

# PHYSIS

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Vol. VII Spring 2010

# Φύσις Physis

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CIEE Research Station Bonaire  
Tropical Marine Ecology &  
Conservation Program

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Zachary A. Lipshultz- Branching Coral

Foreword: Kelsey Burlingame, Zachary A. Lipshultz, Alicia Reigel

Bonaire Map: Linda Kuhnz

*“It is the worst of times but it is the best of times  
because we still have a chance.”*

*- Sylvia Earle*

The ancient Greeks used the word *Physis* to encompass all living organisms and their environments, essentially, the growth and development of all ecosystems without any external influences. The modern definition is simply “nature,” the phenomena of life, from the smallest atoms to the largest mammals on earth, from every tiny plant to all of the giant redwood trees. *Physis* is described in Rod Fujita’s book *Heal the Ocean* as “natural self healing.”

We are four students who set out on a quest to understand the marine ecosystems of Bonaire, Netherlands Antilles. We have traversed the entire island from the very north to the very south, explored the best dive sites, sailed on a glass bottom boat, kayaked through the mangrove tunnels, and stepped deep into the caves under the island. After all of that, we still felt that the best experiences we’ve had in our 15 weeks on the island were underwater, on the coral reefs. There we watched as tiny damselfish fearlessly defended their homes, stared in awe as a green moray eel dared fish to come near him, developed respect for the tranquil tiger grouper as he glided over the reef, admired the beautiful soft corals waving in the current, laughed as frogfishes attempted to lure their prey, and gazed as spotted moray eels danced along the sandy bottoms.

What we have come to realize is that *Physis* takes into account the interconnectedness of tropical ecosystems and that we as humans, cannot fully understand the extent of this complexity. However, we have each attempted to learn about the coral reef ecosystems in our own way, through our research on the coral reefs and the organisms that inhabit them. Here is the finale of our semester, our attempts to lend understanding to the underwater world around us. We hope this leads the coral reefs on the island towards a state of self healing. So we say, “Bring on *Physis*!”

Cheers!

Alicia Reigel  
CIEE Bonaire, Spring 2010



# FOREWORD

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The Council on International Educational Exchange (CIEE) is an American non-profit organization with 122 study abroad programs in 40 countries around the world. Since 1947, CIEE has been guided by its mission...*to help people gain understanding, acquire knowledge, and develop skills for living in a globally interdependent and culturally diverse world.*

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

The marine research reported in this journal was conducted within the Bonaire National Marine Park with permission from the park and the Department of Environment (DROB). Many of the students were involved in collaborative studies with CIEE's long-term research program, Sea Turtle Conservation Bonaire, the Light and Motion Sensor Program and the Bonaire National Marine Park. Students presented their findings in a public forum on 14 April 2010 at the station for more than 60 members of the general public.

The proceedings of this journal are the result of each student's Independent Research project. The advisors for the projects published in this journal were Rita B.J. Peachey, PhD and Amanda Hollebhone, PhD. In addition to faculty advisors, each student had an intern, Andrew Collins or Lauren Saulino that was directly involved in logistics, weekly meetings and editing student papers.



# TABLE OF CONTENTS

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**Frequencies of coral disease in areas suspected of sewage-contaminated groundwater outflow in Bonaire, N.A.**

Zachary A. Lipshultz .....1



**Using prey fish species as bioindicators of anthropogenic stress and predictors of predator density and diversity on coral reefs in Bonaire, N.A.**

Alicia Reigel .....12



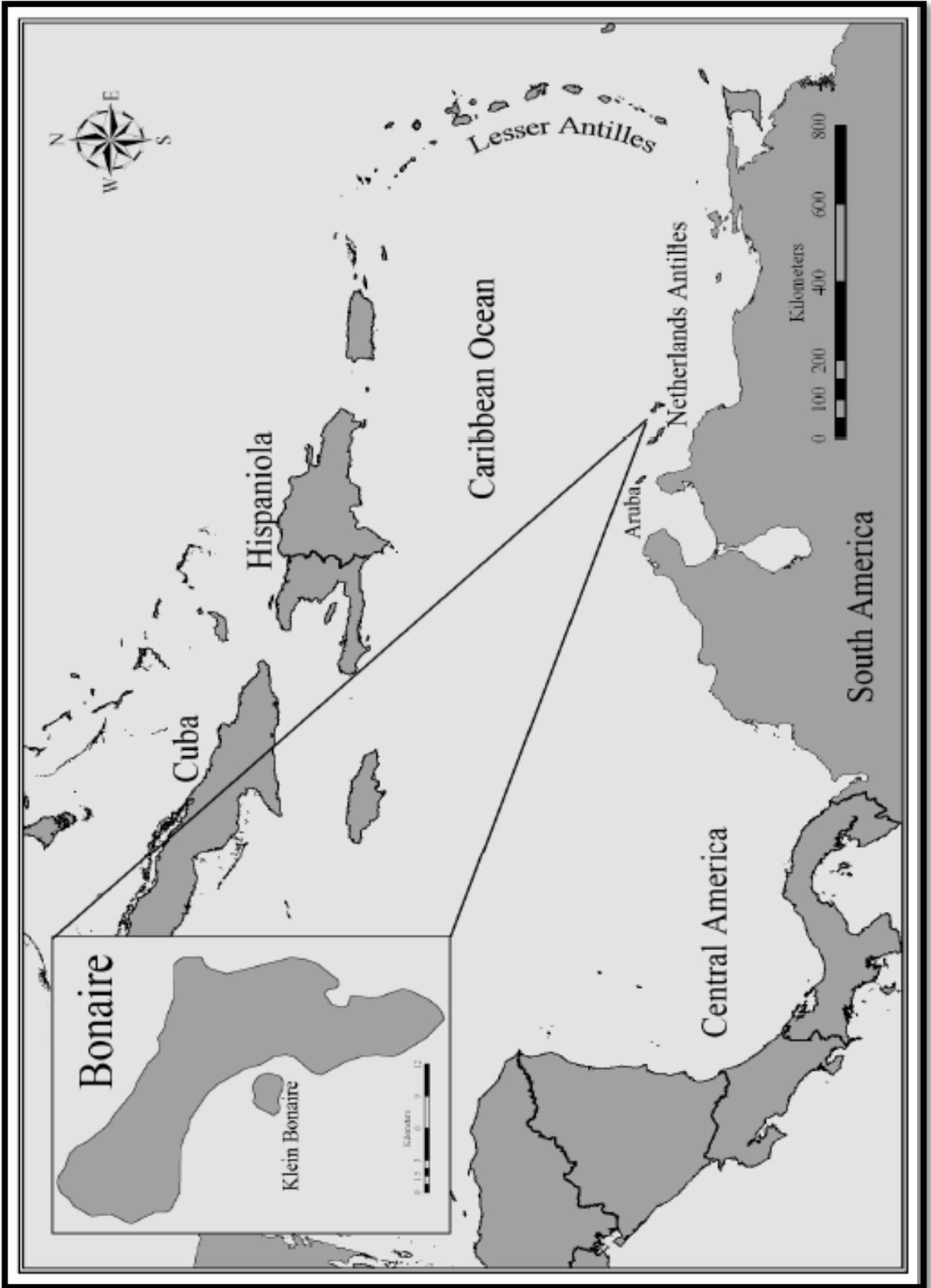
**Fishermen's by-catch: Effect on fish feeding behaviors in shallow sand flats of Bonaire, N.A.**

Colleen Chabot.....21



**Feeding ecology and twilight interspecific interactions of lionfish (Pterois spp.) in Bonaire, N.A.**

Kelsey Burlingame.....27



# Frequencies of coral disease in areas suspected of sewage-contaminated groundwater outflow in Bonaire N.A.

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## Abstract

Anthropogenically induced stressors are degrading coral reefs globally. Nutrients and bacteria present in wastewater increase the frequency and severity of coral disease. As a result of the lack of sewage treatment and poor sewage containment in Bonaire, N.A., the surrounding coastal marine environment is likely the endpoint of sewage-contaminated groundwater, especially near resort areas where water use is high and only a small portion of sewage is trucked away. This study compared the frequency of coral disease at three sites adjacent to resorts (with >100 beds) with three sites in the same region of the leeward coast that are not adjacent to resorts. Because areas where groundwater is entering the near-shore environment have not been identified; physical parameters of the seawater (temperature, specific conductivity, dissolved oxygen, and pH) at the six sites were measured using a YSI multi-parameter probe held directly above the substrate in areas 100 m wide along depths of 3, 6, 9, 12, and 18 m. To detect the presence of enterococci, a fecal indicator bacteria, six water samples were collected at 3, 9, and 18 m isobaths, 0.5 m below the surface and above the substrate. Additionally, water samples were collected twice at 3, 6, 9, 12, and 18 m to determine ammonia concentrations at each site. To determine the frequencies of disease in hard coral ( $\geq 20$  cm) and soft corals (sea fans, sea rods, sea plumes), three 10 x 1 m transects were surveyed at 3, 6, 9, and 18 m at all sites. The relationship between depth and specific conductivity, temperature, dissolved oxygen, and pH were similar for all sites except 18<sup>th</sup> Palm. Significantly higher concentrations of ammonia were found at resort sites ( $p=0.016$ ). Enterococci was detected at the three resort sites in greater concentrations than at non-resort sites. Disease in hard and soft corals did not differ between site types. This study suggests that coral reefs adjacent to resorts have greater concentrations of ammonia and enterococci, common wastewater indicators. Although the frequencies of soft and hard coral disease were not significant between sites, the abundance of hard corals was significantly lower at resort than at non-resort sites ( $p=0.010$ ). Soft corals were less abundant at resort sites than at non-resort sites, but the difference was not statistically significant ( $p=0.059$ ).

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## Introduction

An increase in human populations along coastal lands has led to increases in nutrients, sediments, and pollution discharged into the ocean (Fabricius 2005). Elevated levels of terrestrial loads have contributed to the degraded water quality in coastal and coral reef ecosystems throughout the Caribbean (Burke and Maiden 2004). In Bonaire, Netherlands Antilles, anthropogenically influenced changes in water quality are responsible for the decline of Bonaire's shallow coral reefs since the 1970s (Bak et al. 2005).

Sutherland et al. (2004) reviewed 18 studies on coral disease from 1965 to 2002 and identified eutrophication, sedimentation, pollution, and fecal contamination as stressors that increased the prevalence of coral disease. Bruno et al. (2003) found that nutrient

enrichment increased the severity of disease in *Gorgonia ventalina* and *Montastrea annularis*. Additionally, Kaczmarek et al. (2005) found that coral reefs exposed to sewage effluents high in enterococci had significantly higher frequencies of coral disease.

Kralendijk, Bonaire's capital, lies on coral limestone bedrock deposited during a higher sea level stand (Borst and de Haas 2005). In 1965, a water desalination plant was installed, providing a source of freshwater to the inhabitants of Bonaire (Borst and de Haas 2005). This water supply allowed the island to grow as a tourist destination, and tourism grew from 1,555 stopover tourists in 1955 to 74,332 in 2007 (Abel 2000; DEZA 2007). This growth consequently increased water use on the island and thus, the amount of wastewater produced.

The island of Bonaire does not have a wastewater treatment plant. With coral reefs

on Bonaire in decline, a connection between the effects brought on by growing residential and tourist populations, including increases in untreated sewage, is suspected (Bak et al. 2005). The practice of storing wastewater in septic tanks and cesspits is widespread in Bonaire. However, the porous nature of Kralendijk's limestone bedrock allows for groundwater to easily flow through it (van Sambeek et al. 2000), permitting untreated sewage to leach into the groundwater and eventually into the marine environment (van Sambeek et al. 2000; van Kekem et al. 2006).

The Bonaire National Marine Park conducted a water balance study in 2008 to identify areas of high water use and to measure how much of the water being purchased by resorts (R) was being trucked away from their septic tanks. There were large discrepancies in the amounts of water used compared with what was trucked away, especially at major Rs in Kralendijk (De Leon 2008). De Leon (2008) found that 82% of the water used in major Rs on Bonaire is unaccounted for when considering the amount being trucked from their septic tanks. This observation has led to the assumption that this unaccounted for wastewater is leaching through the groundwater and into the ocean, especially in areas adjacent to major Rs (De Leon 2008).

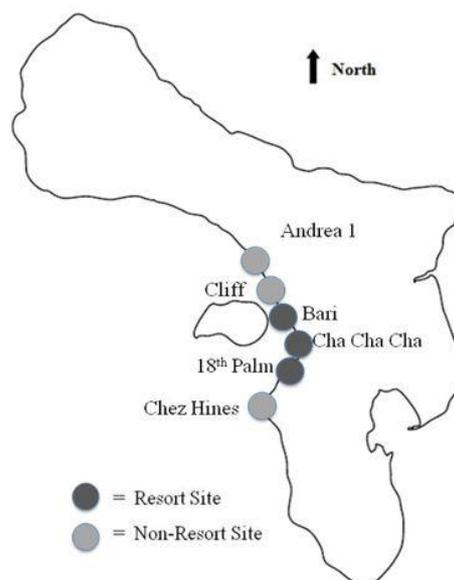
Van Kekem et al. (2006) sampled septic tanks at numerous Rs along Kralendijk's west coast. Results revealed nitrogen concentrations consistently occurring around 70 mg/L in the form of ammonia ( $\text{NH}_4^+$ ). Kralendijk's western coastal waters had higher nitrogen concentrations than did coastal waters of the undeveloped east coast of the island (van Kekem et al. 2006).

Based on the absence of a wastewater treatment plant and the large quantities of unaccounted for wastewater at Bonaire's major Rs, the following hypotheses were tested;  $H_1$ : Temperature (T) and specific conductivity (Sp. Cond.) in shallow seawater adjacent to Rs would be lower than at non-resort (NR) sites;  $H_2$ : Levels of  $\text{NH}_4^+$  and enterococci bacteria would be higher at R than at NR sites; and  $H_3$ : Frequencies of disease in soft (SC) and hard corals (HC) would be higher on reefs adjacent to R than NR sites.

## Materials & Methods

### Site Selection

Study sites were selected for their proximity to Rs containing the top three numbers of available beds (van Kekem et al. 2006) and known to use large quantities of water. The reefs chosen for investigation of groundwater discharge include 18<sup>th</sup> Palm Reef (12.13861°N 068.27644°W), Bari Reef (12.16768°N 68.28634°W) and Cha Cha Cha Reef (12.14420°N 68.27652° W) (Fig. 1). Three NR sites were selected for their proximity to Kralendijk and the R sites (Fig. 1). These sites are located along the central west coast of Bonaire. Two sites were selected north of Kralendijk, Cliff (12.17452°N 68.28983°W) and Andrea I (12.18754°N 68.29590°W), in addition to one site south of Kralendijk, Chez Hines (12.13865°N 68.27645°W).



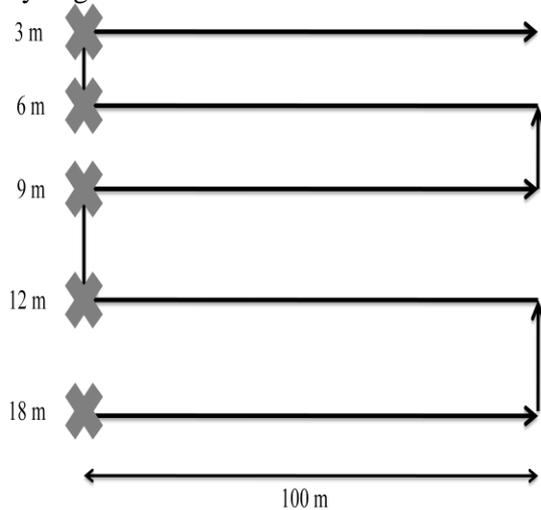
**Fig. 1** Map of Bonaire N.A. indicating the locations of sampling sites

### YSI Multi-Parameter Probe

A YSI multi-parameter probe attached to a data logger by a 30 m cable was used by a scuba diver to measure T, Sp. Cond., dissolved oxygen (DO), and pH at all sites along a U-shaped swimming pattern (Fig. 2). The sampling area was 100 m wide and traversed six depth contours (18, 12, 9, 6, 3 m). The diver guided the multi-parameter probe directly above the substrate as it recorded measurements in the YSI data logger.

A snorkeler operating a global positioning system (GPS) and the YSI data-logger swam

directly above the divers, swimming from the 18 m isobath along the pattern and ending at the 3 m isobath. The GPS and YSI data logger were placed on a Styrofoam platform in a large dry-bag.



**Fig. 2** U-shaped swimming pattern used to measure temperature, specific conductivity, dissolved oxygen and pH along 18, 12, 9, 6 and 3 m isobaths using a YSI multi-parameter probe. X indicates ammonia sampling locations. The same pattern was used to survey coral disease with the exception of 12 m

### Ammonia Sampling

Water samples were collected in 1-l bottles at the ends of each isobath along the U-shaped swimming pattern (Fig. 2).  $\text{NH}_4^+$  concentrations were determined using a LaMotte Salt Water Aquaculture test kit with a detection limit of  $\geq 0.05$  ppm. Sites were re-sampled at a later date to account for temporal variation.

### Enterococci Testing

Six water samples were taken at each site 0.5 m above the substrate and 0.5 m below the surface at 3, 9, and 18 m isobaths to test for the presence of enterococci. Samples were taken in 100 ml sterile bottles (IDEXX 2008). Bottles were filled with sterile saline solution before diving. At depth, bottles were filled with air to flush out the sterile saline solution and inverted to collect ambient seawater. This process was repeated three times before the final sample was taken. Samples were placed on ice and returned to the lab for processing according to the Enterolert enterococci detection protocol (IDEXX 2008). Positive samples were detected through fluorescence under a black light. The most probable number (MPN) of enterococci colony forming units was determined using Enterolert's MPN table.

### Coral Disease Survey

The frequency of coral disease was estimated at all sites. A U-shaped swimming pattern (Fig. 2) was surveyed at 18, 9, 6, and 3 m isobaths. The starting points of three 10 x 1 m transects were randomly selected for sampling at each depth. Distance between transects was measured in kick cycles. Quantities of diseased and healthy HC ( $\geq 20$  cm) and SC (sea rods, sea plumes, sea fans) were recorded.

### Statistical Analyses

$\text{NH}_4^+$  concentrations were compared between R and NR sites at five different depths (3, 6, 9, 12, 18 m) with two-way analysis of variance (ANOVA). The frequencies of disease in SC and HC between R and NR sites were compared at four different depths (3, 6, 9, 18 m) by two-way ANOVAs. Frequencies of disease in SC and HC were arcsine-transformed prior to analysis to obtain more normal distributions to meet the assumptions of ANOVA (Sokol and Rohlf 1969). Fisher's Protected Least-Significant Difference (PLSD) post hoc analyses for pair-wise comparisons determined significance within factors ( $\alpha=0.05$ ).

## Results

### Hard Coral Abundance

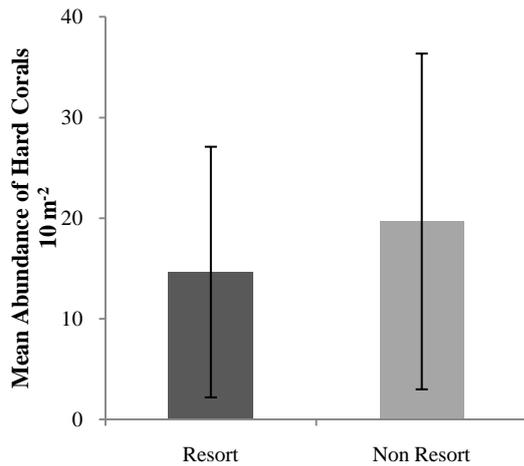
There were significantly more HC colonies at NR than at R sites (two-way ANOVA, Fig. 3, Table 1a). On average, there were 5 more HC colonies  $10 \text{ m}^{-2}$  at NR than R sites. NR sites had more HC colonies  $\geq 20$  cm than did R sites. Additionally, depth had a significant effect on the abundance of HC colonies (Fig. 4, Table 1a). The results of pairwise comparisons indicate that there were significantly fewer HC colonies at 3 m than at all other depths, and that there were significantly fewer HC colonies at 6 m than 9 m or 18 m (Table 1b).

### Soft Coral Abundance

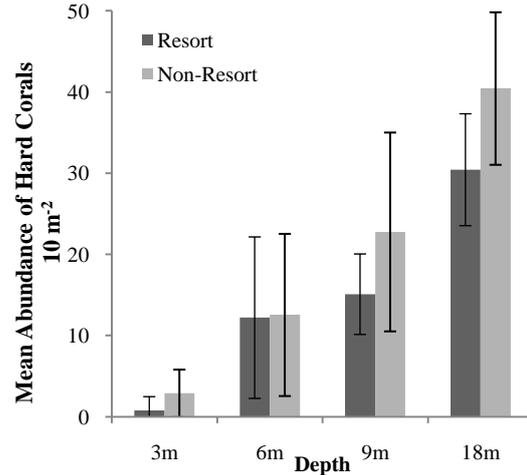
There were more SC colonies at NR sites, but the difference was not significant (Fig. 5, Table 2a). On average, there were 1.8 more SC  $10 \text{ m}^{-2}$  at NR sites than at R sites, but variability around the means was high. Depth had a significant effect on the abundance of SC colonies  $10 \text{ m}^{-2}$  (Fig. 6, Table 2a). The results

**Table 1 (a)** Two-way ANOVA testing the effects of depth (3, 6, 9, 18 m) and site type (R, NR) and their interaction on abundance of hard corals ( $\geq 20$  cm) **(b)** Fisher's PLSD post-hoc comparisons within factors (depth and site type,  $\alpha=0.05$ , S=significant)

(a)						(b)			
Source of Variation	DF	SS	MS	F	P	Depth	Mean Diff.	Crit. Diff.	P-Value
Depth	3	10,713	3571.051	54.872	<0.001	3 m, 6 m	-10.556	5.372	<0.001 S
Site Type	1	455.014	455.014	6.992	0.010	3 m, 9 m	-17.111	5.372	<0.001 S
Depth x Site						3 m, 18 m	-33.611	5.372	<0.001 S
Type	3	280.042	93.347	1.434	0.241	6 m, 9 m	-6.556	5.372	0.018 S
						6 m, 18 m	-23.056	5.372	<0.001 S
						9 m, 18 m	-16.5	5.372	<0.001 S
						NR, R	5.028	3.799	0.010 S



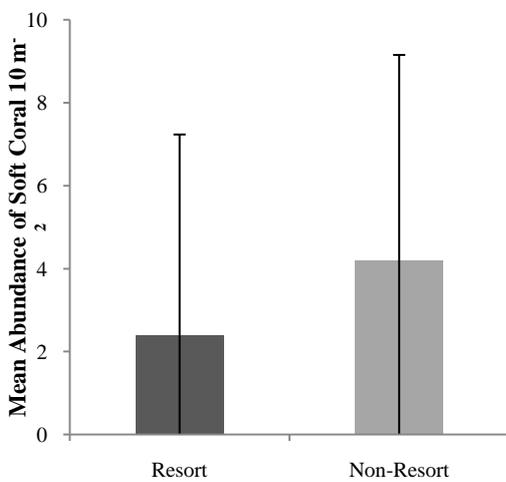
**Fig. 3** Mean abundance ( $\pm$  SD) of hard coral colonies ( $\geq 20$  cm) at R and NR sites



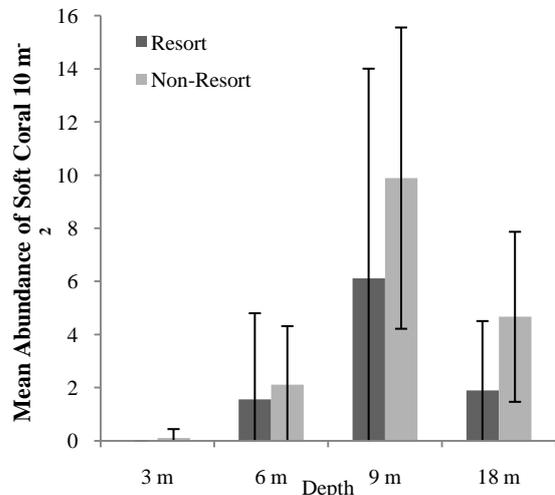
**Fig. 4** Mean abundance ( $\pm$  SD) of hard coral colonies ( $\geq 20$  cm) at 4 isobaths comparing R and NR sites

**Table 2 (a)** Two-way ANOVA testing the effects of depth (3, 6, 9, 18 m) and site type (R, NR) and their interaction on abundance of soft corals **(b)** Fisher's PLSD post-hoc comparisons within factors (depth and site type,  $\alpha=0.05$ , S=significant)

(a)						(b)			
Source of Variation	DF	SS	MS	F	P	Depth	Mean Diff.	Crit. Diff.	P-Value
Depth	3	625.819	208.606	13.132	<0.001	3 m, 6 m	-1.778	2.654	0.186
Site Type	1	58.681	58.681	3.694	0.059	3 m, 9 m	-7.944	2.654	<0.001 S
Depth x Site						3 m, 18 m	-3.222	2.654	0.018 S
Type	3	41.708	13.903	0.875	0.459	6 m, 9 m	-6.167	2.654	<0.001 S
						6 m, 18 m	-1.444	2.654	0.281
						9 m, 18 m	4.722	2.654	0.007 S



**Fig. 5** Mean abundance ( $\pm$  SD) of soft corals at R and NR site



**Fig. 6** Mean abundance ( $\pm$  SD) of soft corals at 4 isobaths comparing R and NR sites

of pair-wise comparisons indicate that there were significantly fewer colonies at 3 m than at 9 m or 18 m (Table 2b), and that there were more SC colonies at 9 m than at 6 m (Table 2b).

**YSI Multi-Parameter Probe**

T decreased slightly or remained the same as depth increased, with the exception of 18<sup>th</sup> Palm, where it increased with depth (Fig. 7). R and NR sites had similar values, with the exception of 18<sup>th</sup> Palm. All sites showed a small increase in Sp. Cond. Measurements (<0.1 mS/cm<sup>-1</sup>) as depth decreased, with the exception of 18<sup>th</sup> Palm where the increase was 0.4 mS/cm<sup>-1</sup> (Fig. 8). Sp. Cond. decreased as depth increased in all cases. DO increased with depth at all sites (Fig. 9). pH remained stable (8.10 to 8.25) at all sites except for 18<sup>th</sup> Palm, where it increased with depth and varied between 7.60 at the 3 m isobath to 7.99 at 18 m (Fig. 10).

**Ammonia Concentrations**

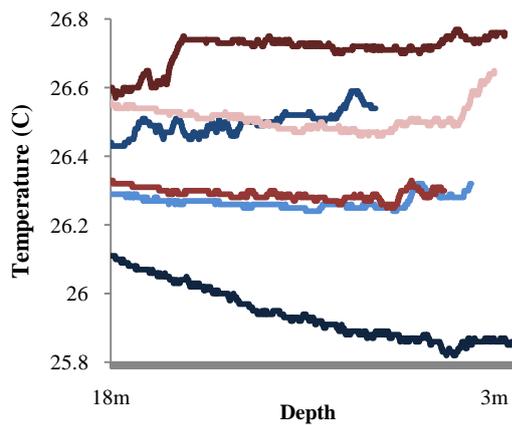
NH<sub>4</sub><sup>+</sup> concentrations in water samples were significantly higher at R sites than at NR sites (Fig. 11, Table 3). Depth had no significant effect on NH<sub>4</sub><sup>+</sup> concentrations (Table 3).

**Enterococci Testing**

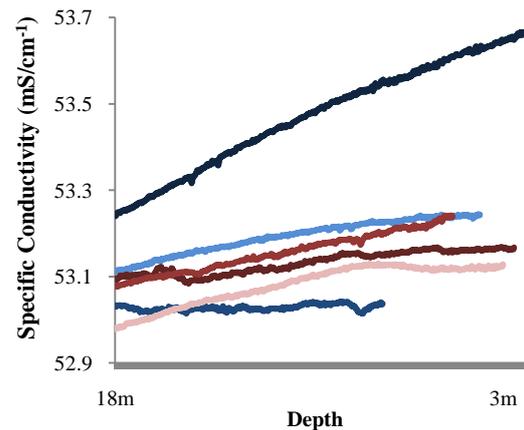
Three of 18 (16.7%) water samples collected from R sites tested positive for enterococci; one occurred at each site. The MPN determined for these samples ranged from 2.0 to 4.2 colony-forming units (data not shown). At NR sites, 1 of 18 (5.6%) water samples collected tested positive for enterococci. Cliff was the only NR site to test positive for enterococci, at a depth of 18 m. The sample's MPN was 1.0 colony-forming unit.

**Hard Coral Disease**

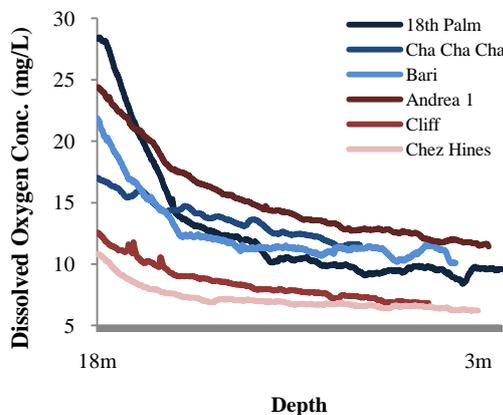
Frequency of disease in HC was slightly higher at R sites than at NR sites (Fig. 12).



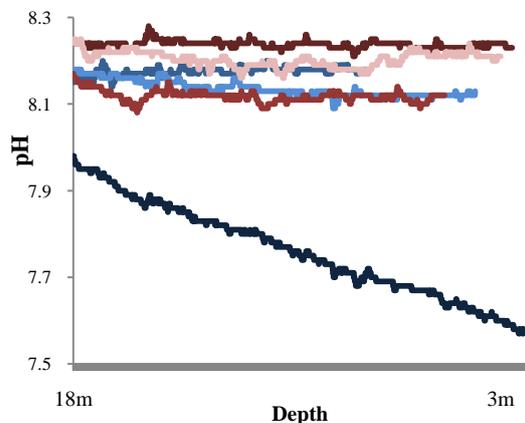
**Fig. 7** Temperatures from 18 to 3 m recorded by an YSI multi-parameter probe along a U-shaped swimming pattern at 3 R (blue lines) and 3 NR (red lines) sites



**Fig. 8** Specific conductivity from 18 to 3 m recorded by an YSI multi-parameter probe along a U-shaped swimming pattern at 3 R (blue lines) and 3 NR (red lines) sites



**Fig. 9** Dissolved oxygen from 18 to 3 m recorded by an YSI multi-parameter probe along a U-shaped swimming pattern at 3 R (blue lines) and 3 NR (red lines) sites



**Fig. 10** pH from 18 to 3 m recorded by an YSI multi-parameter probe along a U-shaped swimming pattern at 3 R (blue lines) and 3 NR (red lines) sites

**Table 3** Two-way ANOVA testing the effects of depth (3, 6, 9, 18 m) and site type (R, NR) and their interaction on ammonia concentration ( $\alpha=0.05$ ). Samples were taken twice per depth

Source of Variation	DF	SS	MS	F	P
Depth	4	0	0.005	0.818	0.520
Site Type	1	0.038	0.038	6.215	0.016
Depth x Site Type	4	0.017	0.004	0.708	0.590

There was no effect of site type (R, NR) on the arcsine-transformed frequencies of HC disease (Table 4a). However, depth had a significant effect on the frequency of disease of HC (Fig. 13, Table 4a). Results of pair-wise comp-

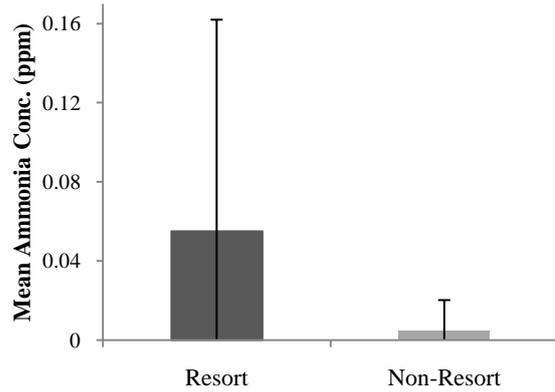
**Table 4 (a)** Two-way ANOVA testing the effects of depth (3, 6, 9, 18 m) and site type (R, NR) and their interactions on frequency of disease in hard corals ( $\geq 20$  cm) **(b)** Fisher's PLSD post-hoc comparisons within factors (depth and site type,  $\alpha=0.05$ , S=significant)

<b>(a)</b>						<b>(b)</b>			
Source of Variation	DF	SS	MS	F	P	Depth	Mean Diff.	Crit. Diff.	P-Value
Depth	3	4	1.495	16.809	<0.001	3 m, 6 m	-0.473	0.199	<0.001 S
Site Type	1	0.096	0.096	1.076	0.304	3 m, 9 m	-0.674	0.199	<0.001 S
Depth x Site Type	3	0.705	0.235	2.642	0.057	3 m, 18 m	-0.494	0.199	<0.001 S
						6 m, 9 m	-0.201	0.199	0.047 S
						6 m, 18 m	-0.021	0.199	0.834
						9 m, 18 m	0.18	0.199	0.075

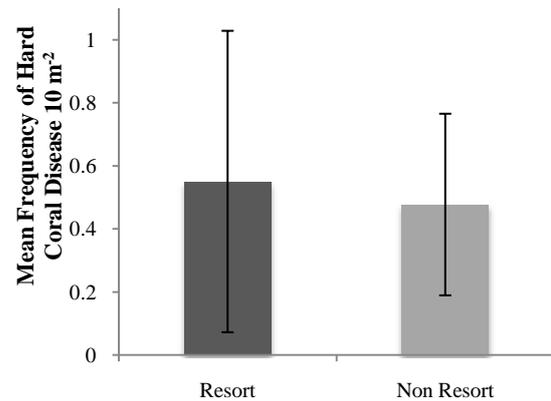
arisons indicated that there was significantly less disease at 3 m than at 6, 9, and 18 m (Table 4b). Additionally, the frequencies of HC disease were lower at 6 m than 9 m (Table 4b). The lower frequency of disease in shallow-water HC may be the result of only highly resistant species living at those depths, as there were on average <5 HC colonies  $10\text{ m}^{-2}$  at 3 m and ~10 HC colonies  $10\text{ m}^{-2}$  at 6 m, whereas the mean abundance of HC colonies at 9 and 18 m was between 15 and 37 colonies  $10\text{ m}^{-2}$  (Fig. 4).

**Soft Coral Disease**

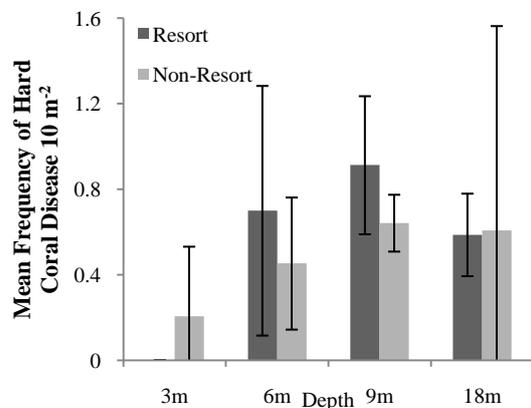
The frequency of disease in SC was higher at NR than R sites (Fig. 14). The arcsine-transformed frequencies of SC disease did not differ significantly by location (Fig. 14, Table 5a). However, depth had a significant effect on the frequency of disease in SC (Fig. 15). Pair-wise comparisons revealed significantly less disease at 3 m than at 6, 9, and 18 m (Table 5b). The frequency of disease at 3 m depth may be the result of only one SC being surveyed at that depth regardless of site type.



**Fig. 11** Mean ammonia concentrations ( $\pm$  SD) of water samples from R and NR sites



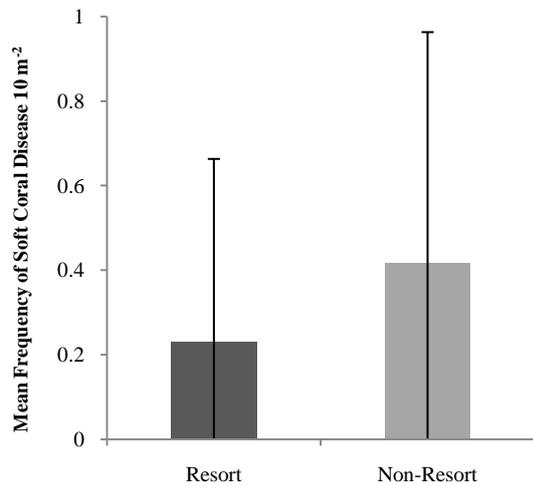
**Fig. 12** Mean frequency ( $\pm$  SD) of disease in hard coral colonies ( $\geq 20$  cm) at R and NR sites



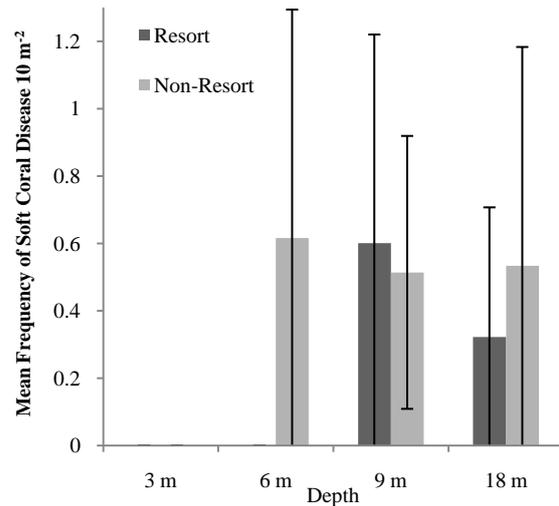
**Fig. 13** Mean frequency ( $\pm$  SD) of disease in hard coral colonies ( $\geq 20$  cm) at 4 isobaths, comparing R and NR sites

**Table 5 (a)** Two-way ANOVA testing the effects of depth (3, 6, 9, 18 m) and site type (R, NR) and their interactions on frequency of disease in soft corals **(b)** Fisher’s PLSD post-hoc comparisons within factors (depth and site type,  $\alpha=0.05$ , S=significant)

(a)					(b)				
Source of Variation	DF	SS	MS	F	P	Depth	Mean Diff.	Crit. Diff.	P-Value
Depth	3	3,071	1.024	5.194	0.003	3 m, 6 m	-0.308	0.296	0.042 S
Site Type	1	0.616	0.616	3.126	0.082	3 m, 9 m	-0.557	0.296	<0.001 S
Depth x Site Type	3	1.327	0.442	2.244	0.092	3 m, 18 m	-0.428	0.296	0.005 S
						6 m, 9 m	-0.25	0.296	0.097
						6 m, 18 m	-0.121	0.296	0.419
						9 m, 18 m	0.129	0.296	0.386



**Fig. 14** Mean frequency ( $\pm$  SD) of disease in soft coral at R and NR sites



**Fig. 15** Mean frequency ( $\pm$  SD) of disease in soft coral at 4 isobaths, comparing R and NR sites

## Discussion

### YSI Measurements

Areas of groundwater outflow were unable to be identified using YSI T and Sp. Cond. measurements. Therefore,  $H_1$ , that lower T and Sp. Cond. would be detected at R sites, was not supported in this study. Although it was hypothesized that lower T would be found in areas of groundwater outflow, groundwater T measurements by Borst and de Haas (2005), indicate that the groundwater in Bonaire is warmer than oceanic water. Groundwater T varies with time; however, T measurements of groundwater by Borst and de Haas (2005) were never below 27°C.

Ts recorded at all study sites never exceeded 26.8°C. At R sites, where groundwater may be entering the marine realm, an increase in seawater T is more likely where oceanic and groundwater mix. There was no consistent pattern between depth and T in this study. Two NR and one R site had higher T at the 3 m isobath, one NR and one R site had no difference and one R site showed a decrease in T as depths decreased.

The hypothesis of decreased Sp. Cond. at R sites was refuted because Sp. Cond. was highest at 18<sup>th</sup> Palm. Sp. Cond. measurements increased gradually as depth became shallower, especially at 18<sup>th</sup> Palm, where it started out higher at 18 m than at the other five sites and increased 8x more than any other site (Fig. 8).

Sp. Cond. is a measure of how well water can conduct an electrical current. Affecting these measurements are all dissolved solids in the water (Pilgrim et al. 1979). An increase in dissolved solids in the water could explain the observed increase in Sp. Cond. at shallower depths at all sites, in addition to the unexpected results occurring at 18<sup>th</sup> Palm.

YSI sampling occurred during the ebb of a spring tide at 18<sup>th</sup> Palm. Izbiki et al. (2009) found that the coastal waters of Santa Barbara, CA had a similar increase in Sp. Cond. during the ebbs of spring tides. Therefore, greater quantities of dissolved solids may have been transported through groundwater into the marine environment at 18<sup>th</sup> Palm that day, because the depth of the water table varies according to the tides in Bonaire (Borst and de Haas 2005). This variation could account for

the sharper rate of increase in Sp. Cond. at 18<sup>th</sup> Palm; however, the overall Sp. Cond., regardless of depth, could be related to the addition of highly saline waters from a man-made inlet that drains a salina directly south of the site.

The depth profiles of sites studied for DO and pH may prove valuable in interpreting the effects of contaminated groundwater on biotic and abiotic processes occurring on Bonaire's shallow-water coral reefs. Higher levels of nutrients in shallow water could explain the decreases in DO observed as depths became shallower. Increased nutrient levels promote algal growth in marine systems (Lapointe 1997). The death and microbial decomposition of algal blooms have been shown to induce sharp declines in concentrations of DO at affected sites, most notably the "Dead Zone" occurring each summer in the Gulf of Mexico (Dodds 2006). Pastorok and Bilyard (1985) found that increased nutrients and organic matter present in sewage effluents reduced DO levels in coral reef communities as a result of microbial decomposition of increased primary productivity. Although there was no distinguished pattern between R and NR sites, DO decreased as depth decreased at all sites and may indicate higher nutrient levels in shallow water along the central, leeward coast line of Bonaire.

#### **Ammonia Concentrations at Resort Sites**

H<sub>2</sub>, that there would be higher concentrations of NH<sub>4</sub><sup>+</sup> at R than at NR sites was supported by this research. Mean NH<sub>4</sub><sup>+</sup> concentrations were 11x higher at R sites. R sites had significantly higher (Table 3) mean concentrations of NH<sub>4</sub><sup>+</sup> than NR sites, suggesting that the source of NH<sub>4</sub><sup>+</sup> is localized (Fig. 11). NH<sub>4</sub><sup>+</sup> concentrations were highest at 18<sup>th</sup> Palm, where they increased as depth decreased. However, mean NH<sub>4</sub><sup>+</sup> concentrations at R sites did not differ with depth. Nutrient enrichment of coral reef ecosystems is known to contribute to localized coral reef degradation (Szmant 2002). In this comparison of R and NR sites, HC were less abundant at R sites. A similar trend in abundances of SC was observed at R sites.

Nutrient-loading has been suggested to increase the severity of disease in SC and HC (Bruno et al. 2003). Additionally, Lapointe (1997) suggests that nitrogen-enriched

wastewater entering coral reefs through discharged groundwater has contributed to a phase-shift from HC to algal-dominated reefs in southeast Florida. To prevent Bonaire's coral reefs from realizing a similar fate, measures need to be taken to decrease loads of excess nutrients from entering the marine environment.

#### **Enterococci as an Indicator of Sewage Contamination**

Enterococci, a common fecal indicator bacteria, has been widely used to identify areas of sewage contamination (Davies-Collie et al. 1994; Kaczmarsky et al. 2005; Izbicki et al. 2009). Griffin (2003) observed declines in coastal surface water quality and increases in fecal-indicator bacteria in areas of septic tank use in the Florida Keys.

Data collected in this study suggests that enterococci bacteria contamination is related to the widespread use of septic tanks and leaching holes at major Rs on Bonaire. This conclusion is supported by its presence at all R sites in greater abundances and densities (MPN 2, 2, 4, 2) and its absence at two of three NR sites and a lower MPN (1). Additionally, all positive samples taken from R sites occurred at 3 m, with two occurring at the surface and one at depth. If the source of enterococci is groundwater, then enterococci should be found near the surface in areas of groundwater outflow because freshwater is less dense than seawater. After mixing occurs, enterococci could be found at any depth in the surface-water mixing zone because it can persist in the marine environment. The presence of enterococci at 3 m could indicate that groundwater is entering the marine environment in very shallow water. Future studies should include sampling at depths <3 m.

The above explanation cannot be extrapolated to the enterococci-positive NR sample, which occurred at a depth of 18 m. Current patterns could potentially explain the presence of enterococci at Cliff, because it is down current from multiple major Rs. Diver-shedding could be a potential explanation as well (Elmir et al. 2009).

The greatest abundance of enterococci (MPN 4) occurred at 18<sup>th</sup> Palm. Griffin et al. (1999) found increases in bacteria and nutrients in areas exposed to wastewater effluents. This study showed greater

abundances of enterococci at R sites, where the concentrations of  $\text{NH}_4^+$  were significantly higher. The site with the greatest concentration of enterococci was found to be 18<sup>th</sup> Palm; the same site with the greatest densities of  $\text{NH}_4^+$ . In addition, the concentration of  $\text{NH}_4^+$  increased in the shallow water samples, as did the MPN of enterococci.

The United States Environmental Protection Agency recommends measuring enterococci levels to determine whether marine recreational waters should be closed as a result of health risks (Griffin et al. 2003). However, Wetz et al. (2004) suggests that fecal-indicator bacteria, such as enterococci, are poor predictors of enteric viruses, which produce health risks in those infected. Questions regarding the ability of enterococci to accurately predict the presence of infective enteric viruses in marine environments remain, but enterococci bacteria have been associated with coral disease (Griffin et al. 1999).

### **HC Disease Frequency**

H<sub>3</sub>, that frequencies of disease in HC would be greater at R sites was refuted because no significant difference was found. Variability of disease frequency around the means was large, indicating the need for a greater number of samples to increase the statistical power for detecting differences if they exist. The low abundance of HC (Table 1b) at 3 m could explain the lower frequency of disease at that depth. The mean frequency was decreased due to transects containing no HC  $\geq 20$  cm at this depth. There were no HC colonies at 3 m at R sites, which means there was no potential for disease. Transects with no HCs also influenced the mean frequency of disease at 6 m. At 9 m, there was more HC disease than at other depths, including at 18 m.

The overall pattern of fewer HC at all depths and more disease at 6 and 9 m, could be explained by the effects of groundwater entering the marine environment at shallow depths at R sites. Over time, HC at shallow depths at R sites may have been killed by disease associated with higher levels of nutrients and bacteria. The HC at 9 m are currently experiencing significantly more disease than those at 18 m. Other factors, such as bleaching or hurricanes, could explain this pattern with the possible exception that there is significantly less HC at R sites.

### **SC Disease Frequency**

The mean abundance of SC at NR sites was nearly double that at R sites. Small sample size, a high number of replicates with no SC, and high variability indicate that a greater number of replicates are needed to detect meaningful differences if they exist. The highest mean abundance of SC occurred at 9 m at R and NR sites. Frequency of diseased SC colonies peaked at 9 m for R sites, but was fairly constant (with the exception of 3 m, where there were none) at the other depths (6, 9, 18 m).

Analysis of sea plume abundances and disease frequencies can be viewed as a microcosm to better understand the plight of SC in Bonaire. Significantly greater abundances of sea plumes were found at NR than at R sites (T-test,  $p=0.019$ ; Fig. 16). However, sea plumes at NR sites had significantly greater frequencies of disease (T-test,  $p=0.042$ ; Fig. 17). The lower abundance of sea plumes observed at R sites could be explained by poor water quality at R sites in past years. Poor water quality may have resulted in higher frequencies of disease and death in sea plumes at R sites previously, affecting their abundances observed today. If the difference in abundance of sea plumes is due to disease, any remaining sea plumes may be disease resistant (Kim and Harvell 2004). Large-scale degradation of water quality in Bonaire (Wieggers 2007) may now be affecting sea plumes and other SC surveyed at NR sites. Therefore, either degraded water quality at NR sites or the presence of disease resistant colonies at R sites could contribute to the higher frequency of SC disease at NR versus R sites.

Lapointe et al. (1990) showed that nutrient levels in the surrounding marine environments were greater in areas on the Florida Keys using septic tanks. Septic tank use on Bonaire has been associated with a decline in coastal water quality, spurring the installation of organic content monitoring programs such as the Light and Motion Sensor Program (LMSP) (Jones et al. 2008). Steps need to be taken immediately to decrease loads of nutrient and microbial-rich wastewater from entering the coastal marine environment, as phase shifts on coral reefs appear to be a rapid and non-linear phenomenon (Hughes 1994). This study provides an initial assessment of areas that are

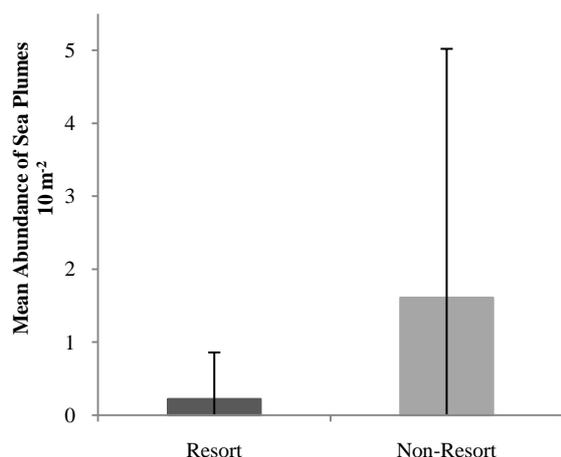


Fig. 16 Mean abundance ( $\pm$  SD) of sea plumes at R and NR sites

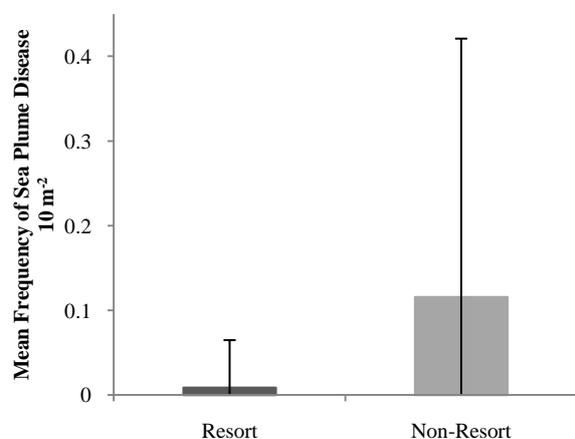


Fig. 17 Mean frequency ( $\pm$  SD) of disease in sea plumes at R and NR sites

likely to have large amounts of contaminated groundwater entering the near-shore marine environment on Bonaire. The most convincing result of this study is that there are lower abundances of HC and SC at R sites. A more comprehensive study with improved replication is necessary to determine the relationships among nutrients, enterococci and disease.

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# Using prey fish species as bioindicators of anthropogenic stress and predictors of predator density and diversity on coral reefs in Bonaire, N.A.

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## Abstract

Bioindicator species have been used to determine changes in water quality and the effect of pollution at sites of environmental concern. Increasingly degraded water quality throughout the Caribbean is leading marine park managers and scientists to use bioindicator organisms to rapidly detect differences in water chemistry by determining connections between environmental parameters and changes in reef fish communities. This study sought to determine bioindicator prey species that could provide early detection of changes as a result of anthropogenic activities in the coastal waters of Bonaire, N.A. The effects of these parameters on the density and diversity of reef fish species was compared between 4 sites of “more (MI)” and 4 sites of “less (LI)” anthropogenic impact (<200 m and >200 m from of coastal development, respectively). Fish communities were surveyed using a modified version of the AGRRA methodology during the morning and evening. Two 30x2 m transects at 12 m depth were used at each site to survey both prey and predator fish species. Water chemistry including nutrient, bacterial and sedimentation levels were also analyzed to attempt to determine the factor(s) driving the changes. This study revealed significantly greater densities and a higher diversity of prey and predatory fish species at MI sites versus LI sites during the morning and the evening. The species that was found at greatest densities for both LI and MI sites was *Stegastes partitus*, with significantly more *S. partitus* at MI sites during both the morning and evening. Thus, *S. partitus* may be a possible bioindicator of stressors on the reefs in Bonaire. The use of *S. partitus* as a bioindicator of anthropogenic stress may help increase the effectiveness of marine management protocols in Bonaire and provide a basis for determining bioindicator species for monitoring coastal water quality throughout the Caribbean. None of the water chemistry parameters studied differed between MI and LI sites, therefore, the driver(s) of the differences in prey species (e.g. *S. partitus*) may be unaccounted for in this study as a result of time lags in the coral reef ecosystem.

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## Introduction

In the last decade, concern over the impacts of human population growth and development on coral reef systems has grown worldwide. Coral reefs are quickly disappearing due in large part to destructive fishing, pollution, global climate change and coastal development (Norse and Crowder 2005). These anthropogenic pressures have led to a phase shift from coral to algal dominated reefs in the Caribbean (Burke and Maidens 2004). Coastal development has been cited as one of the main threats facing the Caribbean due to the increased nutrient and sediment runoff associated with it (Burke and Maidens 2006). The unprecedented rate of coastal development is increasing sedimentation in near-shore waters, smothering reef-building corals and reducing light necessary for photosynthesis (Rogers 1990). Losses of reef-building corals can have ecosystem-wide cascading effects by altering

the reef's structural framework, thus reducing the abundance or changing the biodiversity of fish species (Rogers 1990). Anthropogenically induced decreases in biodiversity are plaguing coral reefs globally (Markham 1996). Nutrients, heavy metals, pesticides and hydrocarbons from runoff and development have also been shown to degrade coral reefs at local scales (Fabricius 2005). As coral reefs thrive in oligotrophic waters, excess nutrients may increase macroalgal growth resulting in a phase shift from coral dominated reefs to algal dominated reefs (Burke and Maidens 2004).

It has been suggested that the direct (e.g. chemical analyses) monitoring of pollutants in marine systems may not provide enough information about their ultimate effects on the biology of coral reef ecosystems (Naranjo et al. 1996). Therefore, indentifying biological entities, or bioindicator species, that readily respond to environmental stressors may be useful in the early detection and monitoring of pollutants and their effects on reef com-

munities. Marine bioindicator species have previously been used to detect environmental stressors. Burger et al. (2007) identified and utilized bioindicator fish and bird species at multiple trophic levels to detect radionuclides in the waters of the Aleutian Islands, AK. They found that 80% of the fish species and 20% of the bird species studied had elevated levels of Cesium<sup>137</sup> in their tissues. These data were used as a baseline for the continued monitoring of the pollutant's spread and effects on other local marine organisms. In New Caledonia, SW Pacific, the tropical brown alga *Lobophora variegata* showed promise as a bioindicator species for excess silver (Ag) in coastal waters. *L. variegata* rapidly concentrates Ag within its tissues. Metian and Warnau (2008) found that after only 28 d the alga retained 7000x more Ag than ambient water. Since excess Ag is often associated with sewage sludge, *L. variegata* may be useful as a bioindicator of improper wastewater treatment. In Bonaire, Netherlands Antilles Williams (2009) looked at Christmas tree worms (*Spirobranchus giganteus*) as potential bioindicators of environmental stress. These sessile, filter feeding worms were documented at significantly greater densities in living coral near commercial and residential sites (<200 m away) with elevated phosphates and finer sediment particles possibly attributed to eutrophication and/or run-off caused by increased coastal development (Williams 2009).

Sessile organisms have commonly been used to assess the utility of bioindicators to identify marine pollutants and stressors (e.g., Cooper et al. 2008; Metian and Warnau 2008; Williams 2009). The stationary nature and modes of nutrient uptake in these organisms (e.g., filter feeding) prove useful in determining localized changes in water quality. However, few marine studies have assessed whether mobile organisms are useful bioindicators of stress (except Burger et al. 2007), and, moreover, useful bioindicators without the need for direct handling or tissue sampling. Though coral reef fishes are not sessile, many species maintain fairly permanent territories on the reefs. For instance, damselfishes (*Pomacentridae*) have been documented protecting microhabitats of algal "gardens" within defined areas of the reef, and several species of parrotfish (*Scaridae*), hamlets (*Serranidae*), surgeonfish (*Acanthuridae*) and butterflyfishes

(*Chaetodontidae*) maintain semi-permanent home territories (DeLoach and Humann 1999). Although mobile, such territorially-limited fish species may respond noticeably and rapidly to local or regional anthropogenically induced environmental changes and raises the question, could one or more of these species play the role of a bioindicator prey species? If prey fish are affected by these parameters could predators be affected too? Stevenson et al. (2007) found that several reefs in the Pacific had a greater biomass of predatory fishes and exhibited increased biomass in lower trophic level herbivorous fishes that are often the prey of choice for these predators. These data corroborated with a study performed in the Northern Hawaiian Islands which found that where apex predator biomass was >50% of the total fish biomass there was elevated biomass of herbivorous fishes at lower trophic levels (Friedlander and DeMartini 2002). These studies suggest that areas with higher densities of prey fishes will also have higher densities of predatory fishes. Therefore, if natural and/or anthropogenic stressors affect the composition of prey fish communities on reefs, it is possible that predatory fishes will be indirectly affected by these stressors as well.

The southern Caribbean island of Bonaire, N.A. has been experiencing deterioration of its near-shore fringing coral reefs since the 1970s (Bak et al. 2003). Growing resident and tourist populations and extensive coastal development have been suggested as potential causes of observed increases in sediment, nutrient and bacterial loads in the leeward coastal waters of the island (Rini 2008; Sinnott 2009; Williams 2009). The lack of a proper wastewater treatment system has led to the widespread use of unlined septic tanks and cesspits on the island (van Sambeek et al. 2000). This, coupled with the island's base of porous limestone (BNMP 2006) allows nutrient and bacteria-"heavy" water to enter directly into the ocean. Building and road construction have contributed to nutrient loading and sedimentation in coastal waters (BNMP 2006). As Bonaire economically relies on its coral reefs (e.g., dive tourism, artisanal fishing) losses or changes in coral communities due to anthropogenically-induced stressors could be devastating. The determination of bioindicator reef fish species to effectively identify reef areas high in nutrients and sediments could potentially increase the effectiveness of the

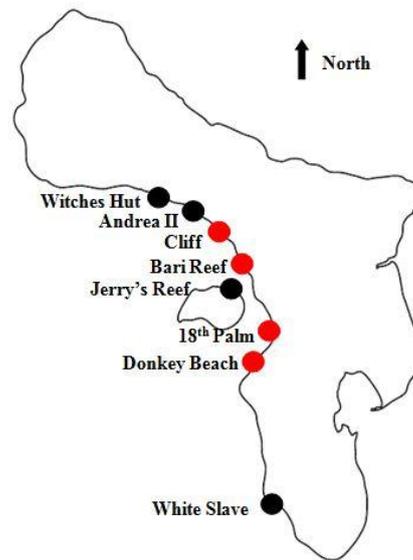
conservation efforts of the Bonaire National Marine Park. As changes in fish communities, particularly densities and diversity of predatory fishes can be easily identified by scientists, diving tourists and fisherman alike, the determination of bioindicator species could serve as an early biological detection system for changes in water quality.

In this study the following questions were addressed: 1) will sites more impacted by coastal human activity have higher densities and diversity of bioindicator prey species than those less impacted by coastal human activity, 2) will sites with higher densities and diversity of bioindicator prey species have higher densities and diversity of Bonaire's common predatory fishes, 3) do the water conditions (e.g., excess nutrients, enterococcal bacterial loads, finer sediment particles) positively relate to bioindicator prey fish densities and thus affect the densities and diversity of predatory fishes?

## Materials & Methods

### Study Sites

In order to assess the effects of stressors such as excess nutrients, sedimentation, and *Enterococcus* bacteria on the densities and diversity of potential bioindicator prey species sites defined as "More Impacted (MI)" and "Less Impacted (LI)" by the long-term water quality monitoring project (LTR) of the Council for International Educational Exchange Research Station Bonaire (CIEE-RSB) were chosen. A MI site is defined as one located <200 m from a commercial establishment such as a resort, restaurant, or store and sites defined as LI are surrounded on all sides by >200 m of rural or residential land (Rini 2008). For this study four MI sites including Donkey Beach, Eighteenth Palm, Cliff and Bari Reef and four LI sites including White Slave, Jerry's Reef on Klein Bonaire, Andrea II and Witches Hut were chosen (Fig. 1). At all LTR sites, excess nutrients (phosphates, nitrates, nitrites, ammonia) and *Enterococcus* bacterial loads are determined through water sampling as well as sedimentation rates and particle size distribution are determined using a permanent sediment trap at 12 m on a biweekly basis.



**Fig. 1** Map of Bonaire, N.A. Red dots indicate "more impacted" study sites and black dots indicate "less impacted" study sites (<200 m and >200 m from sites of coastal development, respectively)

### Potential Bioindicator Fish

In order to identify appropriate bioindicator fish species the process developed by Burger et al. (2006) was used as a guideline. Burger et al. (2006) recommend a literature review on the preferred prey of large predators commonly found at the study sites of interest, as well as *in situ* observations of prey fishes found at each location. Biological, methodological, and societal relevance of the species were considered in accordance with the guidelines provided. A pool of eligible bioindicator prey species was selected from the literature and relevance review and compared with the species documented *in situ*. Those fish considered in the literature to be prey of Bonaire's common predatory fish and found in elevated densities (>20 fish 100 m<sup>-3</sup>) were used as bioindicator species for this study.

### Monitoring

Once potential bioindicator prey fish species were identified through the literature review, their densities and diversity were recorded at MI and LI locations. In order to assess densities and diversity of predator and prey fish species at the study sites, SCUBA was used to complete 30x2x12 m (720 m<sup>3</sup>) transects to both the north and south of CIEE-RSB's permanent sediment trap at 12 m depth. The distance of 30 m was chosen to ensure that observations of fish would be in an area close enough to the permanent sediment traps of

CIEE-RSB and any potential environmental stressors documented through CIEE-RSB's LTR would be viable as parameters for potential differences in fish communities. Following transect deployment, a modified version of the AGRRA fish survey methodology was conducted using a 1 m T-bar (Kramer et al. 2005) to survey the predetermined potential bioindicator prey and predator fish species within the aforementioned volume of space. Predators in Bonaire were predetermined to include: groupers, snappers, moray eels, barracuda, tarpon, bar jack, Caribbean reef squid, boga and black margate. Transects were surveyed in the morning (0730 h) and the evening (1930 h) for all sites in order to determine if densities and diversity of bioindicator prey and predatory fish species varied temporally. Each site was surveyed in the morning and evening on the same day to further avoid temporal variances. During each pass along the transect one diver was assigned to record "very abundant" fishes in order to allow other fish species to be surveyed by the second diver more easily. During morning dives this species was the bicolor damselfish, *Stegastes partitus*, and for evening dives this species was the blackbar soldierfish, *Myripristis jacobus*.

Additionally, during morning dives a 10 min sit-and-observe session was performed at each location prior to transect deployment. This provided an acclimation period for the fish as well as an opportunity to survey each site for any "shy" prey or predatory fishes that tend to avoid the transect line. These observation sessions were performed by two divers positioned back-to-back directly over the CIEE-RSB permanent sediment traps at a 12 m depth so as to encompass a 360° view of the site with their combined range of vision. All fish species observed were identified and recorded.

### **Water Quality**

In order to determine the presence or absence of potential stressors at MI and LI sites the data on excess nutrients, sedimentation and bacterial loads were acquired from CIEE-RSB's LTR data set during the time period of January-March 2010. The collection and analyses of nutrient, bacterial (*Enterococcus*), and sediment samples for the CIEE-RSB LTR data were

done using the methodology described by Sinnott (2009) and Williams (2009).

### **Statistical Analyses**

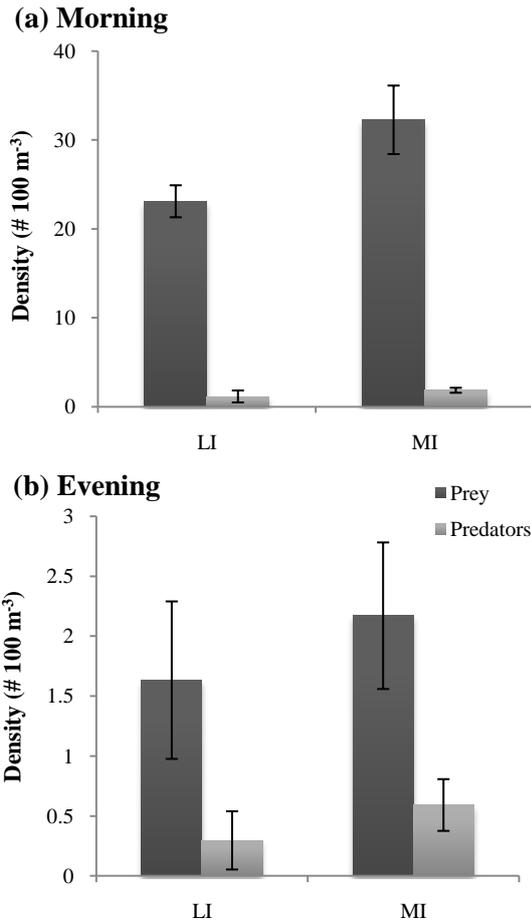
In order to assess any differences between the densities and diversity of prey and predator species at LI and MI sites for morning and evening data two-way analysis of variance (ANOVA) were run. A two-way ANOVA was also used to compare the densities and diversity of bioindicator prey species between LI and MI sites. Additionally, a two-way ANOVA was used to assess differences in the densities of bicolor damselfish, a potentially useful bioindicator species (see results and discussion) between LI and MI sites. Two-tailed T-tests were used to assess differences in nutrient levels and bacterial loads between LI and MI sites. A two-way ANOVA was used to determine differences in sediment particle size distributions and sedimentation rate between site types.

## **Results**

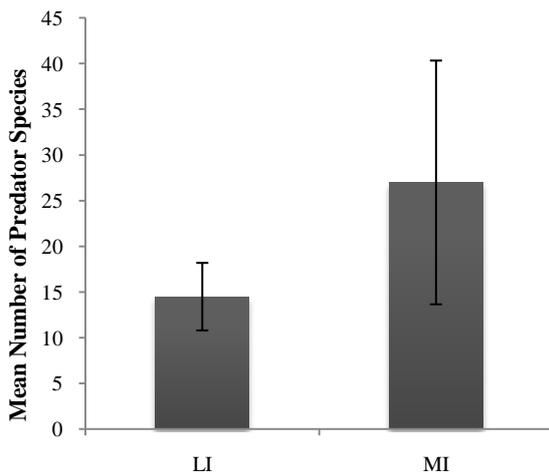
### **Monitoring**

Fish densities were significantly higher at MI sites (two-way ANOVA, site x fish type  $p=0.002$ , Fig. 2a) during the morning. The mean density ( $\pm$  SD) of prey fishes in the morning at MI sites was  $32.256 \pm 3.854$  as compared to  $23.099 \pm 1.799$  at LI sites. The mean density ( $\pm$  SD) of predatory fishes in the morning at MI sites was  $1.840 \pm 0.281$  as compared to  $1.146 \pm 0.672$  at LI sites. In the evening MI sites had higher prey and predatory fish densities ( $2.170 \pm 0.611$  and  $0.590 \pm 0.216$ , respectively) than LI sites ( $1.632 \pm 0.657$  and  $0.590 \pm 0.216$ , respectively, Fig. 2b). The interaction between site and fish type was not significant (two-way ANOVA,  $p=0.621$ ). A higher number of predatory fishes were present at MI sites ( $27.000 \pm 13.342$ , mean  $\pm$  SD), than LI sites ( $14.500 \pm 3.697$ , Fig. 3) during morning sit-and-observe sessions (T-test  $p=0.121$ ).

Overall, predator species were significantly more diverse than prey species during the morning, while prey were significantly more diverse than predators in the evening (two-way ANOVA,  $p=0.003$  and  $p=0.004$ , respectively, Fig. 4a & b).

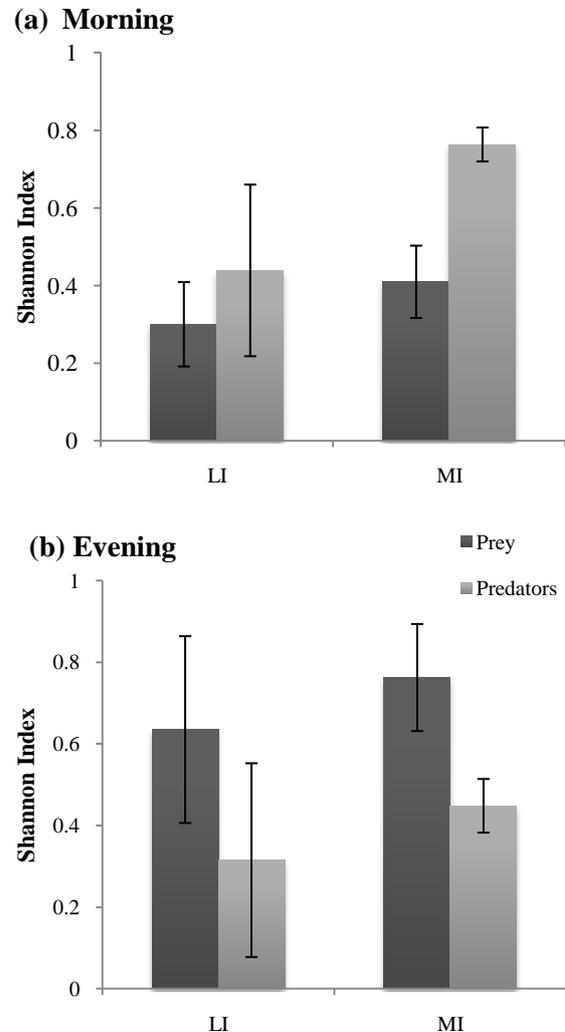


**Fig. 2** (a) Mean density (# 100 m<sup>-3</sup> ± SD) of prey and predatory fishes at less and more impacted sites (>200 m and <200 m from sites of coastal development, respectively) during the morning (two-way ANOVA, site p<0.001, fish type p<0.001, and site x fish type p=0.002) (b) Mean density (# 100 m<sup>-3</sup> ± SD) of prey and predatory fishes at both less and more impacted sites during the evening (two-way ANOVA, site p=0.107, fish type p<0.001, site x fish type p=0.621)



**Fig. 3** Mean total number of predatory fish (± SD) recorded during the sit-and-observe session at less and more impacted sites (>200 m and <200 m from sites of coastal development, respectively) during the morning (T-test p=0.121)

In the morning there was a significantly higher overall diversity at MI sites when compared to LI sites (two-way ANOVA, site p=0.007). This, however, was not found to differ significantly at night (two-way ANOVA, site p=0.174).

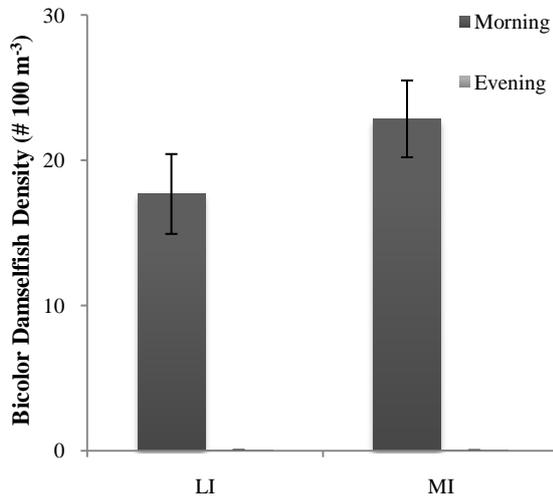


**Fig. 4** (a) Mean diversity (Shannon Index ± SD) of prey and predatory fishes at less and more impacted sites (>200 m and <200 m from sites of coastal development, respectively) in the morning (two-way ANOVA, site p=0.007, fish type p=0.003, site x fish type p=0.133). (b) Mean diversity (Shannon Index ± SD) of prey and predatory fishes at less and more impacted sites in the evening (two-way ANOVA, site p=0.174, fish type p=0.004, site x fish type p=0.976)

### Potential Bioindicator Fish

Upon a more detailed analysis of individual prey species, there appeared to be higher densities of *S. partitus* than any other species encountered. *S. partitus* was observed at densities of >20 fishes 100 m<sup>-3</sup> for three of the four MI sites, thus meeting the previously described requirements for a bioindicator species. Mean densities (± SD) of *S. partitus*

during the morning at LI sites ( $17.682 \pm 2.749$ ) were significantly lower than at MI sites ( $22.856 \pm 2.643$ , two-way ANOVA, site x time  $p=0.019$ , Fig. 5). However, at night *S. partitus* was nearly undetectable at both LI and MI sites ( $0.017 \pm 0.035$  and  $0.026 \pm 0.017$ , respectively).



**Fig. 5** Mean density of *Stegastes partitus* ( $\pm$  SD) at less and more impacted sites ( $>200$  m and  $<200$  m from of coastal development, respectively) during the morning and evening (two-way ANOVA, site  $p=0.019$ , time  $p<0.001$ , site x time  $p=0.019$ )

### Water Quality

Overall, in nutrient levels, bacterial loads, sedimentation rates and sediment particle size distributions were found between LI and MI sites for several weeks prior to and during this study. In fact, nitrate and nitrite were not detected at any sites, while ammonia and phosphate concentration levels (mean ppm  $\pm$  SD) were slightly elevated at MI sites (ammonia= $0.075 \pm 0.150$ ; phosphate= $0.092 \pm 0.010$ ) as compared to LI sites (ammonia= $0.050 \pm 0.058$ ; phosphate= $0.088 \pm 0.025$ ), but were not significantly different (T-test  $p=0.766$  and  $p=0.766$ , respectively; data not shown). *Enterococcus* bacterial loads (MPN  $\pm$  SD) appeared elevated at MI sites ( $0.583 \pm 1.058$ ) compared to LI sites ( $0.338 \pm 0.307$ ), but were not significantly different (T-test  $p=0.671$ ; data not shown)

There were no significant differences in sedimentation rates (mean g day<sup>-1</sup>  $\pm$  SD) between MI ( $0.060 \pm 0.018$ ) and LI ( $0.078 \pm 0.032$ ) sites (T-test  $p=0.375$ ; data not shown) and there was no significant difference among the sediment particle sizes between site type (MI:  $<10 \mu\text{m} \approx 65.7\%$ ,  $10-50 \mu\text{m} \approx 26.8\%$ ,  $51-100 \mu\text{m} \approx 5.2\%$ ,  $101-250 \mu\text{m} \approx 2.1\%$ ,  $251-500$

$\mu\text{m} \approx 0.2\%$ ,  $>500 \mu\text{m} \approx 0.0\%$ ; LI:  $10 \mu\text{m} \approx 63.2\%$ ,  $10-50 \mu\text{m} \approx 28.5\%$ ,  $51-100 \mu\text{m} \approx 5.9\%$ ,  $101-250 \mu\text{m} \approx 2.2\%$ ,  $251-500 \mu\text{m} \approx 0.2\%$ ,  $>500 \mu\text{m} \approx 0.0\%$ , two-way ANOVA, site x particle size  $p=0.974$ ; data not shown).

### Discussion

The goal of this study was to determine bioindicator species that could be useful to scientists and marine park managers to rapidly assess changes in water quality known to be detrimental to coral reefs. The analysis of densities and diversity of prey and predator species was completed between LI and MI sites to determine a potential bioindicator species. The higher densities and diversity of prey fishes (in particular *S. partitus*) at MI sites during the morning suggested that indentifying potential bioindicator prey fishes in Bonaire, N.A. was feasible. Additionally, higher abundances of predatory fishes observed during morning sit-and-observe sessions at MI sites lended support to the idea that higher predatory fish densities may positively relate to higher prey fish densities. Sih (1984) found that for *Notonecta*, an aquatic insect predator, concentrating hunting efforts in areas of higher prey densities increased predator feeding success because the prey was more abundant. Thus, higher densities of predatory fish on a reef may indirectly predict the stressors affecting the site.

The history of environmental stress and/or other parameters not considered in this study may be driving the difference in prey densities, between LI and MI sites. There are several possible explanations why no differences in nutrient concentrations, bacterial loads and sedimentation were seen. The months of November-January is the rainy season in Bonaire. However, the months prior to this study were unseasonably dry (A. Hollebhone personal communication). This has most likely limited the amount of terrestrial runoff containing nutrients, bacteria and sediments entering the coastal waters. However, as time lags and feedback loops are common in natural ecosystems, it is probable that the current water chemistry was not driving the observations made in this study, but possibly it is the water chemistry from months to years past. Time lags in the addition of nitrogen fertilizers exemplify this concept. At the Cedar

Creek Natural History Area in Minnesota a five year study on nitrogen addition revealed an increase in perennial ragweed in year one followed by a decrease to control levels, an increase in bluegrass during year two, and finally, a delayed increase in blackberry during year five (Magnuson 1990). A time lag was additionally observed in meadows dominated by the sedge *Kobresia*, a species that prefers areas free of snow in the winter months. Webber et al. (1976) found that increasing the snow pack in a normally snow-free meadow at first appeared to vigorously increase the growth of the *Kobresia*. However, the plants allocated more energy to growth than reproduction and within 10 yr the large colony had completely disappeared. It is clear that immediate biological responses to environmental conditions are not always common to natural ecosystems.

In sharp contrast to this study, previous analyses of water quality in Bonaire have shown marked differences between LI and MI sites (Rini 2008; Sinnott 2009; Williams 2009). Williams (2009) found elevated levels of phosphates and finer grained sediments at MI sites. Sinnott (2009) found that at a depth of 12 m there were significantly higher concentrations of ammonia ( $\text{NH}_4^+$ ) at MI sites and significantly smaller sediment particle size (characteristic of construction sites) at MI sites at a depth of 12 m. Rini (2008) found significantly elevated concentrations of *Enterococcus* bacteria at MI versus LI sites. Based on the potential for time lags in coral reef ecosystems, it is probable that these previously documented differences in water chemistry between LI and MI sites may be at least partially responsible for the differences in fish densities and diversity observed in this study.

Additional parameters not explored could be solely or in combination responsible for the density and diversity results of this study. Differences in potential substrate type between sites may explain the significantly higher overall densities and diversity at MI sites. Risk (2003) found a positive correlation between fish species diversity and a higher degree of topographic complexity in The U.S. Virgin Islands. Preliminary work in Bonaire comparing reef habitat complexity at six different sites (including three used in this study) suggested no correlation between habitat type and fish species richness (Yanson

2009), but this work did not assess differences in the evenness of the species or the diversity of fish communities. Additional study is needed to investigate these parameters.

Another parameter that could be examined in future studies is the presence of organics in the water column. Preliminary studies on the levels of organics in the water are being performed in Bonaire as part of the Light and Motion Sensor Program (LMSP). There are permanent sensors at several reefs in Bonaire located at 5, 12 and 20 m depths on the LMSP mooring line that monitor temperature and light intensities, as well as detect organic matter in the water column (Jones et al. 2008). Organics can increase surface productivity and fuel changes in benthic environments of marine systems (Suess 1980). These organics, as a direct or indirect food source, could be driving the differences seen in fish densities and diversity, particularly prey fish species, between LI and MI sites.

The determination of a bioindicator fish species for Bonaire specific anthropogenic stressors was an integral part of this study. The only species that met the defined guidelines of a bioindicator species and appeared at densities  $>20$  fishes  $100 \text{ m}^{-3}$  was *S. partitus*. This species was documented at significantly higher densities at MI sites during the morning than LI sites. Sinnott (2009) and Williams (2009) found higher levels of nutrients (ammonia and phosphates), at MI versus LI sites. These compounds may be responsible for increases in macroalgal growth on the coral reefs of the Caribbean (Burke and Maidens 2004). Sinnott (2009) found significantly higher macroalgal cover at MI sites versus LI sites at 12 m in Bonaire, which corresponds with the higher densities of *S. partitus* at MI sites. Alga are “gardened” and fed upon by *S. partitus* (DeLoach and Humann 1999). Thus, it is possible that higher percent of macroalgal cover may be driving the greater densities of *S. partitus* observed at MI sites. This is another potential area of study for future projects on bioindicator species in Bonaire. This determination of *S. partitus* as a possible bioindicator fish may increase the effectiveness of the Bonaire National Marine Park’s efforts to protect reef communities from anthropogenic stressors. Reef sites with elevated densities ( $>20$  fishes  $100 \text{ m}^{-3}$ ) of *S. partitus* may indicate where regulations on coastal development are needed.

## Acknowledgements

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# Fishermen's by-catch: Effect on fish feeding behaviors in shallow sand flats of Bonaire, N.A.

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## Abstract

Worldwide, fisheries produce ~27 million tons of by-catch yr<sup>-1</sup>. By-catch is defined as unwanted fish that are accidentally caught, or discarded carcasses of target species. In Bonaire, N.A., by-catch is produced on a relatively small scale, by artisanal fishermen cleaning fish that they caught and discarding the remains back into the ocean. This study examines the feeding behaviors of fish in Bonaire and the effect fishermen's by-catch has on these behaviors, and also investigates the potential for fish to learn about sites of by-catch input. This study was performed in two parts, the first assessed the effects of established by-catch sites, while the second part focused on learning behaviors of fish. Bites per minute, time of arrival to the food source and fish population data was collected at sites along Kaya Playa Lechi where fishermen were present daily. The same data was collected 30 m away from the fishermen at simulated by-catch sites, as well as at control sites where no by-catch was present. In part II, the discarding of fish was simulated at sites that do not receive it on a normal basis, to obtain observations on fish reactions to a new food source. It was found that densities and biodiversity were significantly greater at by-catch treatments vs. control treatments for part I. Bites min<sup>-1</sup> was greatest when by-catch was present and the time of arrival data showed that the greatest proportion of fish arriving for the fishermen's by-catch was within the first 15 s. For part II, densities and biodiversity were greater at control treatments than at simulated by-catch treatments. Bites min<sup>-1</sup> was greater at simulation treatments, and there was a decrease in the time of arrival over the three day observation period. This data shows that relatively greater fish densities were seen at sites of food input, that fish were more aggressive when by-catch was present, and that fish can learn that to respond to sites with a consistent input of by-catch.

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## Introduction

Animal behavior is influenced by how food is acquired (Orams 2002). In today's global fisheries ~27 million tons yr<sup>-1</sup> of fish discards or by-catch is tossed back into the ocean thus providing a "free meal" to many marine organisms (Alverson et al 1994). Ultimately, the provision of these "free meals" can alter the skills and abilities of the consumers to obtain food by themselves (Orams 2002). At commercial fishing sites in the Catalan Sea, hourly consumption rates of discarded fish by benthic scavengers were compared between day and night and from shelf to slope (Bozzano and Sarda 2002). This study showed that by-catch induces a higher rate of non-selective predation, where predatory fish feed on the by-catch rather than their normal diet of live fish. This can ultimately alter trophic levels in the ecosystem because predatory fish become scavengers (Bozzano and Sarda 2002).

A case study done in French Polynesia recorded sting ray populations at areas where

tourists were allowed to feed them as well as control areas where no feeding was allowed (McCoy 2008). It was found that population densities of sting rays were greater at areas where they were being fed (McCoy 2008). Baited cameras used in a study performed in the Irish Sea, found that the densities of benthic scavengers were greater at areas of fisheries derived discarded by-catch (Ramsay et al 1997). Discards from prawn trawling boats in the Great Barrier Reef, Australia have been shown to last only 30 min on the sea floor before being eaten by various predatory fish such as sharks and snappers, as well as dolphins and seabirds (Hill and Wassenberg 2000). In reef systems, it has been suggested that fish by-catch attracts scavenging and predatory fishes (Jenkins et al. 2003). Aggression between predators and scavengers frequently occurred as individuals competed for by-catch (Jenkins et al. 2003).

Fishing is an integral part of the community, culture and history on the southern Caribbean island of Bonaire, N.A. (Hoetjes et al. 2002). Fishing and fish by-

catch, which includes the dumping of unwanted fish and their remains, is driven by ~30 artisanal fishermen (Hoetjes et al. 2002). The impacts of fishing and fisheries by-catch activities on reef fish behavior are currently unstudied on Bonaire.

For this study it was hypothesized that 1) there would be greater fish densities at areas with by-catch than areas without by-catch, 2) more predatory fish would be represented at areas of by-catch than areas without by-catch, 3) fish would be more aggressive (higher number of bites  $\text{min}^{-1}$  and a shorter arrival time) at areas with by-catch than areas without by-catch and 4) fish could learn that certain areas will have consistent food additions. The goals of this research was to compare sites with by-catch to those without and see how this food source affects fish densities, biodiversity, and behavior, as well as determine if fish can learn and respond to alternative sources of food through an experimental simulation of fishermen's activities.

## Materials & Methods

### Part 1: Effects of Established By-catch Sites

The disposal of by-catch and the remnants of fish cleaned in Bonaire, N.A. primarily occurs along the west coast of the island at several sites near Playa Lechi. For this study, three sites were identified where fishermen clean and discard fish daily. These sites are located between  $12^{\circ} 9' 36.03'' \text{ N}$ ,  $68^{\circ} 16' 54.97'' \text{ W}$  and  $12^{\circ} 9' 26.32'' \text{ N}$ ,  $68^{\circ} 16' 47.82'' \text{ W}$  at intervals of ~180 m (Fig. 1). Preliminary research determined that fishermen used the southernmost site between 1000 h and 1200 h, the middle site was occupied between 1300 h and 1500 h, and the northernmost site was used between 1700 h and 1900 h.

At each of these sites three treatments were created, and centered around the location where fishermen were observed discarding by-catch. Data was collected while snorkeling directly outside of the plots created. Treatments were defined as: 1) fishermen discarding by-catch, 2) simulated by-catch discard (a research assistant stood on shore and threw out fish remains of similar kind and quantity to those disposed of by fishermen in an area where such activity is not usual) and 3) a no by-catch control. The position (north or



**Fig. 1** Map of Kralendijk, Bonaire, N.A. showing the three study sites where fishermen frequently discard fish remains on the leeward side of the island. Site 1 is the most northern site

south of fisherman) and order of treatments was randomly determined prior to each sampling period in order to eliminate bias as to the order or type of treatment observation. Treatments were located within a demarcated area of  $10 \times 15 \text{ m}$  varying in depth from site to site; where the width ran along shore and the length ran perpendicular to shore. A 15 min observation period was used for all treatments during which identification and abundance of fish present, time of arrival into study area, and bites  $\text{min}^{-1}$  were recorded. Fish densities were found by dividing the total number of fish present at each treatment by the total volume of the plot. Biodiversity was determined using the Shannon Index. Fish behavior was documented using a Sony Handycam video camera. Bites  $\text{min}^{-1}$  data was determined by analyzing the videos and time of arrival was defined as the amount of time during which a fish reached the study area, starting when the by-catch entered the water.

### Part II. Learning Behavior Experiment

In order to assess the potential for fish to learn about sites where fishermen consistently provide by-catch, experimental simulations were initiated at and to the north of  $12^{\circ} 9' 36.03'' \text{ N}$ ,  $68^{\circ} 16' 54.97'' \text{ W}$ . Simulations (as previously described) were  $10 \times 15 \text{ m}$  plots which were paired with a control site the same size ~30 m away ( $n=2$ ). Observations of fish feeding behavior were documented and recorded as described above. The placement of study areas for each of the simulated and control treatments was randomly assigned prior to sampling and was maintained throughout the experiment. The two study areas were visited once per day for three days, between 1500 h and 1700 h. There was an interval of two d between sampling periods.

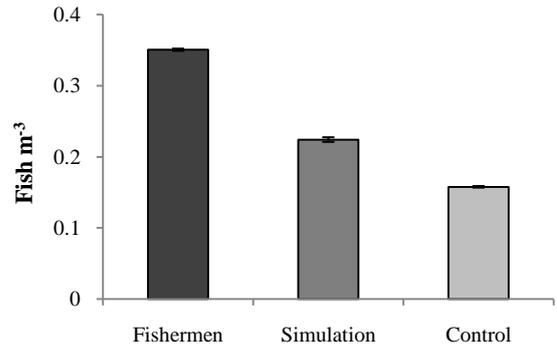
**Data Collection and Analysis**

For Part 1 of this study, data was analyzed using a one-way ANOVA. Times of arrival of fish for both studies were allocated to five time intervals (0-15 s, 16-60 s, 61-180 s, 181-419 s, and 420-900 s). The proportion of all fish in a particular treatment found entering the area within each determined time interval was calculated. Proportions were used to analyze this data as the number of fish entering each treatment varied. Proportions were arcsine-transformed and compared among treatments using a two-way ANOVA. For the learning behavior portion of the study, data were analyzed using a paired T-test.

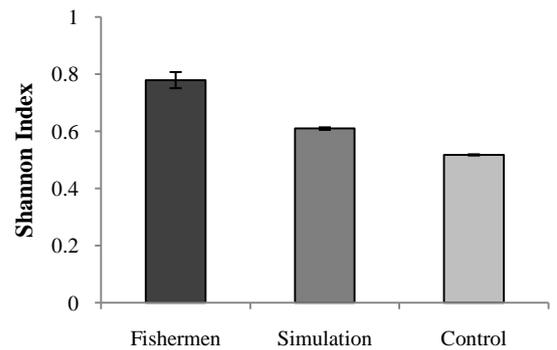
**Results**

**Part 1: Effects of Established By-catch Sites**

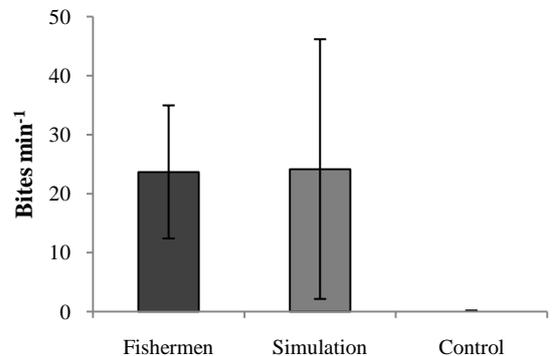
Overall, mean fish densities (fish m<sup>-3</sup> ± SD) were significantly higher for the fishermen’s by-catch treatments (0.351 ± 0.001), intermediate for simulation treatments (0.224 ± 0.003) and lowest for the controls (0.158 ± 0.001) (one-way ANOVA p=0.005, Fig. 2). Fish with the greatest densities (0.751 m<sup>-3</sup>) at fishermen’s by-catch treatments were French grunts (*Haemulon flavolineatum*). At simulated by-catch treatments Slipperydicks (*Halichoeres bivittatus*) had the highest densities (0.510 m<sup>-3</sup>). Mean biodiversity (± SD) was also significantly higher for fishermen’s by-catch treatments (0.779 ± 0.028) intermediate at simulation treatments (0.610 ± 0.004), and lowest for controls (0.518 ± 0.002) (one-way ANOVA, p=0.005, Fig. 3). There were significantly higher mean bites min<sup>-1</sup> (± SD) at fishermen and simulated treatments than for the controls (one-way ANOVA, p<0.001, Fig. 4). Fish with the greatest bites min<sup>-1</sup> (31.167 bites min<sup>-1</sup>) at the fishermen’s by-catch treatments were ocean surgeonfish (*Acanthurus bahianus*) which are know herbivores (Lukoschek and McCormick 2000). For the first time of arrival data there was a significant difference among time intervals (p<0.001). The time interval with the greatest proportion of fish at the fishermen’s treatment was 0-15 seconds, which had a proportion of 0.360 fish arriving to feed on the by-catch (Fig. 5).



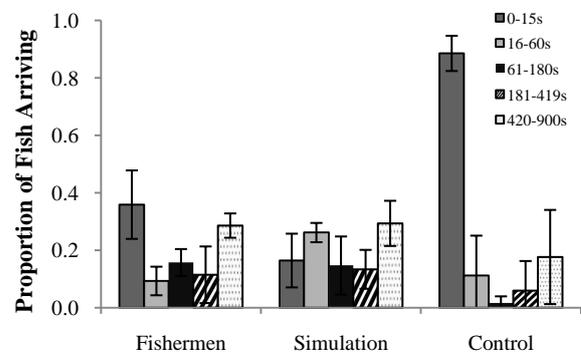
**Fig. 2** Mean fish densities (fish m<sup>-3</sup> ± SD) at the three treatments plots (Fishermen, Simulation, and Control)



**Fig. 3** Mean biodiversity (Shannon Index ± SD) of at the three treatments plots (Fishermen, Simulation, and Control)



**Fig. 4** Mean bites min<sup>-1</sup> (± SD) by fish at the three treatments (Fishermen, Simulation, and Control)



**Fig. 5** Mean proportion of fish (± SD) entering the Fishermen, Simulation and Control treatments in five time intervals (0-15 s, 16-60 s, 61-180 s, 181-419 s, and 420-900 s)

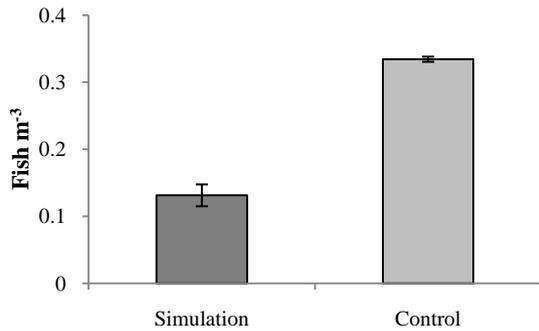


Fig. 6 Mean densities (fish m<sup>-3</sup> ± SD) in the simulation and control treatments of the learning behavior portion of the study

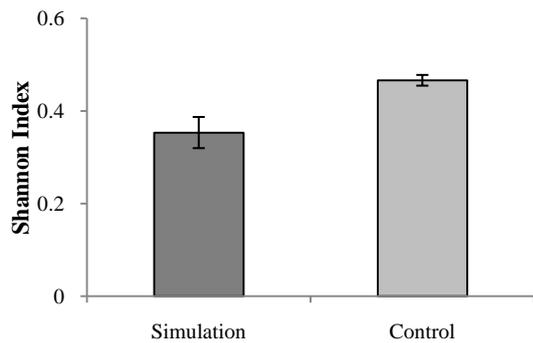


Fig. 7 Mean biodiversity (Shannon Index ± SD) of fish in the simulation and control treatments of the learning behavior portion of the study

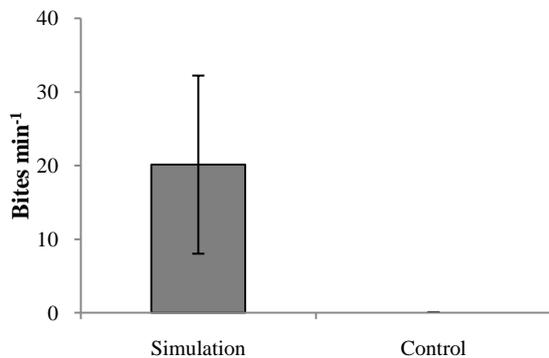


Fig. 8 Mean bites min<sup>-1</sup> (± SD) by fish at the simulation and control treatments of the learning behavior portion of the study

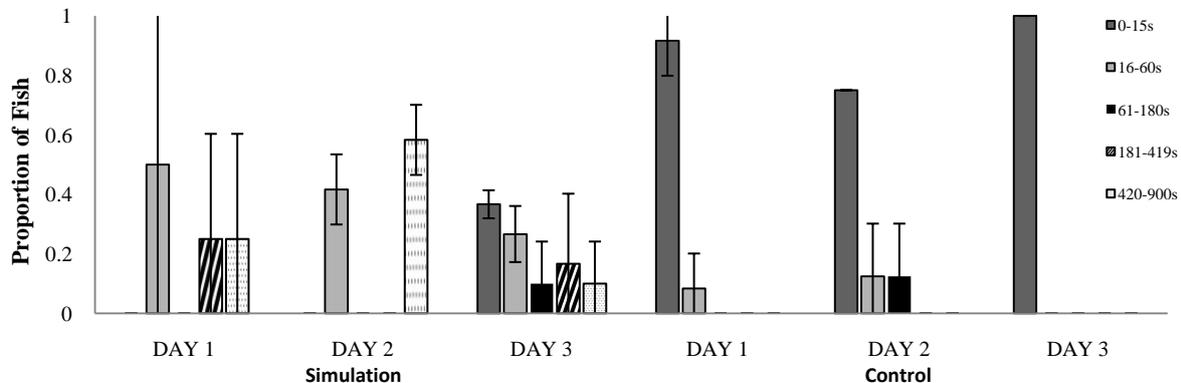


Fig. 9 Mean proportion of fish (± SD) entering the simulation and control treatments during the learning behavior portion of the study in five time intervals (0-15 s, 16-60 s, 61-180 s, 181-419 s, and 420-900 s)

## Part II. Learning Behavior Experiment

Data was combined from the three days this portion of the experiment took place, as well as with the two pairings of treatments. Mean (fish m<sup>-3</sup> ± SD) fish density at the simulation treatments (0.131 ± 0.016) was lower than at the control sites (0.334 ± 0.004, Fig. 6). Mean biodiversity (± SD) was also lower at simulation sites (0.353 ± 0.034) than at the paired control sites (0.466 ± 0.012, Fig. 7). Mean bites min<sup>-1</sup> (± SD) by fish at the simulation treatment was 20.12 ± 12.106 as compared to the control sites where no bites min<sup>-1</sup> were recorded (Fig. 8). Over the three study days, the time of arrival by fish in the simulation treatments clearly changes (Fig. 9). During the first two days, no fish arrived in the first time interval (0-15s), but the third day the proportion of fish arriving in this time interval was 0.37. Whereas, times of arrival in the control treatments stay relatively consistent across the three days as most fish were already present in the plot before the treatment began.

## Discussion

The results from the first part of this study supported the hypothesis that fish densities would be greater at areas of by-catch than at areas without. *H. flavolineatum* was the most prevalent fish species at the fishermen's treatment, which supports the hypothesis that predators had the highest densities in areas which resulted in an increase in fish population (Ramsay et al 1997). Following the pattern observed in densities, biodiversity was highest for fish, at the fishermen's treatments, followed by the simulation treatments, and was with by-catch. Scavenger densities recorded at

sites in the Irish Sea, found that fisheries discards provided a significant source of food lowest for controls. Similar results were found in a study of prawn trawler by-catch, where the scavengers ranged from dolphins, sharks, and birds to benthic scavengers such as echinoderms (Hill and Wassenberg 2000). These results suggest that biodiversity increases when by-catch from the trawling boats is present. A potentially negative outcome of fish consuming by-catch could be a shift in ecological roles. Herbivorous fish, such as surgeonfish (*Acanthuridae*) and parrotfish (*Scaridae*), were feeding on the by-catch. This finding is confirmed by a study on ocean surgeonfish (*A. bahianus*) where it was found that these fish are “opportunistic feeders” (Lukoschek and McCormick 2000). Shifts in ecological roles may also occur when predatory fish turn into scavengers, such as French grunts (*H. flavolineatum*) which had the greatest densities at fishermen’s treatments where they are effectively becoming scavengers and feeding opportunistically on the by-catch. The effects of these shifts in ecological roles are currently unknown and are a potential subject of further studies on fisheries by-catch.

Mean bites  $\text{min}^{-1}$  was greatest at sites with by-catch present, and almost non-existent at areas without the food source, thus supporting the hypothesis that fish will be more aggressive when discards are present. The time of arrival at the fishermen’s treatment was greatest in the first interval (0-15 s) with 36% of the fish arriving within the first 15 s. Comparing this percentage to the first interval in the simulation treatment, which shows that only 16% of the fish arrived for the simulated by-catch within the first 15 s, may suggest that fish have learned about the fishermen’s regular discarding of by-catch. A study done in the Catalan Sea found that snake eels had the shortest time of arrival suggesting that this could reflect the importance of the by-catch in the eel’s diet (Bozzano and Sarda 2002). This finding could be applied to the results of this study; fish with the shortest arrival time could be the most dependent on the by-catch as a food source.

During the learning behavior part of this study, the densities and biodiversity of fishes visiting the experimental plots for all days and treatments was greater for controls than simulation treatments, thus refuting the

original hypothesis. This may be explained by the limited number of replicates as well as the location of the control sites. The control sites included natural and anthropogenic structure which may provide greater habitat availability than the simulation sites. This was confirmed by a study done in the Irish Sea that found substratum type and other environmental factors can influence fish population sizes (Ramsay et al 1997). For overall time of arrival data, the third day was the only day of the simulation treatments where fish arrived in the first time interval (0-15 s). This may suggest that fish learned about the food input within only one week of these simulated treatments. Throughout the three day study, a spotted moray eel (*Gymnothorax moringa*) was present at the same simulation-control pairing for every release of by-catch (whether it was the same individual is unknown). This species showed an overall decrease in time of arrival from 780 s on day 1, to 300 s on day 3. Bites  $\text{min}^{-1}$  was greater at sites of simulated by-catch than control sites, which may be a result of a food source being present in the simulated area but not in the control areas. In conclusion, this study showed that fish were feeding on by-catch, fish were more aggressive when by-catch was present, and that fish can learn to respond to food input over time. Future studies based on these results may examine the effects of by-catch input on the ecological roles of fish that consume these discards.

## Acknowledgements

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# Feeding ecology and twilight interspecific interactions of lionfish (*Pterois spp.*) in Bonaire, N.A.

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## Abstract

Bioinvasions are defined as the establishment of a species in an area where it has not existed previously. Usually the result of an anthropogenic introduction, bioinvasions pose a great threat to coral reef ecosystems. One example of an anthropogenically-induced bioinvasion is that of the lionfish (*Pterois spp.*) to the Atlantic basin. First reported in Bonaire, N.A. in 2009, the Indo-Pacific lionfish has spread rapidly, with 177 fish reported as of 31 March, 2010. One of the purposes of this study was to document interspecific interactions of lionfish with prey and non-prey fish species at twilight, when lionfish are reported to be active. Interactions were video recorded for further analysis. Additionally, stomach contents of lionfish on Bonaire were analyzed and compared to lionfish from a similar study in the Bahamas, which determined that as lionfish size increases, so does the % volume of fish in their diet. Lionfish, collected by the Bonaire National Marine Park and volunteers, were categorized according to total length for use in this study. Prey items found in the stomach contents were identified to the lowest possible taxon. It was hypothesized that as the size classes of lionfish increased, an increase in the % volume of fish and a decrease in the % volume of shrimp in their diet would be observed. Lionfish were observed interacting more with potential prey items than non-prey items based on video analysis. Data analysis of stomach content found that as lionfish size increased, the amount of fish by % volume increased from 60% volume in the smallest size class to 93% volume in the largest size class. This study showed that as lionfish size increases, they rely more heavily on fish as a part of their overall diet, and the fish they are consuming are those they are observed interacting with most.

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## Introduction

Whitfield et al. (2002) define biological invasions as “the arrival, survival, successful reproduction, and dispersal of a species in an ecosystem where the species did not exist previously.” Invasive species have been shown to contribute to several possibly deleterious effects in invaded areas, such as extinctions of native organisms (Mooney and Cleland 2001), loss of native biodiversity as a result of these extinctions (McNeely 2001; Mooney and Cleland 2001), and reduced resources available for native species (Chornesky and Randall 2003, Morris 2009). Certain invasions are thought to be a result of natural processes, however, a large number of marine invasions are the result of anthropogenic introductions, both purposeful and accidental (Whitfield et al. 2002; Morris et al. 2008).

One of the best examples of a rapid marine bioinvasion by means of anthropogenic introduction is that of the lionfish (*Pterois*

*spp.*) to the Atlantic. Their introduction to the Atlantic is largely thought to be the result of purposeful and accidental releases from aquaria along the southeast coast of Florida (Albins and Hixon 2008; Morris et al. 2008). First documented in 1992 off the coast of Florida (Morris et al. 2008), lionfish spread along the east coast of the United States and throughout the northern Caribbean within a decade. At the present time, they have extended their range throughout the southern Caribbean and are expected to spread to the Gulf of Mexico and as far south as the temperate regions of the east coast of South America (Whitfield et al. 2002; Morris 2009; Morris and Whitfield 2009).

Lionfish were first reported on the reefs of Bonaire in October 2009; as of 31 March 2010, 177 fish had been collected, with a reported 50 more sighted by divers on the reefs (unpublished data). Due to the danger they pose to divers and snorkelers, and their potential threat to native reef fish communities, the introduction of the lionfish is of particular concern. Albins and Hixon (2008) showed that lionfish have a direct

negative impact on native reef fish populations in the Atlantic. A reported 79% reduction in fish recruitment was found on reefs in the Bahamas during a five-week observation period, due mainly to predation on post-settlement reef fish (Albins and Hixon 2008).

Lionfish are highly effective predators on small post-settlement fish, particularly Atlantic species which are naïve to their unique predation techniques (Albins and Hixon 2008). The small bodied teleosts, integral to the diets of larger lionfish, are also an essential dietary component to native reef fish, such as snappers (*Lutjanidae*) and grunts (*Haemulidae*). The establishment of lionfish throughout the Atlantic could result in decreases of available resources for native reef fish. Therefore, this study addresses the following three hypotheses:

H<sub>1</sub>: There is a positive relationship between lionfish behavioral interactions and prey versus non-prey fish species.

H<sub>2</sub>: As lionfish size increases, the % volume of teleosts in stomachs will increase and the % volume of shrimp will decrease.

H<sub>3</sub>: The top three families of teleosts found in lionfish stomachs in the Bahamas (*Gobiidae*, *Labridae*, *Grammatidae*) will be consistent with stomach contents of lionfish in Bonaire.

Although other studies have analyzed the feeding ecology of lionfish in the Atlantic, this study provides the first data on lionfish behavior and diet on Bonaire, contributing to what is currently known about the lionfish that are extending their range throughout the western Atlantic.

## Materials & Methods

### Study Site

Bonaire, a small desert island approximately 50 miles north of Venezuela, and Klein Bonaire, an adjacent uninhabited island, are surrounded by fringing reefs protected by the Bonaire National Marine Park (BNMP). Scuba diving is a major industry in Bonaire. Dive sites are easily accessible from shore along the leeward, west coast of the island. The windward coast of the island is not visited as regularly due to consistently strong winds and wave action. Since the arrival of lionfish in Bonaire, dive shops have been sharing information regarding lionfish

and how to mark areas where they are sighted. Divers also report sighted lionfish to BNMP, which keeps an updated spreadsheet of lionfish locations around the island. The lionfish used in this study were collected by volunteers and employees of the BNMP based on reported sightings by the dive community.

### Behavioral Observations

Sites visited were chosen opportunistically based on reports of marked lionfish at depths shallower than 20 m. To test H<sub>1</sub>, videos of lionfish behavior were recorded using SCUBA between 1730 and 1930 h. Videos were analyzed to enumerate prey and non-prey interactions at twilight, when lionfish are reported to be active (Morris et al. 2008), and the number of interactions min<sup>-1</sup> were compared. An interaction with prey was defined as any time the lionfish flared its fins and advanced towards a potential prey item. A non-prey interaction was defined as any time a lionfish flared its fins in a display towards another fish, which similarly showed agitation and aggression, or when another fish displayed aggression towards a lionfish that was not flaring its fins.

### Stomach Content Analysis

To test H<sub>2</sub> and H<sub>3</sub>, stomachs were removed from specimens and contents were identified to the lowest possible taxon. From the 177 specimens in the lab, ten lionfish were randomly selected from four size classes (6.1-10.0, 10.1-14.0, 14.1-18.0, 18.1-20.0 cm) for dissection. Size classes were the same as those used by Morris (2009), for comparison. Stomach contents were counted and photographed; volumes of prey were determined by displacement.

The contribution of each prey taxon to the overall diet was evaluated using the following three relative metrics of prey quantity: percent frequency of occurrence (%F), percent composition by number (%N), and percent composition by volume (%V) (Akins and Morris 2009). In accordance with Morris (2009), an assessment of prey importance was determined using the Indice of Relative Importance (IRI):

$$IRI_a = F_a * (N_a + V_a)$$

This was calculated for the top three families of teleosts found in stomach content analyses.

The contribution of teleosts and shrimp to the overall diet was evaluated using %F, %V, and %N, and assessed using IRI.

### Data analysis

Prey and non-prey interactions  $\text{min}^{-1}$  were compared using a T-test. %V of teleosts and shrimp from the four size classes were determined and compared to the %V of teleosts in the stomachs of lionfish from the Bahamas (Morris 2009) by plotting a line graph. The relative importance of the different fish families consumed by lionfish was determined using the IRI. The relative importance of fish and shrimp found in the stomach contents of lionfish was also assessed using the IRI.

## Results

### Behavioral Observations

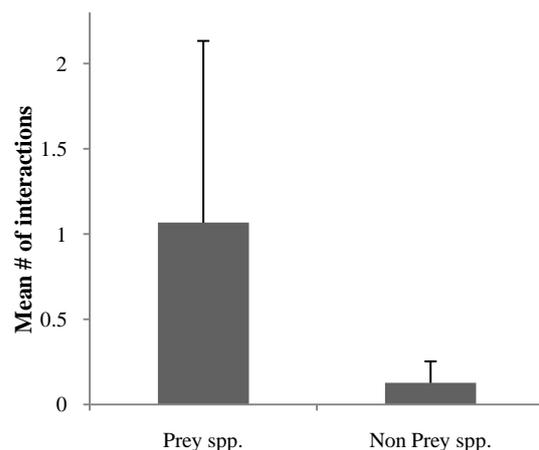
One-hundred seventy-seven lionfish have been collected on Bonaire, 172 from the leeward coast and five from the windward (Fig. 1). The number of lionfish collected on the windward side most likely reflects the low number of divers and dive sites there, rather than the actual amount of lionfish present. The majority of lionfish were collected along Bonaire's southern leeward coast, with the largest abundances occurring at the dives sites Invisibles and Red Slave. Patterns of lionfish collections at dive sites along the leeward coast may reflect differences in the availability of preferred habitat types. Since sites are consistently dove along the leeward coast, with the exception of those located within the boundary of the Washington Slagbaai National Park (WSNP), differences in abundances between sites cannot be explained by a lack of divers. one lionfish was filmed at a depth of 19 m for behavioral interactions that met the criteria 15 min. The second lionfish was filmed at 87% of the lionfish interactions were with potential prey items, primarily wrasses (*Labridae*).

Live lionfish were found and recorded at two of ten sites visited. At Cliff, 1000 Steps for 4 min at 28 m. Thirty-three described above were documented in videos; grunts (*Hamulidae*), and damselfish/chromis (*Pomacentridae*). Teleosts from the families *Pomacentridae* and *Hamulidae* were identified through lionfish stomach content analyses. More interactions were observed with prey

species ( $1.07 \text{ min}^{-1}$ ) than non-prey species ( $0.130 \text{ min}^{-1}$ ,  $p=0.190$ , Fig. 2).

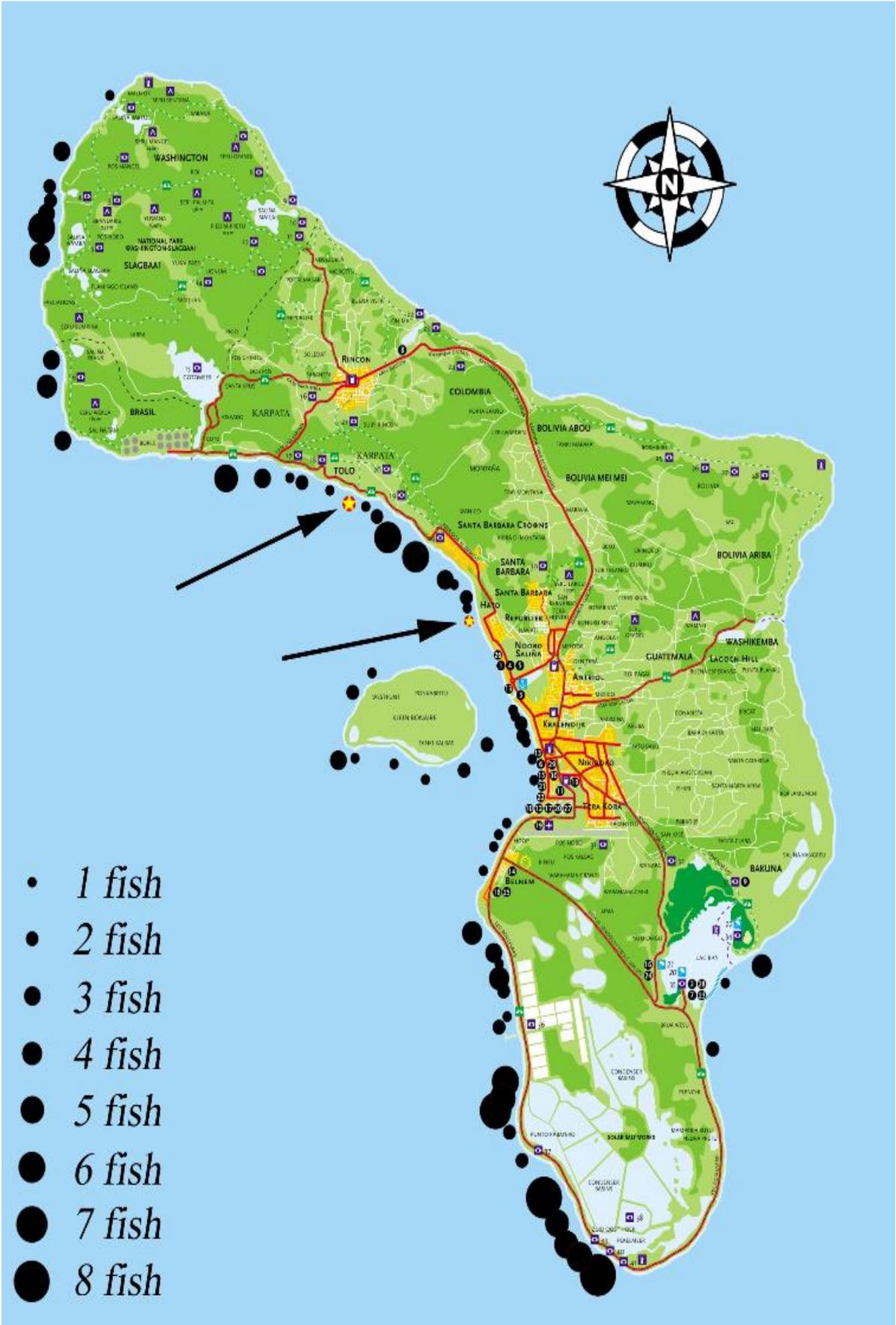
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Although damselfish are potential prey for lionfish, territorial displays by damselfish were observed. Damselfish became agitated when lionfish moved toward their territory, but stayed far enough away from the lionfish to avoid predation. In addition, damselfish displayed aggressive behavior towards the lionfish, which included darting rapidly towards them and moving back and forth, blocking their path.



**Fig. 2** The mean number ( $\pm$  SD) of lionfish interactions  $\text{min}^{-1}$  with prey and non prey fish species. Data compiled from video analysis ( $n=2$ , T-test  $p=0.19$ )

Lionfish were observed displaying territorial behavior towards creolefishes (*Serranidae*) and grunts (*Haemulidae*). Territorial interactions with other non-prey fish consisted of the lionfish flaring its fleshy fins while the non-prey fish directly faced the lionfish at a close distance. During all territorial interactions, non-prey fish swam away from the lionfish. Other non-territorial interactions were observed between lionfish and butterflyfish (*Chaetodontidae*), as well as lionfish and blue tangs (*Acanthuridae*). Additional non-prey interactions were 11 as recorded where non-prey fish, situated around



**Fig. 1** Map of locations where lionfish have been collected in Bonaire, N.A. Black circles indicate collection sites and gold stars represent sites where videos were recorded. Relative size of circles indicates number of lionfish collected

or above the lionfish, swam back and forth towards the stationary lionfish for extended periods of time.

### Stomach Content Analysis

Of the 40 lionfish dissected, nine fish (22%) had empty stomachs. For the purposes of this study, lionfish that were found with empty stomachs were not included in the analysis in order to provide a more accurate representation of prey items consumed by lionfish. Teleosts consisted of 78%V, 38%N, and 50%F of the lionfish diet. Of the 31 fish dissected that contained prey items, only 9.7% of the teleosts were identifiable to family. Of the identifiable families, there was one representation each of *Pomacentridae*, *Apogonidae* and *Holocentridae*, and two of *Gobiidae*. Crustacea, which was composed of only shrimp species, were 24%V, 62%N, and 65%F.

Overall, the mean %V, of shrimp in lionfish diet decreased with size classes from 39%V in the first size class to 6%V in the fourth. Fish comprised 60%V of the lionfish diet for the first size class and 93%V of the fourth (Fig. 3). The %V of fish in the second size class was less than the %V of shrimp, which differed from the other three size classes.

As size class increased, the %N of shrimp in lionfish stomachs decreased, and the %N of fish increased (Fig. 4). The first size class contained 67%N of shrimp, which decreased in the fourth to 33%N. The contribution of teleosts to lionfish diet increased from 32% for the first size class, to 67%N for the fourth (Fig. 4). Change in %N occurred between the third and fourth size classes.

The %F of fish and shrimp was shown to decrease as size class increased (Fig. 5). %F of fish and shrimp overall in lionfish diet increased from 60%F and 80%F, respectively, in the first size class, to 40%F of each in the fourth. For the third and fourth size classes, fish and shrimp comprised the same %F. The widest spread occurred in the second size class, with 40%F fish and 80%F shrimp. The maximum number of crustaceans and fish found per lionfish were ten and six, respectively.

Comparisons between this study and Morris's (2009) in the Bahamas showed an overall positive trend in mean %V in relation

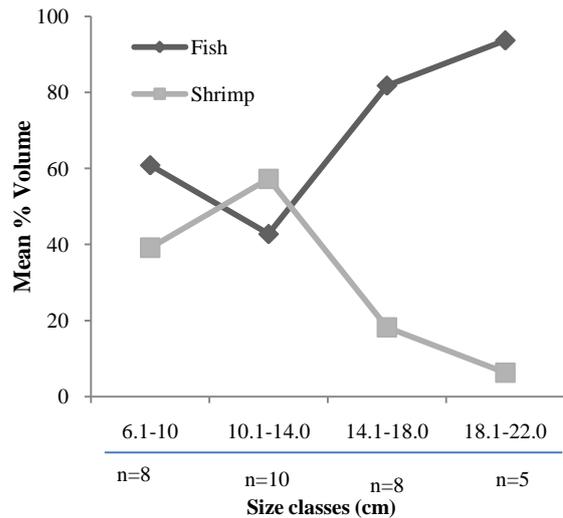


Fig. 3 Mean %V of teleosts and shrimp, for four size classes of lionfish collected in Bonaire

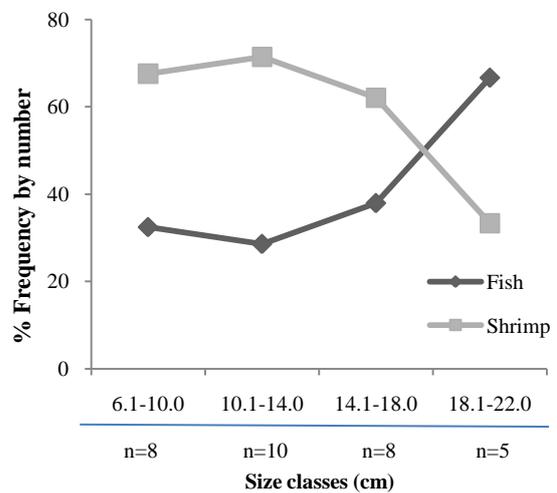


Fig. 4 Mean %N of teleosts and shrimp, for four size classes of lionfish

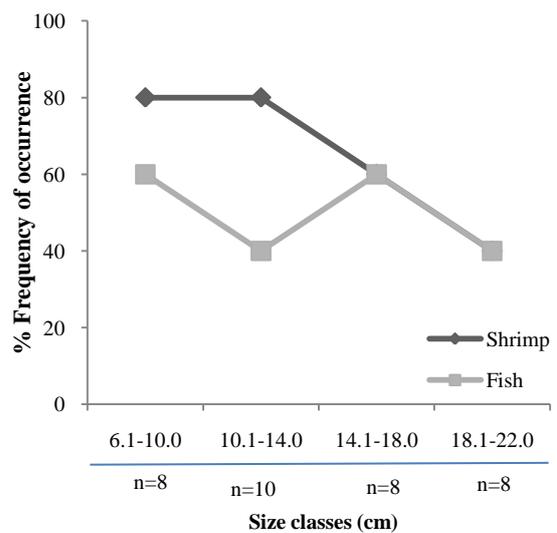
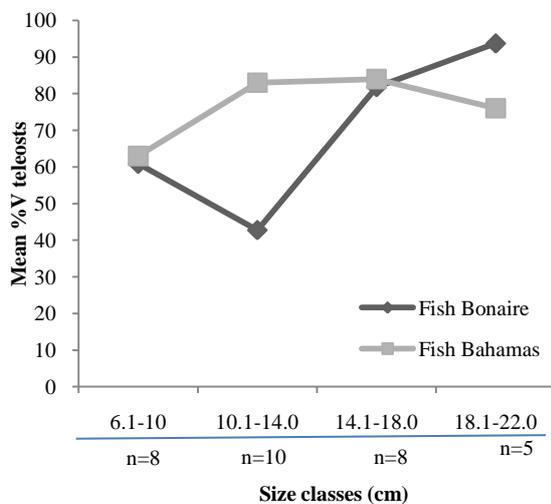


Fig. 5 Mean %F of teleosts and shrimp, for four lionfish size classes

to lionfish size. The first and second size classes displayed almost the same %V of teleosts for both sites. The greatest variance occurred in the second size class, with 42%V in Bonaire and 83%V in the Bahamas.

The IRI ranks the importance of fish and shrimp to the overall diet of lionfish; fish were ranked as a more important contribution. The same family of teleosts (*Gobiidae*) ranked first using the IRI for the Bahamas and Bonaire. The IRI ranked *Apogonidae* and *Holocentridae* second and third for Bonaire and *Labriidae* and *Grammatidae* second and third for the Bahamas (Table 1).



**Fig. 6** The %V of teleosts for 4 size classes in Bonaire compared to the %V of teleosts in the Bahamas

**Table 1** Rankings of importance for teleost families based on IRI results for the Bahamas compared to rankings from Bonaire

Rank	Bonaire	Bahamas
1	Gobiidae	Gobiidae
2	Apogonidae	Labriidae
3	Holocentridae	Grammatidae
4	Pomacentridae	Apogonidae

## Discussion

Based on the results of the behavioral analysis,  $H_1$  was supported; lionfish were shown to interact more with prey items than non-prey items. Although no actual consumption of reef fish was videotaped, several attempts occurred. Lionfish, found to be very opportunistic eaters, have the ability to expand their stomachs to 30-times its normal size, consume prey that are up to 2/3 their own length, and are capable of going long periods without food (Albins and Hixon 2008; Morris

2009). In a study conducted in the Bahamas by Albins and Hixon (2008), a large adult lionfish was observed eating over 20 small wrasses (*Halichoeres bivittatus*) in a period of 30 min. Based on feeding experiments of the lionfish in their home range, one adult lionfish is able to consume approximately 625 small bodied fish per year (Meister et al. 2005).

In Bonaire, lionfish primarily feed on teleosts and crustaceans. The proportion of teleosts found in lionfish stomachs varied by size class, with smaller lionfish feeding more heavily on shrimp, and larger lionfish primarily feeding on teleosts. This is similar to the results found in studies conducted in the Bahamas (Akins and Morris 2009, Fig. 6), which showed a positive trend in relation to mean %V of fish found in lionfish diet as size classes increased. Data from this study supports the hypothesis that as lionfish size increases, a higher %V of teleosts and lower %V of shrimp will be found in their diet. For the second size class, the %V of fish decreased, but was relatively similar to the %V of shrimp. This could be due to the fact that lionfish of that size class are consuming relatively equal amounts of both. Mean %V findings in this study were the same (78%) as ones found in the Bahamas by Akins and Morris (2009).

In the Caribbean, large predators have been overfished (Hoetjes et al. 2002), diminishing the probability of lionfish population growth being curbed by predation. However, due to the popularity of Bonaire as a dive destination, the Bonaire National Marine Park (BNMP) has implemented a community-based program for the early detection and removal of lionfish. The success of the program is uncertain since lionfish are known to live to depths up to 84 m (Meister et al. 2005), which is beyond the depth of recreational divers. BNMP employees and volunteer divers are marking and capturing lionfish on the reef, with 177 removed since October 2009. The ecological impact of lionfish predation is uncertain, but ~ 75% of the lionfish sighted around Bonaire thus far, have been removed.

Due to the limited number of size classes available in Bonaire and the relatively small sample size of video data, future studies will be necessary to determine how lionfish diet in Bonaire relates to their diet in the Bahamas as lionfish in Bonaire reach greater size.

Although the number of identifiable fish families was small, when compared to the Bahamas, two of the five top families were similar. The IRI ranked Gobiids as the most important teleost prey in both the Bahamas study and Bonaire. Although the second and third families differed between the two studies, all families of identifiable prey from Bonaire were included in the top 10 rankings for teleost families in Bahamas, with the exception of *Holocentridae*. The IRI ranked fish as relatively more important to lionfish diet than shrimp, although there was only a small difference between the two. This could be due to the fact that the IRI, when compared to other indices of importance, places higher value on %F, and equal value on %V or %N (Akins and Morris 2009).

Lionfish have been shown to have a direct negative effect on native reef fish populations. Understanding their potential impacts in Bonaire is important due to the fact that lionfish can have potentially deleterious impacts on native reef fish and community structure and may alter reef systems irrevocably throughout the Caribbean.

## Acknowledgements

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University of  
Minnesota- Twin  
Cities  
*Environmental Policy  
and Management  
Marshfield, WI*



**KELSEY  
BURLINGAME**  
Evergreen State  
College  
*Environmental Studies  
Anchorage, AK*



# CIEE FACULTY

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**RITA PEACHEY, PH.D.**  
**Resident Director**

Dr. Rita Peachey is the co-instructor for Independent Research and the Resident Director of CIEE Research Station Bonaire. She earned her B.S. in Biology and M.S. in Zoology from the University of South Florida and her Ph.D. in Marine Science from the University of South Alabama. Her research interests include coral biology and how UV affects the early stages of life in the ocean. In addition, she has studied how pollution can enhance the detrimental effects of sunlight on larval crabs, corals and oysters.

*Primary advisees: Zachary A. Lipshultz and Kelsey Burlingame*



**AMANDA HOLLEBONE, PH.D.**  
**Tropical Marine Conservation  
Biology faculty**

Dr. Amanda Hollebhone is the co-instructor of Independent Research and Marine Conservation Biology faculty at CIEE Bonaire. She received her B.S. in Biology from the UNC Chapel Hill, a Ph.D. in Marine Ecology from Georgia Tech and taught in the Biology Department at Georgia Southern University. Amanda's research interests include reef community ecology and invasive species. Her dissertation research focused on the population dynamics and pre- and post-settlement ecology of a non-native porcelain crab in the oyster reefs of Georgia, USA.

*Primary advisees: Colleen Chabot and Alicia Reigel*



**CAREN ECKRICH**  
**Assistant Resident Director**

Caren Eckrich is the Assistant Resident Director and the Dive Safety Officer for CIEE. She holds a B.S. in Wildlife and Fisheries from Texas A&M University and a M.S. in Biological Oceanography at the University of Puerto Rico in Mayaguez. Caren manages dive planning for the student independent research projects and has a wealth of local experience on the reefs that contributes to the success of student projects. Caren's research interests include fish behavior, seagrass and algal ecology, sea turtle ecology, and coral disease.



**ANOUSCHKA VAN DE VEN**  
**Technical Coordinator**

Anouschka van de Ven is the Technical Coordinator for CIEE. She is a PADI Dive Instructor and underwater photographer/videographer. She has a B.A., First Class Honours Degree in Communications Studies, from the London Metropolitan University and worked in television and advertising in Amsterdam before moving to Bonaire. Anouschka provides administrative support for student projects and participates in the CIEE long-term research program in Bonaire.

# CIEE INTERNS

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Andrew Collins assisted Dr. Rita Peachey's advisees with their independent research projects. He holds a BSc. in Environmental Conservation from the University of New Hampshire.



**ANDREW COLLINS**  
Coral Reef Ecology Intern

Lauren Saulino assisted Dr. Amanda Hollebhone's advisees with their Independent Research projects. She holds a M.A. in Environmental Sciences from Miami University and a B.A. in Biological Sciences and Spanish from Clemson University.



**LAUREN SAULINO**  
Conservation Biology Intern

Lisa Faber is the research intern at CIEE. She is currently working towards a bachelor degree in Coastal Zone Management at van Hall Larenstein University of Applied Sciences in the Netherlands, where she has already obtained a degree in Biological Laboratory Techniques.



**LISA FABER**  
Research Intern





*"People look at the surface, and they think that's the ocean, and because they can't see what's going on below, they think everything's just fine. But those of us with decades of exploration know that the ocean is in trouble, and therefore so are we."*

*Sylvia Earle*

