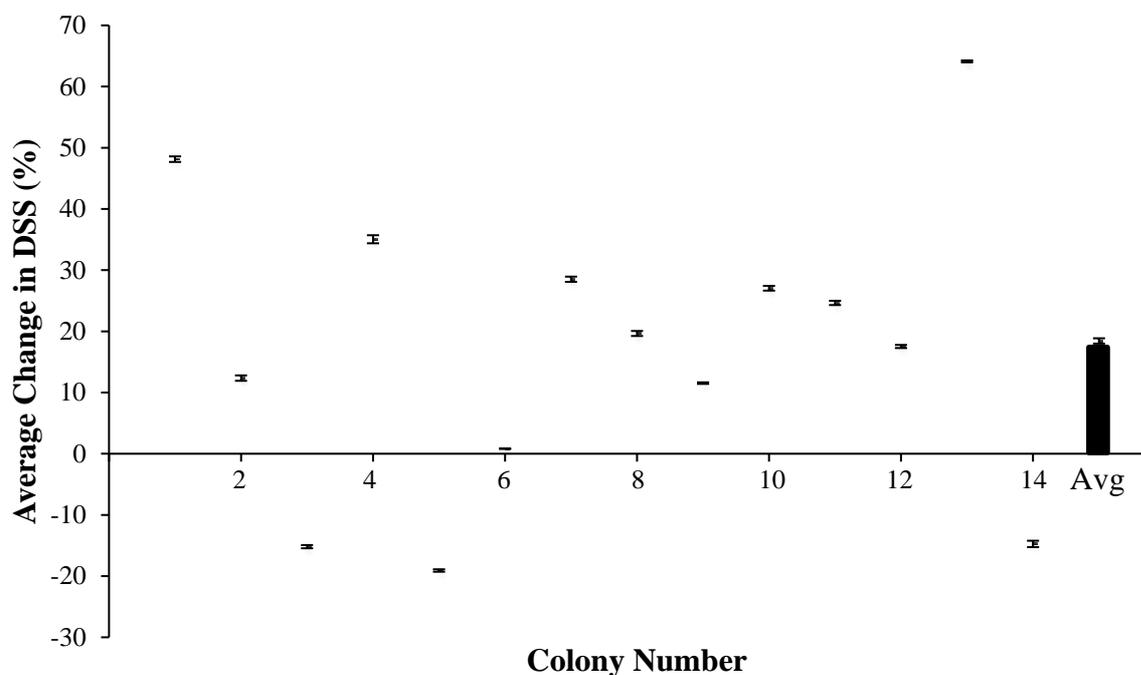


Fig. 2 The relationship between average percent change in number of dark spot syndrome (DSS) afflicted polyps in all lesions per colony by coral colony number ($n = 14$). Average change in DSS per coral was found to be $+18.4 \pm 0.4\%$ indicated by the filled bar. Error bars represent standard deviation. All corals analyzed were *Stephanocoenia intersepta*. All measurements were taken over a minimum of 15 and a maximum of 19 d

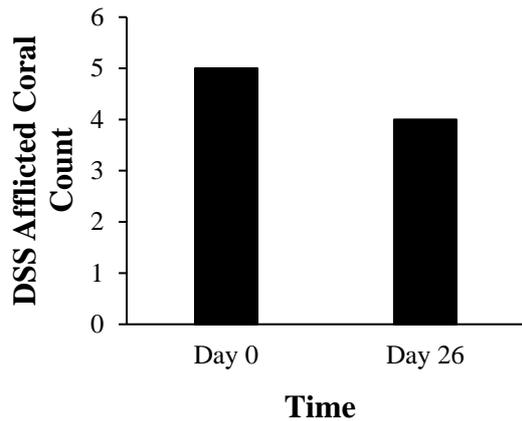


Fig. 3 Depiction of DSS afflicted coral colony counts. Data was collected at two time points, day 0 and day 26. All corals analyzed ($n = 5$) were *Stephanocoenia intersepta* except for one *Orbicella faveolata* coral

Discussion

The first hypothesis was not supported, as DSS progression on coral colonies was observed. Average change in DSS per lesion was found to occur at a rate of $+18.4 \pm 0.4\%$ (Fig. 2). This shows that over the course of 15-19 d, the progression in DSS affliction within a colony, with respect to original lesion size, was greater than initially predicted. Additionally, the second hypothesis was supported, as there was no evidence of DSS transmission from an affected colony to a healthy colony. All previously afflicted corals still exhibited DSS lesions on day 26 except for one coral colony. This coral colony was of a different species, indicating that species may play a factor in response to DSS affliction.

Lesion progression

While the rate of DSS spread has been measured, the findings produced by this study take into account the full nature of the change in area of various lesions per colony. The lesion progression results addressing the first hypothesis offer a different perspective in that they analyze each lesion based on total percent change over time rather than attempting to

measure the rate of progression by distance from a given point over time. Analyzing the entire lesion in terms of percent change allows the observation of DSS as a whole rather than in specific sections to get a more representative measurement of DSS activity. In instances of lesion fusion (Fig. 4), the percent change measurement proved to be the best method in analyzing lesions together rather than attempting to determine which growth was associated with a particular lesion. While there was an average increase of $18.4 \pm 0.4\%$, this finding does not apply to all lesions and some corals displayed signs of DSS recovery. However, this average increase in percent cover of DSS must be closely monitored as afflicted coral tissue is more susceptible to infection by other coral diseases, which could potentially lead to tissue mortality (Borger 2005).

Ailment transmission

There was one coral which completely recovered from DSS by day 26 (Fig. 3). Other afflicted corals did not show signs of recovery. The recovered coral was an *O. faveolata*, while all other afflicted corals were *S. intersepta*, suggesting the possibility that different coral species may be more resilient or respond to DSS differently. Further investigation is necessary to determine if coral species play an integral part in DSS change or whether the observed recovery was due to some other factors such as varying environmental conditions or proximity to other afflicted corals.

There was no evidence supporting transmission of DSS from afflicted colonies to healthy colonies. The 26 d time frame of this study can be compared with the 28 d time frame of *ex situ* experiment yielding no evidence of transmission (Randall et al. 2016). However, it is possible that with a longer data collection period, DSS transmission among corals could be recorded. It is also possible that DSS transmission is not possible, but rather is a consequence of a scleractinian stress response to changing environmental conditions (Borger 2005).

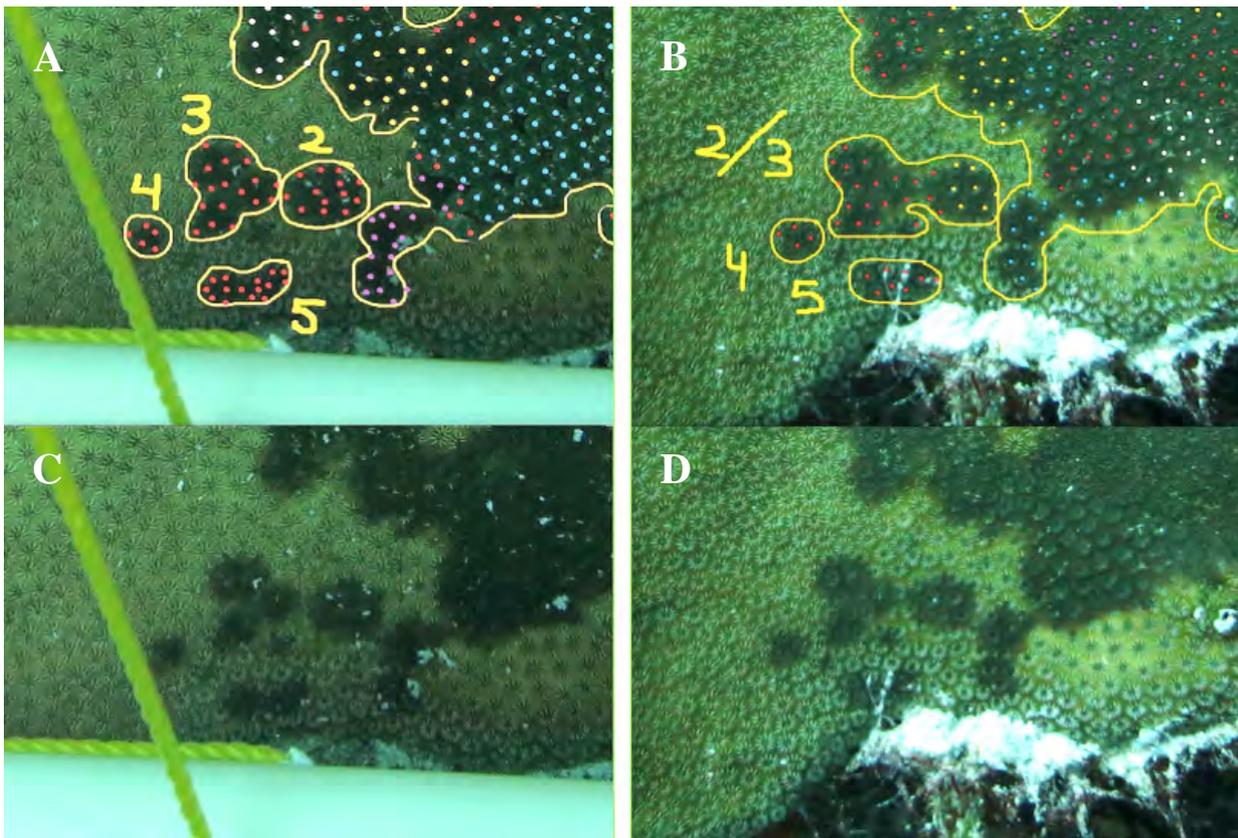


Fig. 4 Photographs of dark spot syndrome (DSS) lesion fusion. Visual observation shows the fusion of lesions two and three over the course of 15 d. 8th March photographs shown on the left with (A) the analyzed image and (C) the raw image. 22nd March photographs shown on the right with (B) the analyzed image and (D) the raw image.

Potential data collection errors include discrepancies in lighting due to collection at different times of day and varying current strengths, resulting in blurry photographs. The differences in lighting may have caused some regions on the coral to appear as DSS at one time period and appear different at another. Blurry photographs necessitated polyp estimation which may not necessarily be a true count of the afflicted polyps for each coral. Potential data analysis errors include subjectivity in counting afflicted polyps based on percent afflicted and polyp color. Extensive measures were taken to minimize these errors, such as using tools in Adobe Photoshop to sharpen and add contrast to images to clearly distinguish polyps from one another. This example was exclusively used for the day zero photographs of corals 7-14, in which the underwater camera mode was not used to take the photographs. These photographs were edited in Photoshop to increase redness and sharpness. No in-depth

analysis was done to determine whether a polyp was afflicted or healthy other than visual confirmation. There was also no standard set for polyp color to compare with and therefore visual confirmation of relative color difference was the sole tool in distinguishing healthy polyps from afflicted polyps.

Modeling was utilized to estimate and compare the rate of DSS progression in the southern Caribbean with the calculated rate of DSS progression in the eastern Caribbean, 0.12 mm d^{-1} (Borger 2005). With the assumption of a perfectly circular polyp, an average polyp radius of 1.22 mm was calculated from five *S. intersepta* polyps photographed in 1982 in Belize (Cairns 1982). This average radius was used to calculate the average area of lesions ($n = 113$) on both the initial and final data collection days. These areas were converted back to radii, with the difference between the radii portraying southern Caribbean linear DSS progression, which can then be compared to

Borger's eastern Caribbean rate. The rate of linear progression of DSS in the southern Caribbean was found to be 0.96 mm over the full data collection interval or 0.06 mm d⁻¹. This calculated rate was found to be about half that of Borger's eastern Caribbean rate, potentially illustrating that DSS progression rates may change with geographic location. This model is useful in that it facilitates analysis of a much greater lesion sample size, providing a more thorough look at DSS progression rate.

Furthermore, this study established a new methodology for calculating DSS activity. The methodology used in the lesion progression study takes into account distinct lesion activity, including localized lesion changes as well as the morphing of multiple lesions, which would have been excluded by pre-existing methodologies. This methodology, with refinement, can be used in future studies to accurately record DSS lesion area changes in response to different variables, such as depth, proximity to other afflicted corals, and warmer temperatures. Further and more detailed analysis is necessary to understand what factors influence DSS spread the most and what can be done to mitigate these stressors. As DSS was recently discovered, there are still many unknown aspects to it; however, with the use of newer technologies and more precise collection instruments, the ability to study this ailment in much greater detail is more feasible now than ever. Utilizing methodologies, such as the one established in this study, enable scientists to closely monitor changes on the reef. By analyzing corals at the polyp level, we can understand the rate at which coral diseases are spreading and get an estimate for the magnitude of impact, enabling us to work to mitigate the effects earlier when chances of recovery are greatest.

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REPORT

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Assessment of sea urchin assemblage as a function of substrate composition

Abstract Sea urchins are essential in marine ecosystems as herbivores and bioeroders that can drastically alter reef benthic structure and dynamics through removing algae, mediating sediment buildup, and increasing reef rugosity. There have been numerous studies conducted to determine biotic factors that influence their spatial distribution; however, focus on abiotic factors, such as substrate type, is comparatively understudied. This study was conducted at Playa Lechi, Bonaire, measuring the mean number of sea urchins m^{-2} on six different dominant substrate types to determine if their assemblage was a function of the benthic composition. Data collection took place in both the intertidal zone and on the coral reef. Results showed that dominant substrate type affected the assemblage of *Tripneustes ventricosus* ($p = <0.001$) and *Echinometra lucunter* ($p = 0.001$). Tukey HSD analyses revealed that the mean number of *T. ventricosus* recorded on concrete dominated substrate ($0.29 m^{-2}$) was significantly higher than on rubble or sand ($0.04 m^{-2}$ and $0.01m^{-2}$ respectively). *E. lucunter* counted on rock dominated substrate ($2.74 m^{-2}$) was significantly higher than on rubble or sand ($0.32 m^{-2}$ and $0.07 m^{-2}$ respectively). The mean number of *Diadema antillarum*, *Eucidaris tribuloides*, and *Echinometra viridis* did not differ statistically across various dominant substrate types. These results suggest that substrate composition can affect the distribution of sea urchins and should therefore be considered an important abiotic factor while delineating management efforts to maintain sea urchin populations within ecologically healthy limits.

Keywords Echinoderm • Species assemblage • Substrate preference

Introduction

Sea urchins play a pivotal role within their marine habitat, working as both efficient herbivores and bioeroders. They can drastically alter reef benthic community structure and dynamics through removing algae, mediating sediment accumulation, and increasing reef rugosity (McClanahan and Muthiga 2013). Often associated with reef degradation during population explosions, their presence in low densities or their complete absence in coral reef habitats can lead to a top-down cascade effect, causing the reef to shift from a topographically complex environment to a community dominated by fleshy macroalgae (Hughes 1994; McClanahan and Mutere 1998; McClanahan and Muthiga 2013). Although the functions sea urchins perform in marine ecosystems have been thoroughly examined, it is essential to better understand the abiotic factors that influence their spatial distribution to properly monitor and protect habitats that foster healthy urchin populations (Grigg and Dollar 1990).

Physical complexity of a habitat is an influential abiotic factor that can affect species assemblage. Sea urchin spatial assemblage is likely related to the structural properties of the substrate they reside on (Entrambasaguas et al. 2008; Alvarado et al. 2012). This study aimed to assess sea urchin assemblage within the intertidal and reef zones as a function of substrate composition at Playa Lechi, Bonaire.

Commonly observed substrates in these zones include: concrete, rock, rubble, sand, live coral, and dead coral. Concrete substrate was characterized by its obvious anthropogenic construction and smooth surface, often covered in turf algae. Live and dead coral were easily identified by their natural structures, both

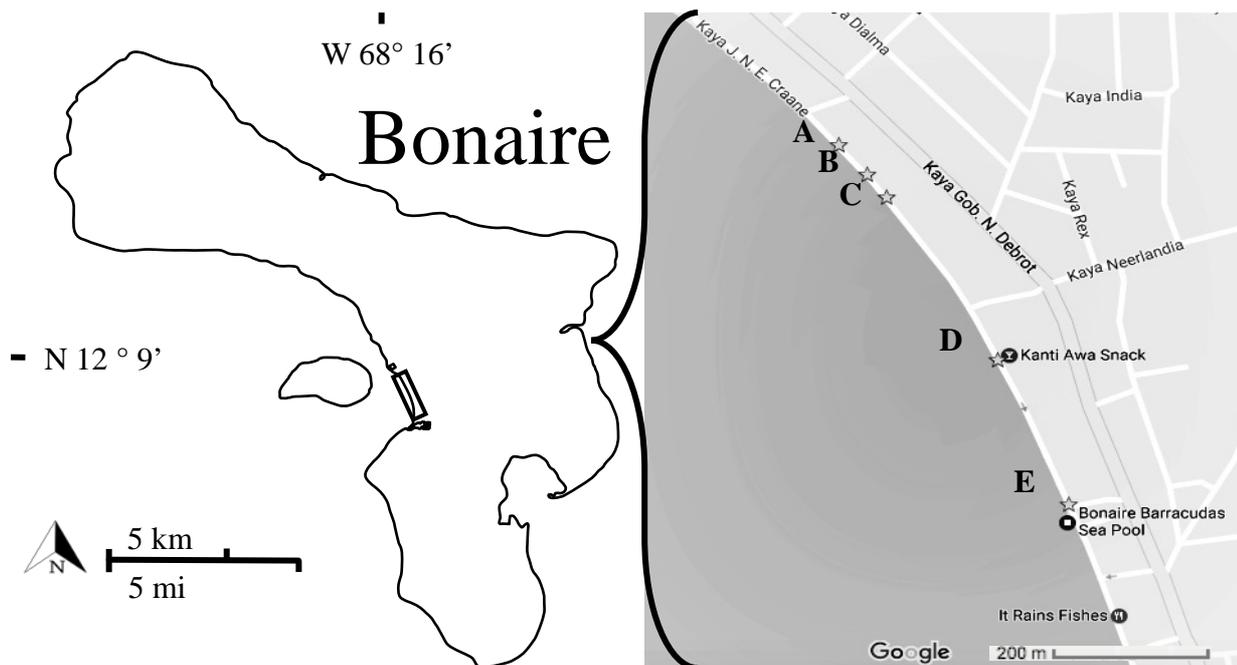


Fig. 1 Map of Bonaire, Netherlands Antilles. Playa Lechi indicated by black rectangle in image on left, each of the five study sites labeled A – E in image on right (Map credit Google Maps)

resembling the form of a typical hermatypic coral species. Six sea urchin species commonly found in Caribbean waters were considered for assessment: *Echinometra lucunter*, *Echinometra viridis*, *Lytechinus variegatus*, *Tripneustes ventricosus*, *Eucidaris tribuloides*, and *Diadema antillarum* (Rodríguez-Barreras et al. 2016; Table 1). A previous study found that only *T. ventricosus* exhibited preference to a substrate, specifically seagrass beds, but the study did not focus heavily on other substrate types, such as coral reefs and intertidal zones (Rodríguez-Barreras et al. 2016). The purpose of this assessment is to gather sufficient data on the species assemblage of these commonly found sea urchins and their substrate preferences on and near the coral reef. By obtaining the baseline data for this location, information will be available for future reference to monitor the changes in species distribution and abundance over time. The hypotheses tested were:

- H1: There is a correlation between sea urchin species assemblage and dominant substrate type
- H2: Sea urchin species will not share the same dominant substrate preference with another species

Establishing baseline data on the patterns of sea urchin distribution at Playa Lechi, Bonaire will provide more accurate estimates of their susceptibility to changes in substrate type. Therefore, if necessary, anthropogenic alterations to important substrate zones can be prevented by proper management plans and natural shifts in substrate type can be addressed effectively.

Materials and methods

Study sites

Five data collection surveys were performed in the intertidal and subsequent coral reef area between the Yellow Submarine dive shop (12°09'37.5"N, 68°16'54.8"W) and Street “Kaya Aruba” (12°09'11.8"N, 68°16'41.4"W) on the island of Bonaire, Dutch Caribbean Netherlands (Fig. 1). A random number generator was used to select five sites along the 900 m distance where each collection was performed (Table 2). Each study site was composed of a littoral zone, reef crest, and reef slope. At the three southernmost sites (C, D, E), seawalls were the boundary of the littoral zone

instead of the natural shoreline. Data collection took place over the course of five weeks from March to April 2017.

Urchin assemblage assessment

Data collection in the intertidal

Exactly 1 m from the shoreline at the starting coordinates of the collection site, a 30 m transect was laid parallel to the shore from north to south (Fig. 2). Along the transect, a 1 x 1 m quadrat,

divided into a 100-box string grid, was laid with the top corner of the quadrat aligning with the meter mark on the transect. This alignment remained consistent to remove bias. With the quadrat held in a fixed position, dominant substrate type was estimated by counting the sections that each substrate type filled (with each square equaling 1% of the total quadrat). The substrate types measured were: rock, rubble, sand, live coral, dead coral, and concrete. Rock was categorized by any continuous substrate composed of natural rock.

Table 1 The six species of sea urchin observed during the study with identification photo, scientific name, and common name. Known habitat preference, Humann and Deloach (2002)

Identification Photo	Scientific Name	Common Name	Known Habitat Preference
	<i>Echinometra lucunter</i>	Rock boring urchin	Most common in shallow, rocky areas, but occasionally found much deeper.
	<i>Eucidaris tribuloides</i>	Slate pencil urchin	Occupy seagrass beds, reef rubble, and reefs.
	<i>Echinometra viridis</i>	Reef urchin	Found on shallow reefs; often on <i>Undaria</i> spp.
	<i>Tripneustes ventricosus</i>	West Indian sea egg	Found in seagrass beds; occasionally on shallow reefs
	<i>Diadema antillarum</i>	Long-spined urchin	No known preference (found in all habitats)

Table 2 The five study sites located at Playa Lechi, Bonaire, where sea urchin abundance and substrate percentage data were collected alongside their distances from Yellow Submarine (12°09'37.5"N, 68°16'54.8"W) and their respective 30 m transect start and end coordinates

Site	Distance (m)	Start Coordinate	End Coordinate
A	72	12°09'35.1"N 68°16'53.5"W	12°09'34.3"N 68°16'52.9"W
B	120	12°09'33.9"N 68°16'52.5"W	12°09'33.2"N 68°16'51.9"W
C	150	12°09'33.2"N 68°16'51.9"W	12°09'34.2"N 68°16'51.1"W
D	450	12°09'24.3"N 68°16'46.4"W	12°09'24.1"N 68°16'46.4"W
E	640	12°09'19.6"N 68°16'44.4"W	12°09'18.4"N 68°16'43.8"W

Rubble was considered to be substrate covered in rock and coral fragments that varied in size and shape. Sand was identified as substrate covered in loose, natural, granulated particles. The number of each species of urchin observed within the quadrat was counted and recorded. The methodology outlined above was repeated every meter along the 30 m transect. At 3 and 5 m parallel to shore, the established intertidal survey methodology was repeated. Each intertidal survey was completed in approximately 90 min.

Data collection on the reef

The diver entered the water from a shore platform at the starting coordinates of the study site and dove perpendicular to the shoreline, directly out to the reef slope, following a compass. After reaching the reef slope at a depth of 12.2 m, a 30 m transect was laid from north to south, parallel to shore. The diver swam along the transect with a 1 x 1 m quadrat with a 100-box string grid to survey for sea urchins on the same six substrate types, as for the intertidal zone. The belt surveyed was 2 m wide with 1 m on either side of the transect. Extensive care was taken to check within crevices of live and dead corals because of the increase in structural complexity on the reef. If a sea urchin was located within the 2 m belt on either side of the transect, the right hand corner of the quadrat closest to the diver was oriented to the nearest whole meter mark to remove bias from quadrat placement. Sea urchin species and the dominant substrate within the quadrat were recorded. The

same methodology was repeated on the reef crest at a depth of 9.1 m. On average, the reef surveys were completed in 20-30 min.

Data analysis

An analysis of variance (one-way ANOVA) compared the means between the number of each species of sea urchin counted on the various dominant substrate types (concrete, rock, rubble, sand, live coral, and dead coral) and determined whether any of those means were significantly different from one another. In any case where the quadrat recorded a substrate cover that was divided exactly by 50% of one substrate and 50% of another, the data were duplicated so that the urchin count for that specific quadrat was considered in each dominant substrate type category for analysis.

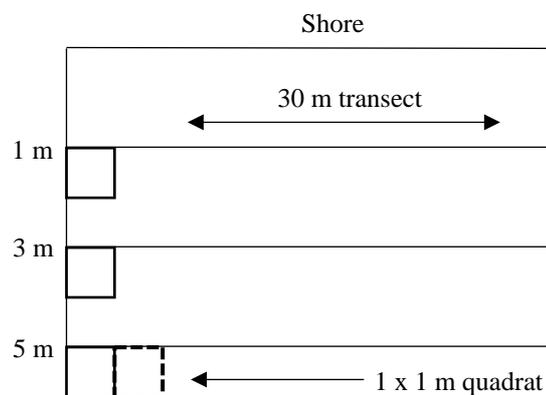


Fig. 2 Diagram of the intertidal zone data collection methodology for sea urchin species assemblage and substrate percentage. Each line parallel to the shore is representative of a 30 m transect

Post-hoc analysis consisted of running a Tukey HSD test for the species of sea urchin with statistically significant p-values to determine where the differences occurred between dominant substrate groups.

Results

Although methodology accounted for a total of six sea urchin species: *E. lucunter*, *E. viridis*, *L. variegatus*, *T. ventricosus*, *D. antillarum*, and *E. tribuloides*, the species *L. variegatus* was not observed during the data collection process and is therefore not included in analysis. Dominant substrate type had a significant effect on the number of *T. ventricosus* ($p < 0.001$) and *E. lucunter* ($p = 0.001$). Post-hoc analyses revealed that the mean number of *T. ventricosus* counted on concrete dominated substrate ($0.29 \pm 0.02 \text{ m}^{-2}$) was higher than on rubble or sand dominated substrate ($0.03 \pm 0.00 \text{ m}^{-2}$ and $0.01 \pm 0.00 \text{ m}^{-2}$ respectively, Fig. 3). *E. lucunter* counted on rock dominated substrate ($0.02 \pm 0.00 \text{ m}^{-2}$) was higher than on rubble or sand dominated substrate ($0.01 \pm 0.00 \text{ m}^{-2}$ and $0.07 \pm 0.00 \text{ m}^{-2}$ respectively, Fig. 3). The mean number of *D. antillarum*, *E. tribuloides*, and *E. viridis* did not differ statistically across various dominant substrate types (Fig. 3). The mean number of individuals m^{-2} was found to be higher on live coral (Fig. 3E) and dead coral (Fig. 3F) than others. Concrete as a dominant substrate type showed a high mean of individuals m^{-2} of *T. ventricosus* and *E. lucunter* (0.29 individuals m^{-2} and 0.33 individuals m^{-2} respectively, Fig. 3A). Rock dominated substrate showed presence of *E. lucunter* was slightly higher than other species (0.02 individuals m^{-2} , Fig. 3B).

Discussion

This study aimed to gather baseline data of the spatial distribution of the five most commonly found sea urchin species at Playa Lechi, Bonaire. This data could potentially contribute to future analyses to determine healthy population densities and distributions based on the

substrate preferences of the various sea urchins examined. Hypothesis one was supported by the statistical results of the one-way ANOVA test, which showed a correlation between sea urchin species assemblage and dominant substrate type. The second hypothesis, that sea urchin species will not share the same dominant substrate preference with another species, was not supported by the results.

Substrate preference

Two out of the five sea urchins studied, *T. ventricosus* and *E. lucunter*, showed preference to a dominant substrate type, specifically concrete and rock, respectively. The results showed that *D. antillarum*, *E. tribuloides*, and *E. viridis* did not prefer any specific substrate over another. The mean number of individuals m^{-2} was found to be higher on live coral (Fig. 3E) and dead coral (Fig. 3F) dominated substrates than others. However, the total number of sea urchins counted on these dominant substrate types (live coral, $n = 3$ and dead coral, $n = 10$) were much lower than the overall number of individuals counted across all substrate types ($N = 796$). Other studies have found similar results that indicate lower sea urchin densities in areas with complex coral cover (Dumas et al. 2007). Results obtained on *T. ventricosus* over the duration of this study at Playa Lechi differ from those found in studies conducted on other Caribbean islands; on Culebra Island, Puerto Rico, *T. ventricosus* is more commonly found in seagrass beds (Rodríguez-Barreras et al. 2016). Whereas this study showed *T. ventricosus* to have a preference for concrete dominated substrate. This difference is most likely due to the fact that there was no seagrass habitat present at the study site, and therefore it could not be included as a dominant substrate type. A future study could be conducted on Bonaire including seagrass habitats to compare the results with this baseline data alongside the Rodríguez-Barreras et al. (2016) evaluation on Culebra Island.

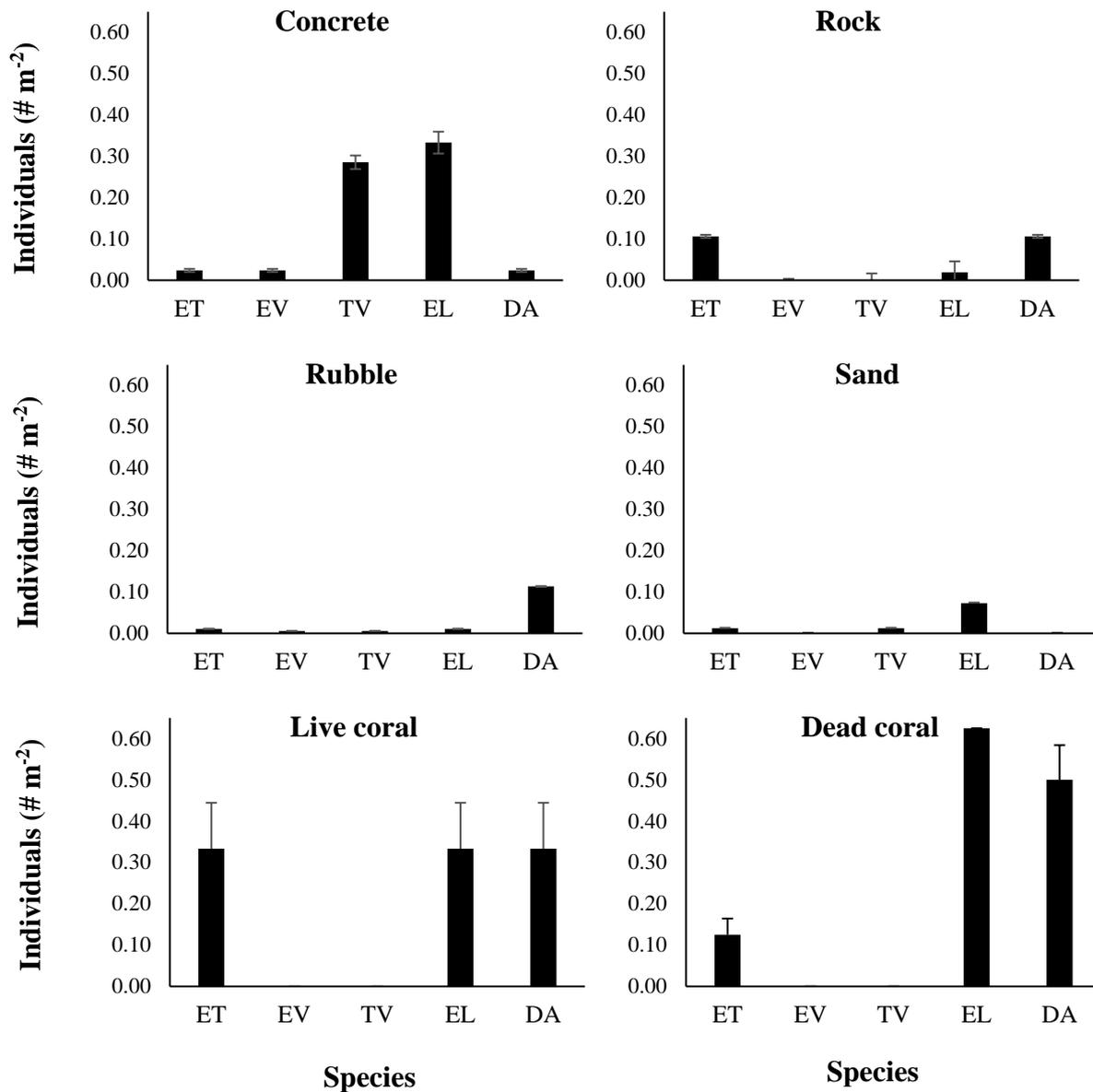


Fig. 3 Mean density of sea urchins m⁻² by dominant substrate type. Species scientific names are represented by ET = *Eucidaris tribuloides*, EV = *Echinometra viridis*, TV = *Tripneustes ventricosus*, EL = *Echinometra lucunter*, DA = *Diadema antillarum*. Standard deviation represented by error bars. *Echinometra lucunter* standard deviation (± 0.06 m⁻²) on “Dead coral” extended past y-axis maximum and was removed to preserve quality of other graphs

Overlap of spatial distribution

There was a significant amount of overlap between species and the dominant substrate they were counted on (Fig. 3). One factor that may have contributed to this variation in results between sites (Playa Lechi, Bonaire and Culebra Island, Puerto Rico) is the duration and season of the data collection period. This study took place over the course of five weeks from March to April, compared to the other evaluation that was conducted in October. Other research that

spanned over the course of an entire year suggests that urchin abundance can have a strong correlation with seasonality, showing highest abundances in the summer and lowest in the winter months (Bauman et al. 2016).

The seasonal factor may also be considered a limitation on the scope of this study; if it was possible to repeat this study at the same sites (A-E at Playa Lechi) during different months throughout the year, then it is possible that the extended time span of collected data would show the influence of seasonality on sea urchin

assemblages. Another limitation of the study is simply personal error during data collection, the reef slope and reef crest are highly rugose environments, and it is possible that there were urchins present within the survey area that were not accounted for if they were not visible to the diver. The scope of this study could also be broadened by addressing time of day as an important variable in the data collection process; if one were to sample the same site multiple times per day, interesting temporal patterns may be derived from the data.

The results of this assessment concur with those found by Hermosillo- Nuñez et al. (2016), which suggested that abiotic factors, such as structure of the habitat, play an important part in the spatial distribution of sea urchins. The larger implications of this study highlight the immediate need for more thorough knowledge on ecological factors influencing sea urchin assemblages. In order to address their vulnerability to anthropogenic and environmental changes and assess our ability to develop effective management plans (Grigg and Dollar 1990). The suggestion that substrate type plays a role in species distribution draws attention to the abiotic variables that may contribute to these observed patterns, rather than simply focusing on biotic environmental factors that are thought to largely control habits of marine species (Dumas et al. 2007). Through effectively identifying urchin substrate preference, efficient intertidal and reef zone monitoring can be implemented. Alongside conservation strategies, this will aid in avoidance of both sea urchin population decimation and explosion events. Consequently, it will keep macroalgae growth within healthy ecological limits leading to overall increased reef vitality.

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REPORT

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The effect of habitat type on foraging tactics of *Caranx ruber*

Abstract Coral reefs are comprised of many fish species occupying a wide variety of ecological niches and trophic levels. Understanding the ecology and foraging behaviors of commercially significant piscivores, such as *Caranx ruber*, allows for the implementation of conservation measures to maintain sustainable fisheries. It also identifies the degradation of reef habitats as primary foraging tactics shift. Previous studies on *C. ruber* have disproportionately focused on following association and little has been done to understand other aspects of their feeding ecology. This study aims to analyze differences in foraging tactics of *C. ruber* among different habitat types at Playa Lechi, Bonaire, Dutch Caribbean. Observational studies were conducted on 29 individuals with 34 distinct foraging events. Data were collected on observed foraging tactics (i.e. following association, temporary localized attack behavior, and roving), habitat, attack frequencies, and average search time between attacks. The results show that habitat type affects the foraging tactics utilized by *C. ruber*. No significant differences in attack frequency or average search time between attacks were found between different foraging tactics. As coral reefs continue to follow phase shift trends towards macroalgae dominated reefs, observed foraging tactics of *C. ruber* may serve as bio-indicators of degradation and loss of habitat complexity. As fisheries of larger bodied piscivores become depleted, shifts toward smaller reef-dwelling piscivores may occur. Better understanding all aspects of *C. ruber* ecology will be important to the proper management of the species as a viable fishery option.

Keywords Trophic levels • Feeding ecology • Following association

Introduction

Fish occupy a wide variety of ecological niches and trophic levels in marine ecosystems. Fish species are classified into different trophic categories on coral reefs including piscivores, invertivores, omnivores, planktivores, and herbivores (Ferreira et al. 2004). It is important to maintain a balance between all trophic levels in order to maintain a healthy ecosystem (Harborne et al, 2017). Global fisheries have preferentially focused on the removal of large bodied piscivores and often exploit coral reefs as they are important habitats for commercially significant fish species (Harborne et al. 2017). An example of top-down controls are the effect predators have directly on the abundance of their prey species and indirectly on the abundance of other species in lower trophic levels. (Jennings and Polunin 1997). Top-down controls, or trophic cascades, are the controls set in place by predators through direct predation and their effects on the relative abundances of species occupying lower trophic levels within an ecological community (Heithaus et al. 2008). One consequence of reducing certain top-down controls is predatory release, which is defined by an increase in prey populations due to decreases in top predator abundance (Heithaus et al. 2008). When a prey species lacks natural predators their abundance will increase due to the reduced predation mortality. Predator-prey relations have even been shown to affect the distribution of adult prey species as expressed by the Predation Hypothesis (Hixon 1991). The hypothesis states that patterns in abundance of

prey species are determined by predation, resulting in low population and greater competition for resources among these species. The removal of predatory species can cause trophic cascades leading to overall degradation of vital reef habitats.

Piscivores fall into five distinct categories based on the different predatory modes utilized. These categories include: (1) species that attack prey within crevices, (2) cryptic species that ambush their prey, (3) species that habituate prey to an illusion that they are non-predatory, (4) species that slowly stalk their prey, and (5) open-water species that pursue their prey (Hixon 1991). *Caranx ruber*, commonly known as the bar jack, have been observed to exhibit foraging tactics that would classify them as open-water species that pursue their prey (Hixon 1991).

Caranx ruber is an important predatory fish within the Caribbean. It employs a number of different foraging strategies to maintain its diet including following association, roving, and ambushing (Silvano 2001). Following association has been seen and observed in a variety of fish species. This behavior has been defined as one follower species, the attendant, following around another species, the nuclear, foraging along the benthic substrata (Silvano 2001). *Caranx ruber* bites to search time ratio were over three times greater when individuals exhibited following association compared to hunting alone (Baird 1993). *Caranx ruber* also employ temporary localized attack behavior (TLAB) defined by recurrent attacks and cyclical swimming patterns within an observable, localized area (Yi, pers. obs.). Although *C. ruber* utilizes different foraging strategies, studies have disproportionately focused on observed following association and little has been done to study other aspects of their foraging tactics (Baird 1993; Silvano 2001; Sazima et al. 2007).

While advances in remote tracking technology have expanded our knowledge of piscivores' movements and foraging behavior, research has focused largely on better understanding shark movement patterns (Harborne et al. 2017). Little research has focused on the movements and foraging

behaviors of less charismatic teleost piscivores, which is critical for fisheries management, conservation initiatives, and the general understanding of their functional roles in coral reef ecosystems (Harborne et al. 2017). This study aimed to provide insight on a previously unstudied aspect of *C. ruber* foraging tactics and to determine what might drive individuals to employ one tactic over another. It has been suggested that high habitat complexity increases the survivorship of prey species by decreasing encounter rates, enabling easier avoidance, and reducing predator efficiency (Beukers and Jones 1998). However, evidence also suggests that areas of high habitat complexity still have high concentrations of piscivore reef fish since these habitats provide important refuge areas for prey species which may then aggregate to these areas (Stewart and Jones 2001). The reef system surrounding the leeward side of Bonaire is classified as a fringing reef that transitions abruptly from sand flats to sloping reef. This stark change in habitat complexity enables one to study the effects of different habitat types on *C. ruber* foraging tactics. Since following association is usually observed when the nuclear species forage along and turn up the benthic substrata, it is predicted that this behavior will typically occur more in habitats where the substrata is not as complex.

H₁: *Caranx ruber* will use following association more frequently on sand flats than on the sloping reef

H₂: Attack frequency will be greater when *C. ruber* utilizes following association rather than TLAB

H₃: The average time between attacks will be shorter when *C. ruber* utilizes following association rather than TLAB

Caribbean reefs have shown significant, widespread declines in architectural complexity within the last four decades due to disease and climatic events such as hurricanes. These events led to the shift towards structural decline (Alvarez-Filip et al. 2009). This suggests that degradation of reef habitats may result in a complete shift in foraging tactics as following

association becomes more advantageous. If following association were to become the primary foraging tactic utilized by *C. ruber*, this may be an indicator of poor reef health. As fishing pressures increase globally, many large bodied piscivore fisheries will begin to collapse, causing the fishing industry to shift and focus their efforts on more abundant, smaller bodied piscivores such as *C. ruber*. By better understanding the feeding ecology of *C. ruber*, stakeholders and conservation agencies will be better able to implement management strategies for the viable and successful fishery of the species.

Materials and methods

Study site

The dive site Playa Lechi ($12^{\circ}9'36.2''\text{N}$, $68^{\circ}16'55.8''\text{W}$) is located on the leeward west coast of Bonaire, Dutch Caribbean (Fig. 1). This dive site is more sheltered than those on the east coast. This offers more stable and favorable conditions for following motile species, such as *C. ruber*, for extended periods of time. Playa Lechi consists of both sand flats and fore reefs, making it a prime location for conducting habitat comparison surveys. TLAB was also first observed on the sloping reef here, therefore it was assumed that this location would allow for further observation of this foraging behavior.

Study organism

Caranx ruber is a member of the Carangidae family and has a wide distribution including the Caribbean, Florida, the Bahamas, and along the eastern Atlantic coast from Brazil north to New Jersey, USA. They are characterized by a silver body with a bright blue and black line along the dorsal and lower tail fin. They can change to darker colorations, nearly black with yellowish tints, particularly during feeding (Humann and Deloach 2002).

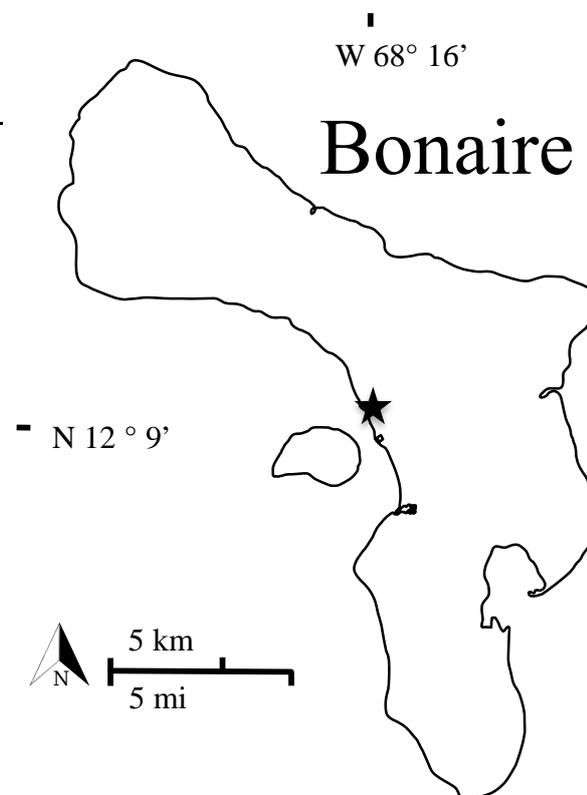
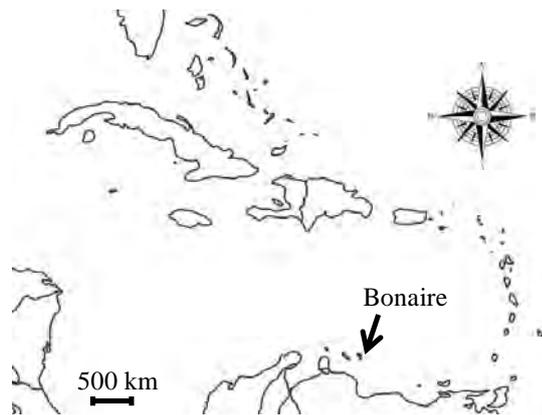


Fig. 1 The location of Bonaire in context of the Caribbean and the study site Playa Lechi ($12^{\circ}09'36.2''\text{N}$, $68^{\circ}16'55.8''\text{W}$) shown by the star on Bonaire, Dutch Caribbean

Data collection

For the study, observation time was split between snorkeling on the sand flats and SCUBA diving on the reef slope. Data were collected during the 5 week period between 1st March and 2nd April 2017. Observations occurred twice a week during this period for a total of eight days of data collection, with the exception of one week due to unfavorable

weather conditions. One snorkel and one dive was conducted on each day of data collection and both lasted 45 min each, for a total of 90 min of observation time per day. It has been suggested that *C. ruber* are more active during the daytime (Harborne et al. 2017), particularly earlier in the day to mid-afternoon (Yi, pers. obs.), so all observations took place between 1100 hrs and 1500 hrs when light does not observably change. Each observation was recorded using an underwater video camera. Raw data collected in the field were later compared to the videos to ensure reliability and accuracy of the in-situ data.

During each observation period, individuals were chosen using a roving diver technique where the first *C. ruber* seen hunting was selected. Once an individual was selected, data were recorded and a stopwatch started at the first observed attack, characterized by darting forward and biting motions (Silvano 2001). Each observation lasted for a maximum time of 10 min or until the individual was no longer able to be observed (i.e. swam away too fast). In cases where one individual displayed multiple foraging tactics within the observation period, each individual behavior was recorded as a different foraging event.

For each foraging event, the displayed foraging tactic, the duration of each foraging event, the habitat type, the approximate size of the individual, the time in seconds from the start of the foraging event that each attack occurred, and number of acts of aggression toward conspecifics were recorded on a slate. Feeding behavior events lasting less than one minute were not included in the statistical analysis.

When a following association was observed, additional information was recorded including the nuclear species, size of the nuclear species, and number of individuals the observed *C. ruber* was following. If another foraging tactic other than following association, roving, or TLAB was observed, a description of the behavior was recorded. For TLAB, no additional data was collected. For instances of TLAB and roving, the foraging tactic was determined 1 min into the observation based on different criteria for each behavior. If the observed *C. ruber*

remained in a discernable limited area, determined by repeated passes over identifiable reef structure (i.e. coral heads, distinct topographic region, large sponges, etc.) and repeated attacks within the area, the observation was recorded as TLAB. When the observed *C. ruber* did not make repeated passes but instead continued along the reef in one direction while attacking along its path, the observation was recorded as a roving foraging event. Once observation was no longer possible or the 10 min period ended the roving diver technique was used again until the next *C. ruber* seen hunting was found.

Data Analysis

A Chi-square test was used to see if there was a significant correlation between foraging tactic and habitat. The number of attacks were counted for each foraging event then divided by the total duration of the foraging event to determine the frequency of attacks per minute. The time in seconds from the start of the foraging event for each attack during the observation period was recorded. These attack times for each foraging event were then used to calculate the search time before each foraging event using the equation $T_n - T_{n-1} = ST_n$, where T_{n-1} and T_n are the successive attack times and ST_n is the search time between the two attacks. The ST_n values were then averaged to get an average search time for each foraging event. ANOVA statistical tests were used to determine the effect of foraging tactic on attack frequencies and average search time between attacks.

Results

Effect of habitat on foraging tactic

A total of 29 *C. ruber* were surveyed exhibiting 34 distinct feeding behavior events ($n = 34$). These events were placed into three distinct behaviors: following association, TLAB, and roving (Fig. 2A). Habitat affected foraging tactics ($\chi^2 = 13.08$ $df = 2$, $p = 0.001$). Following associations were observed more frequently in

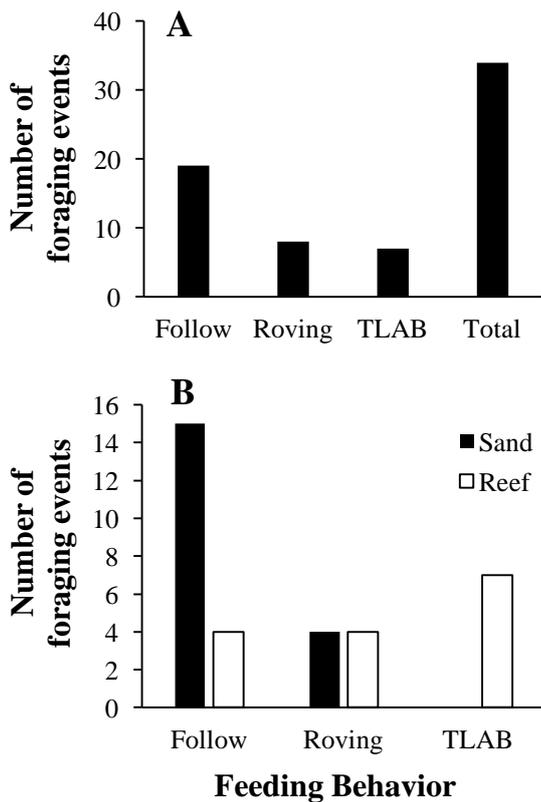


Fig. 2 (A) The distribution of behaviors (Follow: following association, Roving: roving, TLAB: temporary localized attack behavior, and total) displayed by *Caranx ruber* among all observations (n = 34). (B) The distribution of each feeding behavior between different habitats, sand and reef

the sand flats than on the reef whereas TLAB was exclusively observed on the reef. Roving was not observed to differ in frequency between the two habitats (Fig. 2B).

Following association events

The majority of species followed were benthivores that disturbed the substrate in order to locate and uncover food. *Caranx ruber* were seen to follow *Pseudupeneus maculatus* most frequently, followed by *Mulloidichthys martinicus* (Fig. 3). During the observations, multiple instances occurred where an individual *P. maculatus* was observed resting on the benthos and the following *C. ruber* would remain in close proximity until the nuclear was ready to begin foraging again. In instances where a foraging pair was joined by other

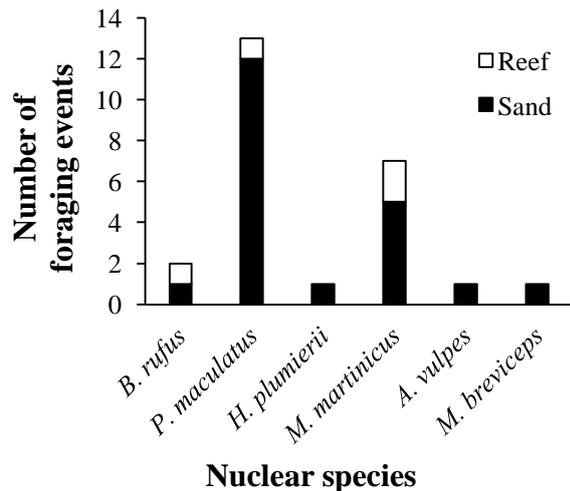


Fig. 3 The nuclear species distribution that individual *Caranx ruber* were observed to follow during following association behavior events, between different habitats: sand and reef (n = 19)

nuclear species (e.g. a group of *M. martinicus*), the *C. ruber* would remain with the original *P. maculatus* if it decided to leave the aggregation of nuclears.

Differences in attack frequencies among foraging tactics

Although the distribution of attack frequencies for each feeding behavior tended to show higher attack frequencies for TLAB and roving than following association (Fig. 4), there was no significant difference in attack frequencies between foraging tactics (mean \pm SD in bites per min for following association: 1.12 ± 0.45 , TLAB: 1.73 ± 0.80 , and roving: 1.57 ± 1.10 , ANOVA: $F = 2.38$, $df = 2$, $p = 0.109$).

Difference in average search time among foraging tactics

The distribution of average search times of each distinct feeding behavior event (n = 34) reflect a trend towards longer average search times for following association (Fig. 5). However, average search time did not significantly vary among foraging tactics (mean \pm SD for following association: 51.70 ± 22.56 s, TLAB: 32.36 ± 18.26 s, and roving: 33.58 ± 25.31 s, ANOVA: $F = 2.91$, $df = 2$, $p = 0.070$).

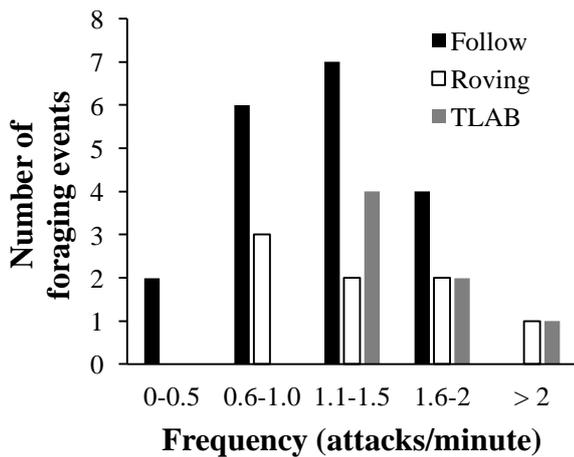


Fig. 4 The distribution of the attack frequencies of each feeding behavior event (n = 34) among different feeding behaviors employed by *Caranx ruber*

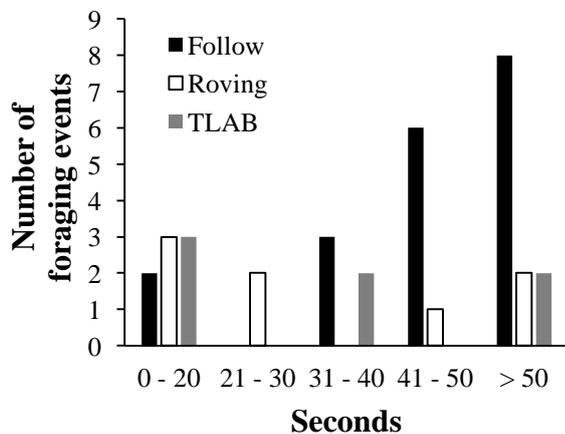


Fig. 5 The distribution of average search time of each feeding behavior event (n = 34) among different feeding behaviors employed by *Caranx ruber*

Discussion

Fish species employ a wide range of foraging strategies in order to fulfill their energetic demands (Hixon 1991). This study found that habitat type affects the foraging tactic used by *C. ruber*. Results showed *C. ruber* preferred to utilize following association on the sand flats more than on the reef. TLAB was exclusively observed on the reef, suggesting that this tactic is not favorable on the sand flats. Therefore, the first hypothesis was supported. Both attack frequency and average time between attacks showed no significant difference between different foraging tactics, leading to the

rejection of both the second and third hypothesis.

Following association on sand flats

Results showed habitat type affected foraging tactics. This suggests that different foraging tactics are more successful in certain habitats than others. For example, following association occurred more frequently on the sand flats, which has a higher percent cover of sand and rubble that is sifted through by the benthivorous nuclear species. Bottlenose dolphins (*Tursiops truncatus*), like *C. ruber* use a variety of different foraging tactics; however, individuals and groups within the Florida Bay population were found to specialize in one tactic and limit their ranges to the habitat (i.e. depth) that their foraging tactic was most successful in, showing that foraging tactics were habitat dependent (Torres and Read 2009). For *C. ruber*, the amount of sand and structural complexity present may determine which foraging tactic is best suited for a said habitat.

The higher frequency of following association on sand flats might also be due to a greater abundance of observed nuclear species present on the sand flats compared to the reef. This could be quantified in future studies through an abundance survey and might provide further insight on the observed differences in habitats. The sand flats also are less structurally complex resulting in less visual obstructions for the *C. ruber* following a nuclear species. If the follower has to take time and energy finding the nuclear, this behavior may be less favored. Future studies may focus on whether it is easier for *C. ruber* to remain in proximity to the nuclear on sand flats than on coral reefs and if this effects the foraging tactic employed.

Following association between *C. ruber* and *P. maculatus*

Pseudupeneus maculatus, the spotted goatfish, was the most prevalent nuclear species during observations. *Pseudupeneus maculatus* are white in color and are characterized by three dark blotches along the body. They are known to change rapidly into a reddish brown color.

Pseudupeneus maculatus are zoobenthivores that sift through sand and rubble using barbels at the base of their mouths to dig up small invertebrates (Humann and Deloach 2002; Sazima et al. 2006). They are diurnal and can be locally abundant, making the species an appealing nuclear for opportunistic follower fish such as *C. ruber* (Sazima et al. 2006).

Pseudupeneus maculatus distributions appeared to differ among habitats and were observed to be more abundant in the sand flats than on the reef. Since there is a greater percent cover of sand and rubble on the sand flats than on the reef, it can be predicted that *P. maculatus*, as a benthivore, would prefer this habitat. For this reason, the preference for sand flats may be caused by the preference for *P. maculatus* rather than the effects of habitat characteristics on *C. ruber* directly. The observations of *C. ruber* waiting for *P. maculatus* also suggests that this species is the preferred nuclear species.

Attack frequencies and search times

Results showed no significant difference in attack frequencies or search times among the different behaviors. From a biological standpoint, these results are reasonable because if one tactic was more successful overall as a foraging method, it would not be energetically viable to rely on the other, less successful tactics. Harbor seals (*Phoca vitulina*) have been shown to change their foraging tactics based on the prey species they are targeting to increase the energetic profitability of the prey (Bowen et al. 2002). In *C. ruber*, it would be interesting to see if the stomach contents of individuals are affected by the foraging tactic utilized. Studies on the energetic profitability of different prey species of *C. ruber* should be conducted to determine if foraging tactics differ among habitats due to different relative abundances of prey species.

Limitations

This study was limited in terms of study sites. In future studies, additional data should be collected at different sites around Bonaire which still have both sand flat and reef habitats. Strong

currents and unfavorable weather also affected data collection. Certain individuals could not be observed due to their motile nature and swimming capabilities of *C. ruber*.

Conclusion

It is important to understand the feeding behavior and ecology of *C. ruber* to better inform conservation and fisheries management. If the effect of habitat on foraging tactics is due to differences in habitat complexity between sand flats and reefs, one may expect that further habitat degradation will shift their hunting strategies to following association if it is found to be more energetically beneficial. *Caranx ruber* foraging tactics might also be used to serve as bioindicators of reef health. Understanding feeding ecology of *C. ruber* is important since they are commercially significant transient predators. Their abundance affects the food chain which can lead to shifts in the whole ecosystem. Due to fisheries of bigger, pelagic piscivores being depleted and over harvested, there may be future shifts in market to smaller piscivore species that are more abundant and still viable as a food commodity.

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