

ECOLOGY AND EVOLUTION OF THE LIONFISH INVASION OF CARIBBEAN
CORAL REEFS: RESISTANCE, ADAPTATION AND IMPACTS

Andrea Anton Gamazo

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Approved by:

Charles Peterson

Karl Castillo

Joel Kingsolver

Johanna Rosman

Paula Whitfield

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ABSTRACT

ANDREA ANTON GAMAZO: Ecology and evolution of the lionfish invasion of Caribbean coral reefs: resistance, adaptation and impacts
(Under the direction of Charles Peterson)

The lionfish (*Pterois volitans*) invasion of the Caribbean is a notable example of the successful establishment of a predatory marine fish outside of its native range. In 20 years lionfish have spread over most of Caribbean and the western Atlantic. Lionfish densities in their new range can be up to fifteen times higher than in their native range. On reefs in the Caribbean, lionfish reduce fish populations to the point that this invasive species is considered one of the top ten most serious emerging environmental issues in the world. Native prey can be vulnerable to consumption by exotic predators with which they lack an evolutionary history. Such prey naiveté has been assumed to be a major cause of extinction for endemic species. Yet prey naiveté has been tested rigorously in few cases and never in the marine environment. In Chapter 1 and 3 of my dissertation I used metrics of predator avoidance by small, native Caribbean and Pacific fishes to quantify their responses to lionfish. Field experiments and observations revealed that Caribbean native prey do not recognize of lionfish as a predator, indicating prey naiveté towards this exotic threat. In Chapter 2 and 4, I tested biotic and environmental resistance to the early success of the lionfish invasion in two Bahamian islands and the Belizean Mesoamerican Barrier Reef. Lionfish abundance was negatively related to large grouper biomass in Belize but not in the Bahamas. Wave exposure and marine protection from

reef fishing were also negatively related to lionfish abundance and field observations suggested that high-energy of exposed environments might be the dominant determinant of the lionfish density pattern. The direct and indirect effects of lionfish on marine ecosystems in the Caribbean are of great concern for conservation. In Chapter 4 I assessed lionfish impacts on abundance and community structure of reef-fish at large spatial scales. Surveys at 15 sites located along the Belizean Mesoamerican Barrier Reef were performed before and after the lionfish invasion. A negative effect of lionfish abundance on the reef-fish abundance and community composition was detected only 2 years after first lionfish detection on Belizean coral reefs.

DEDICATION

Para Nate y Mochuelo

Todo pasa y todo queda.

Caminante no hay camino

sino estelas en la mar...

Caminante no hay camino

se hace camino al andar

- Antonio Machado

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CHAPTER 1

Prey naiveté towards an invasive marine predator as compared to native predators in reef-fish communities

ABSTRACT

Native prey can be vulnerable to consumption by exotic predators with which they lack an evolutionary history. Such prey naiveté has been assumed to be a major cause of extinction for endemic species, particularly on islands and in lakes. Yet prey naiveté has been tested rigorously in few cases and never in the marine environment. We used metrics of predator avoidance by small, native Caribbean fishes to quantify their responses to lionfish and native predators. Field experiments indicated that white grunts generally display shorter average separation distances and a closer minimum approach to lionfish than native predators.

Furthermore, white grunts exhibit separation distances from exotic lionfish that are equal to those displayed in response to two non-piscivorous fishes, a strong indication of naiveté towards the exotic predator. Field observations of free-ranging fish revealed that several other species of small fishes also exhibit limited predator-avoidance behavior towards lionfish. A failure to recognize novel predators as threats is a dangerous form of prey naiveté because it precludes expression of most anti-predator responses, and could contribute to the rapid proliferation of lionfish across the Caribbean Basin.

INTRODUCTION

The evolutionary “arms race” between predators and their prey implies existence of a dynamic equilibrium explaining persistence of predators and prey (Geffeney et al. 2002). Through natural selection, prey species have evolved chemical, structural and behavioral characteristics to reduce predation risk. When exotic predators are introduced into a community containing potential prey that lack a shared coevolutionary history, local extirpation or species extinction can result (Porter and Savignano 1990, Fritts and Rodda 1998, Salo et al. 2007). Predator avoidance behaviors may not be elicited, and, if expressed, may be ineffective against a novel predator with which the prey has no shared evolutionary history (Cox and Lima 2006).

This phenomenon is termed prey naiveté and can take different forms (Banks and Dickman 2007), the most extreme being when prey do not recognize novel predators. Flightless birds in New Zealand that fail to respond to the presence of invasive rats or stoats (Banks and Dickman 2007) represent one example. Prey naiveté also occurs when prey recognize the novel predator as a threat, but respond with an ineffective behavior. Predator novelty in this case can be related to prey being unfamiliar with predator hunting strategy. For instance, small wallabies in Australia freeze in the presence of invasive foxes, a response that only increases their susceptibility to these predators (Banks and Dickman 2007). Another example of ineffective anti-predator response is hiding in burrows by aquatic European water voles when in the presence of the exotic American mink. This response allows effective escape from native European mink, but not from smaller invasive female mink, which can still reach the vole hiding in a burrow (Macdonald and Harrington 2003).

Surprisingly, prey naiveté has been rigorously tested in only a few cases (Russell and Banks 2007, Rehage et al. 2009, Barrio et al. 2010, Kovalenko et al. 2010, Kuehne and Olden

2012). The degree of prey naiveté may often be related to the degree of evolutionary isolation the prey population has experienced (Cox and Lima 2006). Hence, naiveté is expected to be more common on islands, in rivers, and in lakes, and less pronounced within larger terrestrial ecosystems. Circumstantial evidence in support of this hypothesis comes from the introductions of invasive Nile perch into Lake Victoria and of the brown tree snake onto the island of Guam, both of which had devastating effects on endemic fauna resulting in extinctions of hundreds of species (Fritts and Rodda 1998). Invasive predators in marine systems are rare and prey naiveté has been hypothesized to be low because of high connectivity in marine communities (Cox and Lima 2006). For instance, members of the *Serranidae* family (groupers and sea basses) are distributed worldwide in the tropics and subtropics, sharing evolutionary history with prey species. Thus, marine prey may identify a novel grouper as a threat because of similarity of its appearance and behavior to co-familial predators within the native range.

The lionfish (*Pterois volitans*) invasion of the Caribbean is a notable example of the successful establishment of a predatory marine fish outside of its native range (Fig. S1.1). Lionfish were first sighted in 1985 off Florida (Morris and Akins 2009), arrived in the Bahamas in 2004, and in recent years have proliferated over most of Caribbean Basin, Gulf of Mexico and up the Atlantic coast to North Carolina (Whitfield et al. 2007, Schofield 2010). Lionfish densities in their new range can be up to fifteen times higher than in their native range (Kulbicki 2012). On reefs in the Bahamas, lionfish impact native fish populations by reducing recruitment (Albins and Hixon 2008), fish biomass (Green et al. 2012), and species richness (Albins 2013). Invasive species have become a major

conservation concern (Clout 2001), with the lionfish invasion considered one of the top ten most serious environmental issues in the world (Sutherland et al. 2010).

The degree of prey naiveté can be measured by comparing the behavioral responses of prey to invasive versus native predators. Prey naiveté to lionfish has been suggested before (Albins and Lyons 2012, Cure et al. 2012) and the purpose of our study was to experimentally test this hypothesis. In two field experiments, we compared two metrics of predator avoidance by a common prey fish (white grunts) in the presence of invasive lionfish and in the presence of native predators. Additionally, we conducted field observations quantifying these behavioral metrics by several species of prey.

METHODS

Field experiments

We ran two field experiments from September through December 2009 in The Bight of Old Robinson, Abaco, Bahamas (26° 20' 43"N, 77° 01' 21"W; for site descriptions, see Layman and Allgeier 2012). In both experiments we deployed a cage (80 x 18 x 18 cm) that was divided in the middle by one mm mesh to separate predator and prey (Fig. S1.2). We chose this cage size to maintain the predator in close proximity to the prey, yet allow enough space for the three prey individuals to swim inside the cage. The prey side of the cage was marked with pins (every five cm) as a frame of reference to quantify distance (Fig. S1.2). In this experimental arena, prey could use visual and chemical cues to identify the predator, but the predator could not consume the prey. Experiments were performed in 2-4 m deep sandy areas containing sparse *Thalassia testudinum* (turtle grass). We collected predators and prey from

nearby rocky outcroppings and small coral reefs. All fish were collected using baited fish traps (61 x 61 x 46 cm), except for lionfish, which were caught with small aquarium nets. Each predator was starved for at least 24 h prior to experimental use. *Haemulon plumieri* (white grunt) was chosen as the focal prey species for multiple reasons. First, neither *Haemulon plumieri*, nor any of the other 21 species of the *Haemulon* genus, is known to co-occur with lionfish in the Pacific (Rocha et al. 2008). This indicates a lack of recent co-evolutionary history between lionfish and the *Haemulon* genus and meets a criterion for testing naiveté. Second, lionfish are known to consume white grunts in the Caribbean (Green et al. 2011, Munoz et al. 2011). Finally, grunts are ecologically and economically important on Caribbean coral reefs (Meyer et al. 1983, Yeager et al. 2011). White grunts were collected from a population that had been syntopic with lionfish for about four years since lionfish were first observed in Abaco in 2005 (Schofield 2010).

Within field cages, we compared metrics regarding potential predator avoidance by prey. Two experiments provided overlapping and complementary information. Experiment 1 had three treatments: *Pterois volitans* (lionfish); *Epinephelus striatus* (Nassau grouper) as a native predator; and *Halichoeres garnoti* (yellowhead wrasse), an invertivore that feeds mainly in zoobenthos (Randall 1967), as control fish. Inclusion of *H. garnoti* trials allowed us to test for potential responses to a similarly-sized non-piscivore. To test if differences between novel and native predators in experiment 1 were species-specific (e.g., prey respond to Nassau grouper more strongly than to other native predators), experiment 2 was conducted with additional native predator species. Experiment 2 included 6 treatments: lionfish; *Epinephelus guttatus* (red hind grouper), *Lutjanus apodus* (schoolmaster snapper), *Epinephelus striatus* (Nassau grouper), and *Ocyurus chrysurus* (yellowtail snapper) as native

predators (each of which consumed white grunts in captivity; Anton and Simpson personal observations), and *Holocentrus rufus* (squirrelfish) as a non-piscivorous fish control. We used new predators in each replicate (one per day) in both experiments. We used new prey in each replicate in experiment 1, whereas in experiment 2 we sequentially exposed the same set of three prey fish to each predator treatment on a given day, employing a new set of three prey fishes every day to provide replication. The temporal sequence of predator treatments was randomized each day. Neither predator nor prey size varied significantly among predator treatments (Table S1, Table S2); however, because we detected a marginal p-value for predator length in experiment 1, predator size was included as a fixed factor in subsequent analyses.

Videos were taken of the cage during each trial using an underwater Sony Digital Handycam DCR-PC101 MiniDV camera (Sony Corporation of America, New York, USA) mounted on a tripod (Fig. S1.2). Videos were taken for three min during experiment 1 and two min during experiment 2. After starting the recording, researchers left the area to minimize any possible disruption of natural fish behavior. Predator and prey were given one minute to acclimate to the cage before the video recording began. Following a scan sampling approach (Kovalenko et al. 2010), a fixed image was extracted from each video at ten seconds intervals for a total of 12 images per treatment per day.

The methods of assessment of prey naiveté reported in the literature vary among studies (Rehage et al. 2009, Barrio et al. 2010, Kovalenko et al. 2010). Distance to a predator has been considered a reasonable quantitative metric of predator avoidance in fish (Johnsson et al. 1996, Arai et al. 2007) and it is a conspicuous fish behavior (Fig. S1.3). We used two metrics of distance to predator to quantify predator effects on prey avoidance behavior (Table

1). The first response was *separation distance*, defined as the average distance between the mouth of the prey fish and the mouth of the predator. Distances were calculated using ImageJ (Abramoff et al. 2004), yielding a total of three measurements per image and 36 measurements per treatment replicate. The second response was *closest approach distance*, namely the minimum separation distance between any of the three prey individuals and the predator. We selected the shortest of the 36 distance measurements per replicate as the closest approach distance. For both experiments, each replicate was run on a separate day between 11:00-15:00 h (n=7 per experiment).

Field observations of free-ranging fish

Interactions among free-ranging, unconstrained predators and prey were observed in the field to document potential prey naiveté under natural conditions (Fig. 1.1). Prey behavior was assessed through spot-check observations (Rehage et al. 2009) conducted by one observer. We quantified how smaller prey fishes responded to each of five species of larger fish (termed focal predator): lionfish as a novel predator; red hind grouper, schoolmaster snapper, Nassau grouper as native predators; and yellowhead wrasse as a non-piscivorous control fish (Fig. 1.1). We estimated the distance between individual focal predators (for each of the five species) and the small prey fish with the help of an underwater graduated slate. Focal predators were chosen as encountered while snorkeling over extensive shallow reefs (1-10 m depth). The observer swam in one direction only to avoid re-encountering the same focal predator individuals on Abaco (the Bight 26° 20' 43" N, 77° 01' 21" W and Sandy Point 25° 59' 51" N, 77° 24' 12" W) between 9:00-16:00 h.

Focal predators were selected within the range size of 15-45cm TL to control for size effects. The size of the focal predator was estimated with the help of an underwater graduated slate and assigned to one of 6 categories (15-20cm, 20-25cm, 25-30cm, 30-35cm, 35-40cm and 40-45cm). The level of activity of the predator individuals could also affect the behavior of prey fish; hence, we did not take measurements on individuals that were resting inside crevices, swimming away from the observer or otherwise unusually active. In our observations, lionfish, Nassau grouper and red hind grouper species were relatively sedentary, whereas schoolmaster snapper and yellowhead wrasse species were active swimmers.

After examination of the focal predator and smaller fish in its vicinity to ensure that they appeared unaffected by presence of the snorkeler, the measurement period began and lasted for three min. The snorkeler was always at least 2.5 m away from all fishes being observed, a distance considered reasonable to assess fish behavior in the field (Cure et al. 2012). For each focal predator, the observer began the three-min observation period by haphazardly selected one small fish <5 cm TL, within 60 cm of and approaching the focal predator, observed its movements, and recorded the distance at which it turned away from or stopped approaching the focal predator as a metric of minimum approach distance. This distance between the mouth of the small fish (potential prey) and the mouth of the focal predator was visually estimated for each individual prey fish with the help of an underwater graduated slate. Within the three-min period, 11 individual prey fish on average were sequentially observed.

Observed prey were constrained to be <5 cm TL to standardize the size class of potential prey among all focal predator species and sizes. We made observations of only those prey within 60 cm of the focal predator because prey fish at greater distances could fall outside the

observer's fixed field of vision. Observations of different individual small fish were recorded around each species of focal predator, totaling 156, 157, 116, 116 and 176 small individual prey fish observations for yellowhead wrasse, lionfish, Nassau grouper, red hind grouper and schoolmaster snapper, respectively. Observations were conducted on days of light winds and good visibility.

We observed prey fishes around a total of 19 lionfish, 13 yellowhead wrasses, 15 schoolmaster snappers, 10 red hind groupers, and 15 Nassau groupers. The eighteen prey species observed included fifteen reported prey plus 3 suspected prey of lionfish in the Caribbean (Table S3). Distance from prey to focal predator was assigned to one of eight categories (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-60 cm). Because of the mobility of schoolmaster snapper and yellowhead wrasse, distance away from the snorkeler was greater for these species and the snorkeler followed individual focal predators by swimming slowly along a parallel path separated by >2.5 m from the track of the focal predator while taking measurements. Otherwise, the observer remained as still as possible to minimize any influence on fish behavior. All prey species were identified to the species level except for the blennies and gobies that were classified in one group.

Statistical analyses

To determine statistical significance in separation distances between small fish and predators as metrics of predator avoidance behavior in experiment 1, a fixed-effects model was used with predator treatment and predator total length as fixed predictors. In experiment 2, the same set of prey was exposed sequentially to all predator treatments with the order of predator species (termed treatment order) randomized for each set of prey, requiring a mixed-

effects model. To test for an interaction between the fixed and random factor, we compared models with and without the interaction term between predator treatment (fixed factor) and day (random factor) using likelihood ratio test (Crawley 2007). Because we found no significant interaction between predator treatment and day for either of the two dependent variables, the interaction was not included in subsequent analysis. The mixed-effects model included predator treatment and treatment order as fixed factors and prey-group as a random factor to account for repeated use of three individual white grunts across separate treatments. The inclusion of treatment order allowed an assessment of the degree of acclimation or sensitization that could occur to the same prey-group over the course of the multiple predator trials. When treatment order was found significant, we examined the nature of the effect by graphing the data (e.g., order and distance) segregated by treatments. Separation and closest approach distance were best fit by normal distributions, as indicated by AIC (Akaike's Information Criterion) and the examination of the model residuals. From the model's regression table of coefficients, and using lionfish as our reference group, we identified with *a priori* contrasts any significant differences in the metrics of predator avoidance between lionfish and the other predator treatments (Table 2).

To analyze how closely a small fish approached different species of focal predators when free-ranging in the field, we used a mixed-effects model (TableS3). Only prey species that were observed interacting with all five species of focal fish were included in the statistical analysis (Table S3). We included five of the 18 prey species that were observed with the five species of focal predators (Table S3). The average (\pm SD) number of observations of prey species per focal predator species was 20 (\pm 12); hence, our field observational study had an orthogonal design. The mixed-effects full interaction model for the field observations

included focal predator species, prey species and focal predator total length (TL) as fixed factors and predator ID as a random factor because multiple observations were conducted on each individual focal predator.

Stepwise model simplification approach was used to find the best model to explain distance to focal predators. When a fixed factor was not significant, as determined by likelihood ratio test and Akaike's Information Criterion (AIC), it was excluded from the model following the principle of parsimony (Crawley 2007). The dependent variable was distance between the small fish and the focal predator, which best fit a normal distribution model as determined by AIC and graphical examination of the model residuals. Because we found no significant effect of focal predator TL, prey species, or their interactions, these factors were excluded from the final model. From results of the best model's regression and using lionfish as our reference group, we identified with *a priori* contrasts any significant differences in small fish proximity to lionfish and each focal predator species (Table 3). All statistical tests were performed using R version 2.15.0 (R project for Statistical Computing, <http://www.r-project.org>) using packages nlme (Pinheiro et al. 2012) and lme4 (Bates et al. 2011). Significant differences were determined at α of 0.05.

RESULTS

Experiment 1

The average separation distance maintained by white grunts (prey) from exotic lionfish was 30% shorter than from native Nassau groupers but matched the distance separating them from the yellowhead wrasse, a native non-piscivore, used as a non-threatening control fish

(Table 1.2, Fig. 1. 2). The pattern of differences among these three predator treatments for closest approach distance by white grunts was identical to that exhibited by average separation distance (Table 2, Fig. 1.2). Neither behavioral metric was affected by total length of the predator, therefore it was excluded from the analysis.

Experiment 2

White grunts maintained a 22-23% shorter average separation distance from exotic lionfish than from the native Nassau grouper and from the native schoolmaster snapper (Table 1.2, Fig. 1.2). Average separation distance of white grunts from lionfish did not differ detectably from their average separation distances from native squirrelfish (non-piscivorous), yellowtail snapper, and red hind grouper, each of which was similar (Table 1.2, Fig. 1.2). Although non-significant statistically, average separation distance of white grunts from lionfish was 5-10% shorter than from each of these predators. Treatment order had no effect on average distance (Table 1.2).

The closest approach distance of white grunts was closer by 28-51% to exotic lionfish than to the native piscivores - Nassau grouper, red hind grouper, yellowtail snapper, and schoolmaster snapper (Table 1.2, Fig. 1.2). Lionfish and the non-threatening control predator, the squirrelfish, did not differ in the closest approach metric (Table 1.2, Fig. 1.2). We detected a significant effect of treatment order (Table 1.2) on closest approach distance. Visual inspection of the results indicated that closest approach distance increased with treatment order and that this pattern was driven solely by lionfish. When the same analysis was performed after excluding the lionfish trials from the data set, the significant effect of treatment order disappeared (Table 1.2). Closest approach distances exhibited by white

grunts tended to be longer when exposed to lionfish that were tested later in the sequence of experimental trials in field cages.

Field observations on free-ranging fish

Small ($TL < 5\text{cm}$) fish, including five species from three different families (Table S1.3), revealed longer average approach distances to two native predators – the Nassau grouper by 22% and red hind grouper by 37% – than to exotic lionfish (Table 1.3, Fig. 1.3). An analogous 15% longer average approach distance to a third native predator, the schoolmaster snapper, was not statistically detectable. Small prey fish were observed closer to a non-predatory control fish, the yellowhead wrasse, than to lionfish by 46% (Table 1.3, Fig. 1.3). Length of the focal predator and prey fish species did not affect the distance that small fish maintained from the large fish in our field observations.

DISCUSSION

Our experiments indicate that juvenile white grunts in the Caribbean fail to express avoidance behaviors towards exotic lionfish to the same degree that they respond to native predators (Fig. 1. 2). Instead, the white grunts exhibit separation distances from lionfish equal to those from non-piscivorous fishes, implying prey naiveté to this exotic predator. Our observations agree with previous suggestions that prey in the Caribbean are not recognizing lionfish as a predator (Albins and Lyons 2012, Albins and Hixon 2012, Cure et al. 2012). To our knowledge, this is the first documented case of prey naiveté to invasive predators in the marine realm (Cox and Lima 2006).

Our field observations of free-ranging fishes imply only limited avoidance of lionfish by five additional species of prey fishes, in that they make closer approaches to a native, non-piscivorous fish than to lionfish, yet fail to maintain a separation distance from lionfish as great as their separations from two species of native piscivores (Fig. 1.3). Separation distance from a third native piscivore was not significantly greater than from lionfish, but the estimated magnitude of this possible difference was relatively small and the p-value was marginally non-significant (Table 1.2), implying limited detection power.

Some evidence exists for experimental artifacts, although none influence our conclusions. In experiment 2, the effect of treatment order, which led to greater closest approach distances of prey to lionfish when tested later in the sequence of trials, suggests sensitization of the prey fish towards a novel fish. This experimental artifact probably led to overestimates of closest separation distances from lionfish in experiment 2, as compared to an alternative design in which prey individuals were not re-used. This artifact does not influence our conclusions because the closest separation distance from lionfish remains significantly shorter than for all native predators (Fig. 1.2). In addition, this potential bias runs in the opposite direction of the difference between responses to lionfish and native predators, thereby rendering our results conservative. In enclosure experiments, predator and prey movement is restricted, potentially altering predator hunting behaviors and escape responses of prey. Yet we found parallel patterns for the predators in our field observations, with five species from three families of native Caribbean prey fish approaching more closely to lionfish than to native predators. In the results of our second experiment (Fig. 1.2), we show that white grunts exhibit closer average separation distances and smaller closest approach distances to lionfish than to four species of native predators, but for two of the predators only the closest approach metric

reveals statistical significance. This represents our only inconsistency between metrics and may be explained if the closest approach metric is a more powerful tool with finer resolution of differences in anti-predator behavior.

Maintaining a safe distance from predators is a common avoidance response found across the animal kingdom (Milinski et al. 1997, Holmes et al. 2005, Vitousek et al. 2010). Success of predator attacks declines with distance from prey (Cresswell et al. 2010) because greater separation allows the targeted prey to hide or escape. Although we presume, like others before us (Parris et al. 2006, Arai et al. 2007, Takahara and Yamaoka 2009), that shorter separation distance implies higher risk of predation, we have no basis on which to convert our metrics (average separation distance and closest approach distance between prey fishes and potential predators or control fish) to absolute risk of predation. Indeed, risk of predation as a function of separation distance would be expected to differ among predators as a function of their hunting modes. Predator hunting mode, described as the hunting strategy of the predator, has been shown to affect prey behavior (Schmitz 2008). A meta-analysis on predator hunting modes (Preisser et al. 2007) found that cues from stationary predators evoke stronger anti-predator behaviors (e.g., reduced prey activity, growth, fecundity and survival) than cues from actively hunting predators because the presence of sedentary predator cues indicate proximity to the predator and, hence, higher predation risk. Following the Preisser et al. (2007) classification of predator's hunting mode, our predator fish species would fall into two categories: active predators (schoolmaster snapper and yellowtail snapper) and sit-and-pursue predators (lionfish, Nassau grouper and red hind grouper). Hunting mode fails to explain any of the differences among predator avoidance behaviors in either of our experiments. In field observations, we found stronger avoidance by prey fishes of grouper

than of active schoolmaster, as predicted by Preisser et al. (2007), but not stronger avoidance of lionfish (which have a hunting mode similar to grouper) than of schoolmaster.

It is possible that prey fish are not at risk to lionfish predation at the distances reported in this study. However, a recent study (Albins and Lyons 2012) reports that lionfish produce jets of water directed towards the prey while hunting. The study reports that “the maximum distance from the mouth of the lionfish that a visible jet-front reached was 9.6 cm”. Our study documented prey fish swimming less than 5 cm from the mouth of lionfish in experiments and in field observations, which is within the lionfish feeding range reported by Albins and Lyons (2012). Failing to maintain a safe distance from the exotic predator indicates a failure to recognize the invader as a predator (Banks and Dickman 2007) and may qualify as the most dangerous form of prey naiveté because it precludes most anti-predator responses.

Two plausible mechanisms may explain prey naiveté towards invasive lionfish in the Caribbean. First, lionfish and Caribbean fish species lack recent co-evolutionary history because of geographical isolation. The Pliocene formation of the Isthmus of Panama between the Caribbean and the Pacific has segregated marine species for over three million years. Second, prey may still respond to novel predators if the novel predator is similar enough in appearance to native predators to invoke similar predator avoidance behaviors (Blumstein et al. 2009), but there are no predatory species in the Caribbean similar to lionfish. Although the *Scorpaenidae* family is represented in the Caribbean by five species, they are rare and differ from lionfish both behaviorally and morphologically. Unlike native Caribbean *Scorpaenidae*, invasive lionfish have aposematic coloration (Hofreiter 2010), possess large feathery pectoral fins and are able to swim and remain suspended while hovering. Native scorpionfish are cryptic ambush predators, whereas lionfish are not. These differences between exotic lionfish

and Caribbean *Scorpaenidae* (and other native predators) appear to render them different enough that prey fish fail to recognize lionfish as predators. Additionally, the specific hunting strategy of lionfish is novel in the Caribbean, dependent on specialized bilateral swim bladder muscles that allow them to attack from any orientation (Hornstra et al. 2004), large pectoral fins that they flare before striking (Albins and Lyons 2012), and the capacity to project disorienting jets of water towards their prey while hunting (Albins and Lyons 2012). Hence, even if prey species learn or evolve to recognize lionfish as predators and maintain a similar distance compared to native predators, it is possible that lionfish would still possess high predatory effectiveness because of their novel and sophisticated hunting techniques. Lionfish are successful invaders, spreading throughout the Caribbean basin in less than a decade (Schofield 2010). Additional evidence supports the conclusion that naiveté of native prey species towards lionfish is contributing to its invasion success. First, lionfish have a generalist diet in the invaded range and have been reported to feed on at least 63 species and 22 families of teleost fish, one family of molluscs and 9-11 families of crustaceans in the Caribbean (Albins and Hixon 2008, Morris and Akins 2009, Green et al. 2011, Munoz et al. 2011, Layman and Allgeier 2012). Also, stomachs of lionfish (19%) tend to be empty less frequently than those of sympatric native predatory fishes in the Caribbean, such as schoolmaster snapper (44%) and gray snapper (74%, Layman and Allgeier 2012), supporting our findings of prey naiveté towards lionfish.

Long-term impacts of exotic consumers on prey populations can be mitigated by evolutionary adaptation of prey (Strauss et al. 2006). This rapid evolution of prey responses to novel predators can take place in just a few generations (Berger 2001, Freeman and Byers 2006, Langkilde 2009). Predator avoidance towards lionfish might evolve quickly under

intense selection pressure by the lionfish predators. Yet, in the meantime, genetic variation may be substantially reduced, thereby diminishing the potential for prey fishes to adapt to future environmental change (Strauss et al. 2006). Given the wide geographical scale of the lionfish invasion, strong selection within multiple prey species could have widespread consequences for coral reef ecosystems in the Caribbean.

Table 1.1. Metrics of predator avoidance recorded in experiments 1 and 2 to assess predator recognition and prey responses to predators.

Prey avoidance behavior	Defined as	Method	Units
Separation distance	Average distance between the prey and the predator	Measurements from images from videos	mm
Closest approach distance	Closest distance between the prey and the predator	Measurements from images from videos	mm

Table 1.2. Summary of the best regression models for the anti-predator response of white grunts to lionfish, native predators and fish controls for experiments 1 and 2. Table depicts the statistical results of comparing each predator avoidance behavior between lionfish and each native predator species and control treatment. Each predator/control treatment is indicated here by the first letters of the name: full names and results are presented in Fig. 1. 2. Bolding indicates significant p-values.

Metrics of predator avoidance						
	Separation distance			Closest approach distance		
Experiment 1						
	df	F	p	df	F	p
Predator treatment	2	11.07	< 0.001	2	20.47	< 0.001
	df	T	p	df	t	p
LF vs YH	18	-0.02	0.985	18	-0.14	0.885
LF vs NG	18	4.52	< 0.001	18	4	< 0.001
Experiment 2						
	df	F	p	df	F	p
Predator treatment	5	5.81	< 0.001	5	13.73	< 0.001
Treatment order	1	2.31	0.139	1	7.75	0.009 †
	df	T	p	df	t	p
LF vs SQ	28	1.30	0.203	29	1.48	0.148
LF vs YT	28	1.43	0.161	29	2.48	0.019
LF vs SM	28	3.57	0.001	29	6.28	< 0.001
LF vs NG	28	4.05	< 0.001	29	5.46	< 0.001
LF vs RH	28	0.809	0.42	29	3.36	0.002

† When lionfish are removed from the analysis, p=0.14

Table 1.3. Summary of the best mixed-effects model selected for analysis of the field observations of behaviors of free-ranging fishes. The table depicts results analyzing how separation distances between small fish and focal predators differ between lionfish and three native predators and a non-predatory control fish (yellowhead wrasse). Bolding indicates significant p-values.

Mixed effects model for field observations	t-value	P
Random factors		
Predator individual ID (random variable)		
Predictor		
Focal predator species		
Lionfish vs. Yellowhead wrasse	-5.102	<0.001
Lionfish vs. Nassau grouper	2.362	<0.018
Lionfish vs. Red hind grouper	3.661	<0.001
Lionfish vs. Schoolmaster snapper	1.749	0.08

Figure 1.1. Drawing indicating the position of the snorkeling observer relative to each focal predator (replication listed) during field observations of behavioral responses of free-ranging fishes.

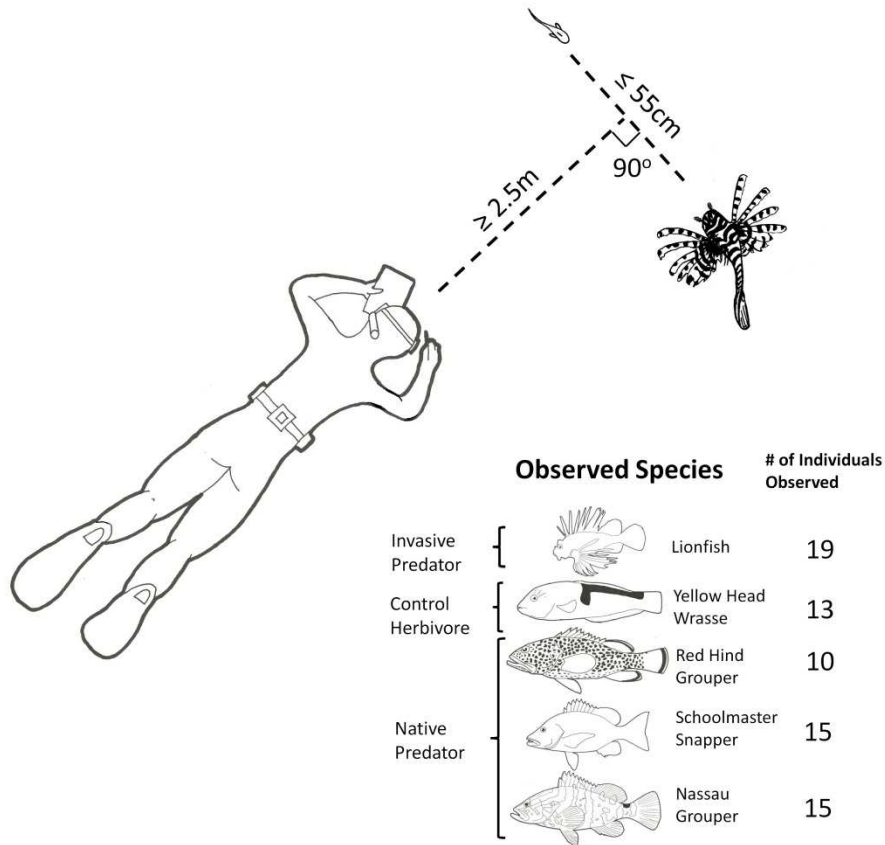


Figure 1.2. Metrics of predator avoidance from the field caging experiments (mean \pm 1SE). Values are the expected values from the best fitting models. Asterisks (*) denote significant differences ($p < 0.05$) in separation distance of prey to predators between a native non-piscivorous fish (black bars), exotic lionfish (white bars), and native predator treatments (grey bars).

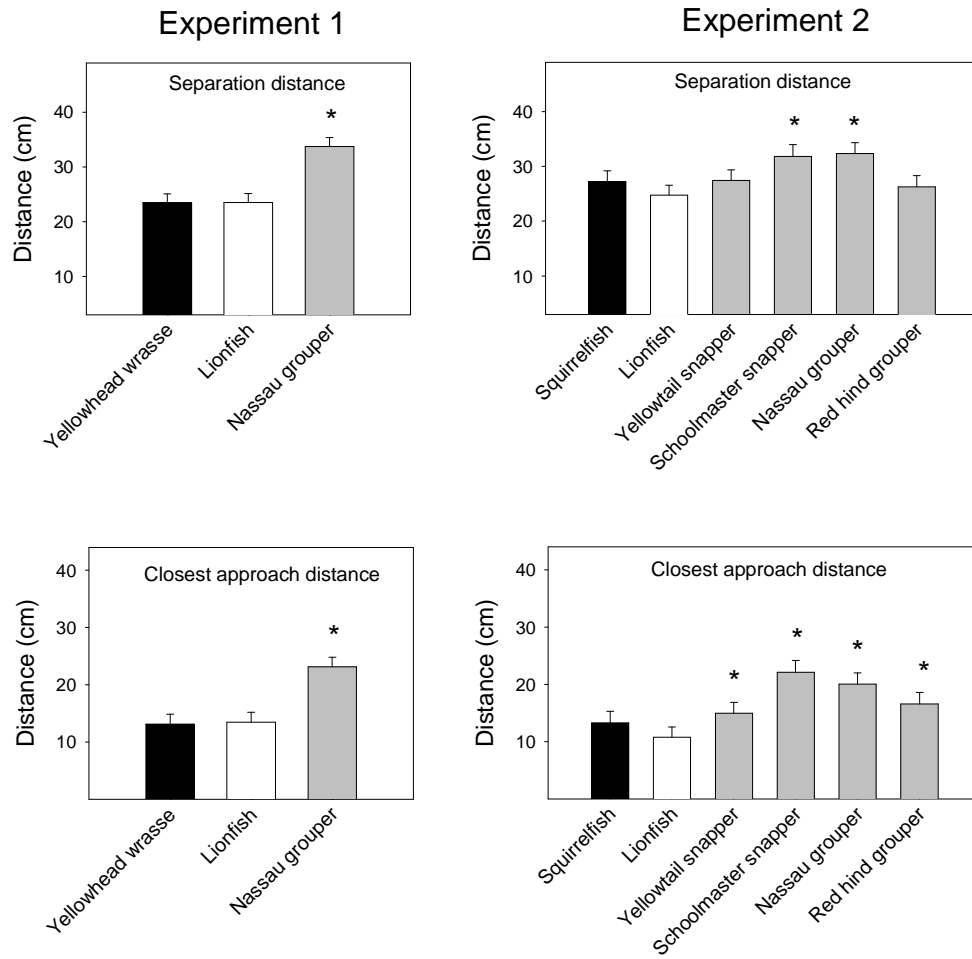


Figure 1.3. Separation distance (mean \pm 1SE) from the field observations of free-ranging fishes. Values are the expected values from the best fitting models. Replication for each treatment provided in Fig. 1.1 Asterisks (*) denote significant differences ($p < 0.05$) in separation distance of prey to focal predators between a native non-piscivorous fish (black bars), exotic lionfish (white bars), and native predator treatments (grey bars).

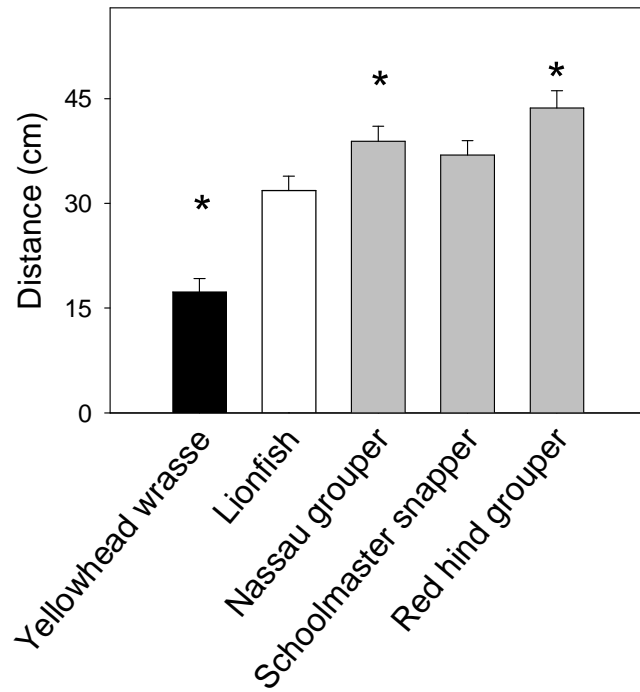


Figure S1.1. Pictures of invasive lionfish (*Pterois volitans*) in a shallow coral reef in Belize (top) and on a seagrass bed in the Bahamas (bottom).



Figure S1.2. Experimental set-up showing the cage and the underwater camera mounted on a tripod (A) and a close-up of the cage (B).

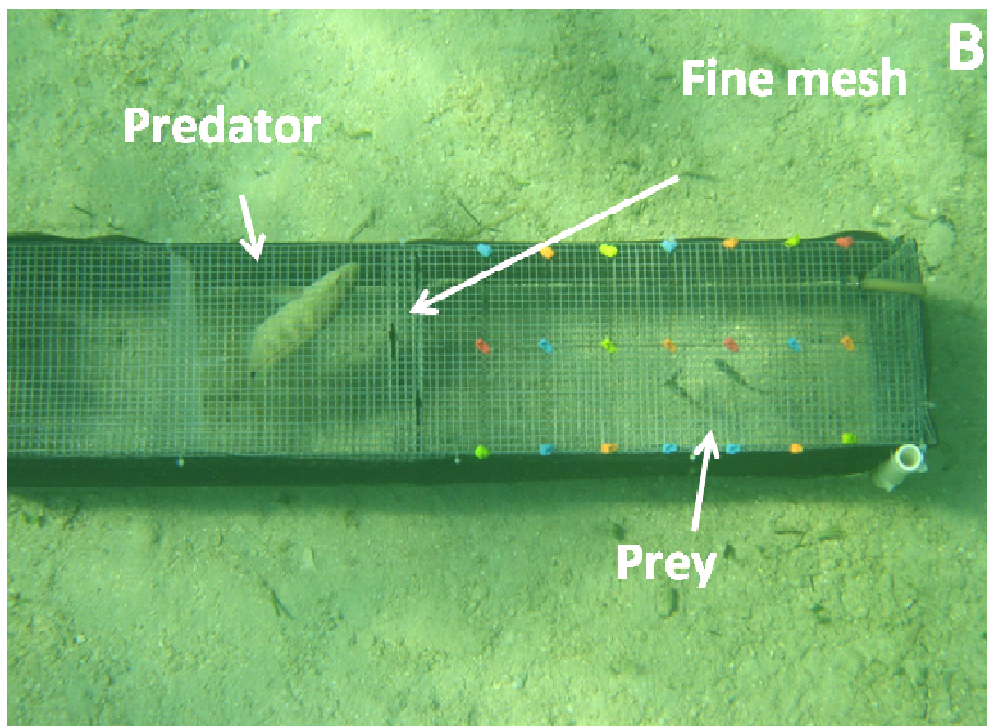
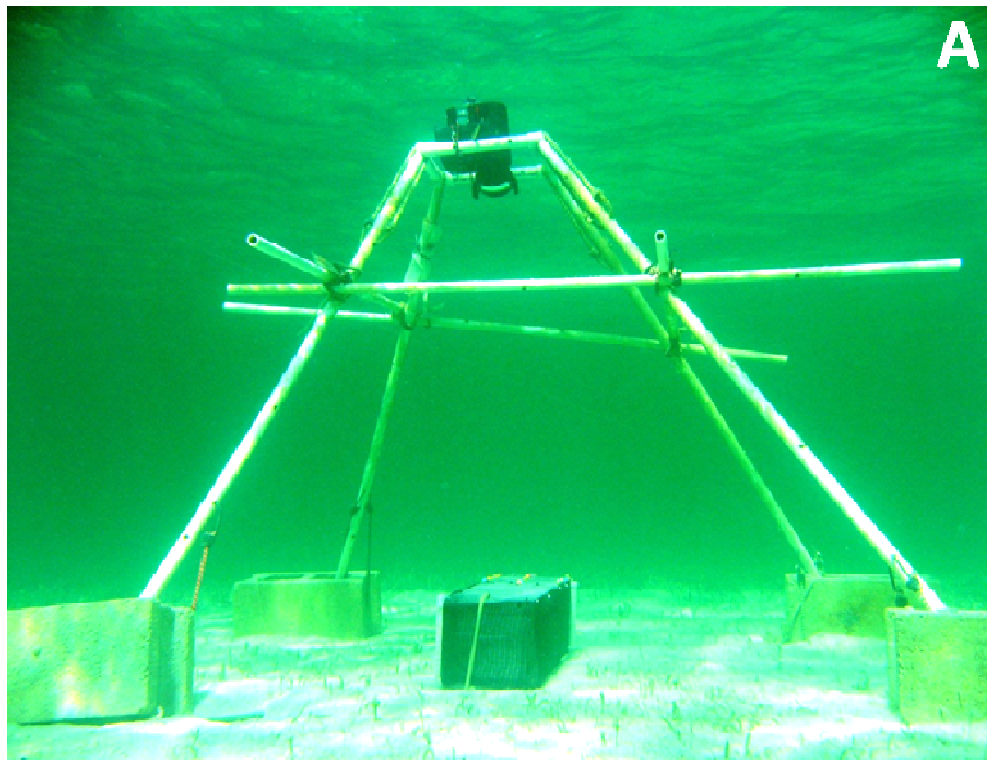


Figure S1.3. Observations of prey fish (grunts) avoiding Nassau grouper (native predator) in shallow reefs in the Bahamas.



Table S1.1. Results of the one-way ANOVAs testing for differences in fish total length for prey and predators among predator treatments for both experiments 1 and 2.

Prey fish total length	MS	F or t	p-value
<i>Experiment 1</i>			
Predator treatment	0.052	0.086	0.918
Lionfish vs. Yellowhead wrasse		0.378	0.71
Lionfish vs. Nassau grouper		0.038	0.97
<i>Experiment 2</i>			
Day	1.262	1.481	0.254
Predator total length	MS	F or t	p-value
<i>Experiment 1</i>			
Predator treatment	5.373	3.317	0.059
Lionfish vs. Yellowhead wrasse		-1.050	0.308
Lionfish vs. Nassau grouper		1.512	0.148
<i>Experiment 2</i>			
Predator treatment	1.570	0.456	0.805
Lionfish vs. Squirrelfish		0.518	0.608
Lionfish vs. Yellowtail snapper		1.209	0.235
Lionfish vs. Schoolmaster snapper		1.019	0.315
Lionfish vs. Nassau grouper		1.223	0.229
Lionfish vs. Red hind grouper		0.648	0.521

Table S2.2. Results of the mean and standard deviation of total length for prey and predators in experiments 1 and 2.

Prey TL	TL mean (cm)	TL SD (cm)
<i>Experiment 1</i>		
Predator treatment		
Yellowhead wrasse	4.9	0.9
Lionfish	4.7	0.7
Nassau grouper	4.9	0.8
Predator TL	TL mean (cm)	TL SD (cm)
<i>Experiment 1</i>		
Predator treatment		
Yellowhead wrasse	18.2	0.9
Lionfish	18.9	1.7
Nassau grouper	19.9	1.1
<i>Experiment 2</i>		
Predator treatment		
Squirrelfish	19.4	3
Lionfish	18.9	1.7
Yellowtail snapper	20.1	1.5
Schoolmaster snapper	19.7	1.1
Nassau grouper	20.1	1.7
Red hind grouper	19.5	1.6

Table S3. List of all fish species and families of small fish whose approaches to free-ranging predators were observed in the field. Fifteen species are known (Yes) or suspected (No) lionfish prey species in the Caribbean. The small fish species included in the statistical analysis are bolded.

Observed species common name	Observed species scientific name	Family	Known lionfish prey species?
Beaugregory damselfish	<i>Stegastes leucostictus</i>	Pomacentridae	Yes (Albins and Hixon 2008)
Bicolor damselfish	<i>Stegastes partitus</i>	Pomacentridae	Yes (Morris and Akins 2009)
Dusky damselfish	<i>Stegastes adustus</i>	Pomacentridae	No
Longfin damselfish	<i>Stegastes diencaeus</i>	Pomacentridae	Yes (Green et al. 2011)
Blue chromis	<i>Chromis cyanea</i>	Pomacentridae	Yes (Morris and Akins 2009)
Sergeant major	<i>Abudefduf saxatilis</i>	Pomacentridae	Yes (Layman and Allgeier 2012)
Blue tang	<i>Acanthurus coeruleus</i>	Acanthuridae	Yes (Green et al. 2011)
Princess parrotfish	<i>Scarus taeniopterus</i>	Scaridae	No
Red band parrotfish	<i>Sparisoma aurofrenatum</i>	Scaridae	Yes (Green et al. 2011)
Stoplight parrotfish	<i>Sparisoma viride</i>	Scaridae	Yes (Morris and Akins 2009)
French grunt	<i>Haemulon flavolineatum</i>	Haemulidae	Yes (Green et al. 2011)
Tomtate	<i>Haemulon aurolineatum</i>	Haemulidae	Yes (Green et al. 2011)
White grunt	<i>Haemulon plumieri</i>	Haemulidae	Yes (Green et al. 2011)
Puddingwife wrasse	<i>Halichoeres radiatus</i>	Labridae	No
Slipery dick wrasse	<i>Halichoeres bivittatus</i>	Labridae	Yes (Morris and Akins 2009)
Yellowhead wrasse	<i>Halichoeres garnoti</i>	Labridae	Yes (Morris and Akins 2009)
Bluehead wrasse	<i>Thalassoma bifasciatum</i>	Labridae	Yes (Morris and Akins 2009)
Caribbean sharpnose puffer	<i>Acanthigaster rostrata</i>	Tetraodontidae	Yes (Morris and Akins 2009)
Blenny/goby <i>sp</i>		Benniidae/Gobiidae	Yes (Morris and Akins 2009)

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CHAPTER 2

Environmental resistance to and biological facilitation of lionfish early invasion success in Bahamian coral reefs

ABSTRACT

Lionfish (*Pterois volitans*), venomous predators from the Indo-Pacific, are recent invaders of the Caribbean Basin and southeastern coast of North America. Lionfish are expanding their invasive range, allowing opportunity for the study of their early invasion success.

Quantification of lionfish abundances 4-5 yr after first detection on coral reefs of two Bahamian islands (Abaco and San Salvador) permitted inferences about factors influencing the invasion process. Lee- and windward environments differed dramatically in lionfish abundance, which was 11 times greater at leeward sites, but abundances of small and medium fishes and of large native predatory fishes were unrelated to environmental type. Lionfish density increased with abundances of large native predators as well as with small and medium fishes. These relationships suggest that (1) higher-energy environments may impose intrinsic resistance against lionfish invasion, (2) predation or competition by native fishes may not provide resistance against lionfish invasion, and (3) abundant prey fish may facilitate lionfish invasion success. These analyses of factors potentially suppressing or facilitating lionfish invasion success can be justified by results of multivariate analyses, which showed that lionfish abundance did not induce detectable changes in the composition

of large predatory, medium or small fish communities, implying a lack of lionfish impact on the fish communities at this early stage of invasion.

INTRODUCTION

Establishment of non-native species in new biogeographic regions can have serious consequences on biodiversity (Fritts and Rodda 1998) and is now recognized as one the world's most critical conservation challenges (Pejchar & Mooney 2009). Both physical and biological characteristics of the new environment affect the fate and success of exotic species (Brightwell & Silverman 2011, Madrigal et al. 2011). Clearly, the physical environment must be physiologically tolerable: harsh environments such as deserts have been shown to be the least invaded worldwide (Lonsdale 1999), perhaps because the suite of non-native species pre-adapted to those extreme conditions is limited. Alternatively, when environmental conditions are tolerable, biotic resistance may alone inhibit local invasion success (Elton 1958). Biotic resistance stems from community diversity (Stachowicz et al. 1999) or from the effects of strong local enemies (e.g. predators, competitors, or pathogens), affecting the fate of the exotic species in the new range. For instance, the native blue crab (*Callinectes sapidus*) provides biotic resistance against invasion by green crabs (*Carcinus maenas*) through direct predation in eastern North America (deRivera et al. 2005). Similarly, communities are more susceptible to invasion if they provide essential resources (Davis et al. 2000) or if the exotic species outcompetes native species in resource acquisition. For instance, invasive Argentine ants (*Linepithema humile*) outcompete native ants for food sources, depressing native ant abundance in northern California (Human and Gordon 1996).

Invasive lionfish (*Pterois volitans*), a native species from the Indo-Pacific, was detected in Florida in 1985 (Morris and Akins 2009) and spread rapidly throughout the tropical Caribbean and subtropical southeast Atlantic coast (Schofield 2010). This particular invasion is now ranked as one of the top-ten most serious environmental issues in the world (Sutherland et al. 2010). Densities of lionfish in their new biogeographic region range up to 15 times those in their native environment (Whitfield et al. 2007; Kulbicki 2012). On reefs in the Bahamas, lionfish consume recruits and are thereby capable of reducing native fish abundances (Albins & Hixon 2008), fish biomass (Green et al. 2012), and fish richness (Albins 2013). These findings are consistent with a meta-analysis that reveals that some novel predators can exert impacts on prey populations roughly double that of native predators (Salo et al. 2010). Possible explanations for the successful lionfish invasion of the Atlantic include its diet breadth, comprising dozens of species of native fishes (Albins & Hixon 2008; Morris & Akins 2009; Green et al. 2011; Munoz et al. 2011; Layman & Allgeier 2012), naiveté of prey towards exotic lionfish (Cure et al. 2012, Anton et al. 2013), and the possibility of a geographic escape from control by natural enemies (Mumby et al. 2011; although see Appendix 2.1). Threats posed by invading lionfish are particularly serious because of the high ecological and economic values of coral reefs in the Caribbean (Barbier et al. 2011). Similarly, lionfish are a threat to reefs in southeastern North America, habitat for valuable reef fishes of the snapper-grouper complex already seriously stressed by overfishing (National Marine Fisheries Service 2008).

Here we utilize the opportunity posed by the initial detection of invasive lionfish on two Bahamian islands in 2005 (Schofield 2010) to sample potentially important physical and biological environmental characteristics to test which factors contribute to the early success

of the lionfish invasion. We operationally define invasion success by either lionfish density or biomass. We assume, on the basis of relatively low lionfish densities, as compared to other Atlantic locations (Green and Cote 2009; Kulbicki et al. 2012), that we are largely evaluating how these environmental factors affect invasion, but we also test whether lionfish abundance has any detectable influence on the natural communities of both native small and medium (potential lionfish prey) and large (potential lionfish predators and competitors) fishes. By assessing the effect of the environment (lee- and windward sites), we test how physical energy relates to lionfish invasion success on coral reefs. By exploring how lionfish density relates to abundances of large native groupers and other predatory fishes, we infer whether resistance to invasion may be provided by natural predators or competitors. Finally, by relating lionfish density to abundance of small and medium fishes, we infer whether prey availability may be inhibiting or facilitating lionfish invasion success.

METHODS

Field Sampling

We conducted field surveys at 22 sites around two Bahamian islands, San Salvador (18 sites) in July-August 2009 and Abaco (4 sites) in August 2010, in coral reef habitat at depths between 9-17 m (Fig. 2.1). Lionfish were detected at both islands in 2005 (Schofield 2010). Sites were separated by more than 1.5 km. Buoys were avoided when selecting sites to minimize possible influences of spearfishermen on lionfish. Replicate (3-4) haphazardly placed transects 50 m long were deployed to perform surveys of benthic habitat cover and fish abundances at each site. Transects were oriented parallel to shore and surveys conducted

between 9:00-16:00 h. On each transect, 3-4 divers working together but on different sections along the transect followed a sequence of sampling protocols. We quantified lionfish and large (> 30 cm in total length, TL) native predatory fish abundances by species (listed in Table S2.1) and estimated TL of each individual within 500m^2 (50×10 m) areas along the transect. Care was taken to include examination of cryptic habitats to avoid underestimating lionfish densities (Green et al. 2013). We counted potentially suitable prey fishes less than 5 cm total length (TL), termed small fishes, in 30m^2 (15×2 m) areas. In addition, we counted potentially suitable prey fishes of 5-10 cm TL, termed medium fishes, in 120m^2 (30×4 m) areas. Species richness of the entire fish assemblage was estimated by merging species presence information, independent of size, from the 30, 120 and 500m^2 survey areas at each site. This included species of fish 10-30 cm in TL, which were recorded in the 120m^2 areas but not used in this paper.

We also quantified benthic habitat cover along 30 m of the transect line placed on the bottom at each site. We classified benthic habitat type as coral or macroalgae. Turf algae, sand, sponges, cyanobacteria, crustose coralline algae and gorgonians were also identified but not used in this paper. These habitat types were either rare (e.g. sponges, gorgonians) or provide no emergent habitat structure (e.g. crustose corallines, cyanobacteria, sand). We estimated proportion of areal cover by each habitat category at 50 cm intervals along and directly below marked points on the transect line. Benthic cover measurements were pooled by transect and then averaged across transects to produce site means for each habitat type.

To compare our findings with previous correlations between grouper and lionfish (Mumby et al. 2011), densities of each taxon were transformed to biomass. Grouper data were converted to mass using allometric scaling relationships with body length (Marks and Klomp 2003).

Lionfish lengths in cm were converted to biomass (g) using empirically fitted, allometric scaling parameters ($a=0.00492$ and $b=3.31016$) obtained by measuring and weighing 137 lionfish from Abaco (Appendix 2.2).

Environmental classification of the sites

We classified sites as windward and leeward (Fig. 2.1) depending on the predominant winds (east-southeast for both islands) and consultations with local fishermen. In order to investigate the accuracy of our leeward-windward categorical designation of the sites, we estimated the average bottom velocity (i.e. velocity of the water near the sea floor) at each site as a metric of the degree of wave exposure to demersal and semi-demersal species. Land masses can modify the wave energy near the bottom and wave exposure was calculated as follows. First, we obtained publicly available maps from Google Earth (<http://www.google.com/earth/>) for both islands. Then we used the maps to determine vectors of the oceanic waves that could strike each site (all directions from which the waves could reach a site). Bottom velocity depends on wave direction, dominant wave period, wave height and depth (Kundu 1990). Wave direction, dominant wave period, and wave height were obtained from data available online (<http://www.ndbc.noaa.gov/>) from two permanent moored buoys (41047-NE Bahamas and 41046-East Bahamas) owned and maintained by the National Data Buoy Center. We assumed that the same waves that were reaching these buoys also reached our study sites, with the exception of Sandy Point, the southwestern site on Abaco, which is surrounded by land masses and fully protected from Atlantic waves (Fig. 2.1). The historical record of wave data from buoys is intermittent but included data from September to December 2007, May to December 2009, January to July 2010 and January to

December 2011 from the NE Bahamas buoy and data from August to December 2010 from East Bahamas buoy. Buoys collect data hourly from which we estimated bottom velocity (Kundu 1990) hourly for each site for all waves that directly reached that site: otherwise bottom velocity was recorded as zero. We then computed monthly average bottom velocities for every study site over all the time periods (above) for which these buoys recorded.

To test the appropriateness of assignments of each site into a leeward or windward classification, we used estimated site means of bottom velocities from May through August (“summer” months) to construct box plots of the hourly velocity distribution by month for each site, allowing visual comparison between the groups of lee- and windward sites. This time period includes the field sampling months of July and August plus the two preceding months, which could also have strong influences on biotic patterns. We further examined the annual patterns of seasonal change over months in average estimated bottom velocity for each site, to obtain additional information about lee-and windward sites.

Statistical analyses

We merged data sets across islands to simplify statistical analysis and because only one leeward site was sampled on Abaco (Fig. 2.1). To determine the statistical significance of the environment (lee- and windward) on each independent biological variable (coral cover, macroalgal cover, fish species richness, small fish density, medium fish density, lionfish density, lionfish biomass, density of large predatory fishes, and grouper biomass), we employed generalized linear fixed effects models. As guided by Akaike’s Information Criterion (AIC), coral cover, macroalgal cover, lionfish biomass, and grouper biomass were best fitted by normal distributions, whereas medium fish density, fish species richness and

density of large predatory fishes were best fitted by negative binomial distributions. Lionfish density was best fitted by a zero-inflated negative binomial (ZINB) distribution because these response variable contained more zeros than expected by chance based on a negative binomial distribution (Zuur et al. 2009).

We employed full generalized linear fixed effects models to determine the effects of the environment and benthic habitat cover (coral or macroalgae) on the small and medium fish densities. Analogous statistical analyses were performed to test for the effects of the environment and fish variables (large predatory fish density, small fish density, medium fish density, and fish species richness) on lionfish density, as well as for the effects of the environment and medium fish on large predatory fish densities. A similar analysis was performed to measure the effects of the environment and grouper biomass on lionfish biomass. As indicated by Akaike's Information Criterion (AIC), small and medium fish density were best fitted by negative binomial distributions on generalized linear models. Similarly, models with lionfish density as dependent variables were best fitted by negative binomial distributions on zero-inflated models and lionfish biomass was fitted by normal distribution in a generalized linear model. The stepwise simplification approach was used to find the best model to explain each dependent variable. When an interaction between the two fixed factors was non-significant, as determined by AIC and likelihood ratio test, it was excluded from the model following the principle of parsimony (Crawley 2007). When this interaction was significant, we examined the nature of the interaction by observing plots of relationships between variables within the model. When the significant interaction was produced because the main effects were not constant across the entire range of the covariate, we centered and scaled the covariate (Schielezeth 2010).

We also compared fish community compositions between lee and the windward environments, separately assessing small fishes, medium fishes and large predators. We first used n-MDS ordination (Kruskal 1964) to display patterns of dissimilarity in fish community composition among of study sites based on lee- vs. windward environments. PERMANOVA (Anderson 2005) produced formal statistical tests of differences among observed clusters of sites. Density data were $\log(x+1)$ transformed to account for the influence of rare species and pairs of sites compared by the Bray-Curtis similarity index. PERMANOVAs were run for each fish category with environment as the independent variable, using default settings with 999 unrestricted permutations.

Our analyses using lionfish density or biomass as the dependent variable assume that lionfish have not impacted the reef communities yet and hence the other biotic variables can be treated as independent predictors. We tested this assumption in nine separate PERMANOVA analyses. We used each combination of (1) an alternative categorical lionfish abundance (presence/absence, density, and biomass) as the fixed, independent variable and (2) a dependent, continuous response variable (fish community composition for small and medium fishes, and large predatory fishes). These analyses used data only from leeward sites because 70% of the windward sites had no lionfish. The categorical lionfish abundances for density and biomass used in the PERMANOVAs were obtained from frequency distributions. All PERMANOVA analyses were performed with PRIMER 6, whereas the other statistical analyses were conducted using R version 2.15.2 (R project for Statistical Computing, <http://www.r-project.org>) with packages MASS (Venables and Ripley 2002) and pscl (Zeileis et al. 2008).

RESULTS

Inspection of the summer-time distributions of monthly estimated near-bottom velocities by site for the 4 years of buoy wave data (Fig. 2.2) revealed virtually no overlaps between lee- and windward sites, implying appropriate classifications. Two windward sites did display somewhat lower average wave-generated bottom velocities, Channel and La Crevasse (Fig. 2.2). The bottom velocities in the windward sites were on average more than 5.4 times higher than in the leeward sites for the summer months and 2.5 times higher for all months (Fig. 2.2 and Fig. S2.1). Plots of mean bottom velocities for each month of the year (Fig. S1) revealed compellingly dichotomous patterns discriminating between east (termed windward) and west (termed leeward). The leeward pattern at all sites except one showing clear summer minima and smooth, monotonic transitions across seasons from summer to winter and from winter to summer (Fig. S2.1). Sandy Point, at which virtually no bottom velocity was evidenced in any month, was the outlier with no evident difference in estimated average bottom velocity over the years. In contrast, while velocities were also generally slower during summer at the windward sites, transitions between months were not smooth, and the seasonal patterns showed higher variability among sites (Fig. S2.1). The bottom velocities in the windward sites were on average more than 5.4 times higher than in the leeward sites for the summer months and 2.5 times higher for all months (Fig. 2.2 and Fig. S2.1).

Lionfish, density and biomass, and macroalgal cover were the only biotic variables that differed significantly between lee- and windward environments (Fig. 3). Average lionfish density was more than 11 times higher on lee- than on windward sites (36.4 versus 3.2 ha⁻¹) (Fig. 3). Lionfish biomass exhibited an even more extreme pattern of a 98-fold higher average level in lee- versus windward environments (Fig. 2.3), a consequence of finding only

small (5-20 cm TL, mean of 10.5 cm) individuals at windward sites as contrasted with a mean size of 22.1 cm TL at leeward sites. Average macroalgal cover of the bottom was almost 2 times higher in lee- (75% cover) than in windward environments (41% cover), whereas average coral cover was indistinguishable between environments at 9.6-10.3% (Fig. 2.3). Observed average densities of small and medium fishes and of native, large predatory fishes were also similar across environments (Fig. 2.3). A subset of native predatory groupers, Nassau grouper (*Epinephelus striatus*) and tiger grouper (*Mycteroperca tigris*), likewise failed to display a significant density difference between environments (Fig. 2.3). Finally, fish species richness did not differ between lee- and windward environments (Fig. 2.3).

Neither of the fish groupings most likely to show relationships with benthic habitat cover did. The negative binomial generalized linear models indicated that abundances of small and medium fish were unrelated to benthic habitat cover of either type (Table 2.1).

Our zero-inflated negative binomial models of how lionfish abundance across sites related to abundances of various groupings of fishes and fish species richness help uncover possible functional relationships affecting lionfish invasion success. Lionfish abundance was positively related to small and to medium fish abundances (Table 2.2). Lionfish were also more abundant where densities of large native predators were high (Table 2.2). Lionfish abundance did not exhibit any response to fish species richness, using fishes from all size classes (Table 2.2). Likewise, in a generalized linear fixed effects model, lionfish biomass exhibited no relationship to large grouper biomass (Table 2.2).

Multivariate n-MDS ordinations displayed and corresponding PERMANOVAs tested whether fish community composition differed between lee- and windward sites for each of

three size classes of fishes. Small and medium fish communities varied between energy regimes, although sites did not fully segregate by energy regime into two separate groupings (Fig. 2.4). In contrast, the community of large predatory fishes did not exhibit significant differences between energy regime (Fig. 2.4). Stress values for the n-MDS ordinations were all sufficiently low to allow inferences from the two-dimensional displays (Fig. 2.4).

In testing for whether lionfish, present on these islands for 4-5 years before our sampling, had already influenced the composition of any fish communities, we employed statistically powerful multivariate PERMANOVA analysis. We report results of analyses on untransformed fish data, which reached identical statistical conclusions to those achieved by square root and logarithm transformations. PERMANOVA demonstrated no evidence of impacts of lionfish abundance on the small fish community composition, with p-values of 0.554 for lionfish presence/absence, 0.533 for lionfish abundance, and 0.506 for lionfish biomass. Lionfish did not impact the community composition of medium fishes with p-values of 0.139 for lionfish presence/absence, 0.333 for lionfish abundance, and 0.169 for lionfish biomass. Finally, lionfish did not affect large (native) predatory fish community composition, with p-values of 0.390 for lionfish presence/absence, 0.863 for lionfish abundance, and 0.334 lionfish biomass.

DISCUSSION

By conducting multivariate community analyses, we learned that lionfish abundance, as operationally defined by any of three metrics, did not have any influence on small fish, medium fish, or large predatory fish community composition on the islands of San Salvador and Abaco. Lionfish had only been present on these islands for a few years, which may not

have been long enough to build population sizes to levels that could cause impacts on potential prey communities, the small and medium fishes, or on possible competitor or predator communities, the large predatory fishes. These demonstrations of lack of impacts of lionfish early in the invasion process allow us to treat effects of the abiotic environment and a suite of biotic variables on lionfish abundance as tests of whether these environmental and biotic variables inhibit or facilitate early invasion success of lionfish.

Environmental influences on biotic variables

We classified our study sites into two environments differing in physical energy, based upon exposure to prevailing winds, insights from experienced fishermen, and multi-year analyses of bottom velocity obtained from oceanic buoys. Environment had an evident and large influence on lionfish and macroalgae, but not on any other biological variable. The leeward sites held an 11-fold higher density and nearly 100-fold greater biomass of lionfish than windward sites. Only 5 lionfish were encountered on the windward sites and they were all small, averaging 10.5 cm in total length. The apparent inhibition of lionfish invasion success in the windward environment may reflect direct impacts of physical stresses on the lionfish or could arise through one of several possible indirect effects of environmentally driven biotic influences. We will turn to our other data to evaluate the evidence for and against direct environmental control versus one of many other possible indirect controls on lionfish. Among other biotic variables analyzed, only bottom cover by macroalgae was related to physical environment. Macroalgae dominated the bottom on the leeward side, on average covering 75% of the bottom as compared to 41% on the windward side, while cover of live coral, the other biogenic habitat provider, was equally low at 9-10% in both environments.

As expected from Krajewski and Floeter (2011)'s demonstration that large, predatory coral reef fishes occupy both physically quiescent and energetic environments equally, large, predatory fishes in our study as well as a subset of those predators, groupers, did differ in abundance or biomass between environments. A similar absence of discrimination between environments existed for densities of potential prey fish (small and medium fishes).

With the noteworthy exception of one study on a physical environmental factor, temperature tolerance of lionfish (Kimball et al. 2004), previous research on processes that may regulate lionfish abundance has assessed biotic interactions (e.g., Mumby et al. 2011). Consequently, our study represents the first demonstration that lionfish abundance and biomass during early stages of invasion are dramatically suppressed in an energetic environment. Some other fishes are scarce in wave-exposed environments, perhaps because the energetic costs of locomotion may be a considerable barrier to occupation (Bellwood et al. 2002). When hunting, lionfish hover over or near their potential prey, they flare and spread their oversized, interconnected pectoral fins before striking (Allen and Eschmeyer 1973; Albins and Lyons 2012). They often blow jets of water at their prey, presumably to disorient them before striking (Albins & Lyons 2012). The practice of these complex and sophisticated hunting behaviors could be rendered difficult or impossible under conditions of high bottom water velocities or strong oscillatory wave surge. Although the leeward sides of Abaco and San Salvador experience conditions of higher wave-generated flow velocities in winter and early spring, such conditions prevail at windward sites except during summer months (Fig. S2.1). Our results are consistent with the interpretation that lionfish invasion success is suppressed on these two islands by physically energetic conditions because of the sensitivity of their complex hunting behavior to hydrodynamic perturbations. If this interpretation is confirmed

by subsequent testing, it would imply some optimism that energetic environments may serve as refuges for coral reef fish biodiversity even as lionfish may fundamentally modify fish communities in more protected environments (Green et al. 2012).

Bottom-up effects on lionfish

The demonstration that, in addition to the physical environment, both small fish and medium fish abundances are positively related to lionfish abundance implies that prey availability can also limit lionfish invasion success - a bottom-up process. Our inability to demonstrate an effect of macroalgal cover on small or medium fish density leads us to conclude that the positive association between lionfish abundance and small and medium fish abundances cannot be explained by a process of algal habitat enhancement of invertebrate foods for small and medium fishes. Hence, coral reefs with high abundances of potential prey fishes for lionfish provide more essential resources to lionfish and may be more susceptible to this exotic invader.

Lionfish abundance is unrelated to native fish species richness

Elton's bioresistance hypothesis states that places with more native species are more likely to inhibit the establishment of non-native species (Elton 1958). The absence of a relationship between native fish species richness and lionfish abundance allows us to reject this bioresistance hypothesis of Elton (1958) as applied to early lionfish invasion of these two Bahamian islands. These results add to a growing body of literature (see review by Levine and D'Antonio 1999) showing that hotspots of biodiversity can harbor numbers of non-native species that equal or exceed those in low-diversity areas (Stohlgren et al. 2003), when

measured at spatial scales that contain sufficient spatial heterogeneity (Davies et al. 2005).

Our lionfish transect area was 500 m², an space large enough to capture environmental heterogeneity within coral reefs, which might explain the lack of relationship between native fish species richness and lionfish abundance (Davies et al 2005).

Lionfish abundance/biomass is not controlled by competition or top-down predation

Our analyses showed that lionfish abundance was positively related to density of large native predatory fishes, but lionfish biomass was not associated with grouper biomass. The positive relationship of lionfish abundance with abundance of large predatory fishes implies a lack of impact of competition and perhaps also predation on early invasion success of lionfish on these two islands. Instead, this positive effect of large native predatory fishes on lionfish abundance may arise indirectly through joint influences of some other variable on both lionfish and large native predatory fishes. However, our study did not gather sufficient data with which to identify such a common driver of abundance of both types of predators. The lack of an effect of grouper biomass (which included only those fish >30 cm in total length) and lionfish biomass supports a conclusion that on Abaco and San Salvador native predators are not providing resistance against lionfish invasion. Low predation on lionfish may not be surprising given the potent venom in their dorsal, anal, and pelvic spines, lethal to animals (Balasubashini et al. 2006). Although the act of any predation on healthy lionfish has not yet been reported, numerous studies of another successful toxic invader, the cane toad invading Australia, show low predation in the newly established range (Shine 2010).

The lack of a negative relationship between grouper biomass and lionfish biomass in our study contrasts directly with the recent conclusions in Mumby et al. (2011) from their study of lionfish and grouper biomass at sites along a chain of the Exuma Cays, also in the

Bahamas. The Exuma reef sites included two sets: one in the Exuma Cays Land and Sea Park (ECLSP), where native grouper biomass is now high after protection from fishing, and another set to the north, where fishing continues and grouper biomass is far lower. By regressing lionfish biomass (y) on grouper biomass (x) across all 12 sites, Mumby et al. (2011) found a strong negative relationship, driven by one cluster of points with high grouper and low lionfish biomass (ECLSP sites) and another cluster with one seventh the grouper and double the lionfish biomass (sites outside ECLSP). From this relationship, the authors concluded that when protected from fishing for long enough to rebuild grouper population biomass, predation by these native grouper populations can suppress the proliferation of lionfish on Exuma reefs.

The effectiveness of predatory resilience to lionfish is contingent on naturally high density and biomass of groupers, a condition met only in the ECLSP protected area and not on Abaco, San Salvador, or the fished area sampled by Mumby et al. (2011). Grouper biomass in the Exuma protected area was on average approximately 9 times what we documented on Abaco and San Salvador, so our failure to detect suppression of lionfish proliferation on these two islands can be explained by their relatively low biomass of native groupers.

However, inferring exposure to oceanic waves from the east by examining the map of the Bahamas suggests that the ECLSP may be more exposed to these energetic waves than the set of control sites to the north. Consequently, high-energy conditions may be confounded with high grouper biomass in the contrast of sets of sites used by Mumby et al. (2011). So our novel insight on the capacity of high-energy conditions to provide resilience against lionfish invasion success may motivate re-examination of the factors producing lionfish biomass patterns on Exuma.

Table 2.1. Best statistical models for the effects of benthic habitat (coral and macroalgal cover) and environment (leeward vs. windward) on fish density (small and medium fishes).

Dependent variable	Independent variable	Coefficient Estimate	SE*	p	Distribution
Small fish density	Intercept	9.667	0.208		NB [†]
	Coral cover	0.029	0.016	0.081	
	Environment	-0.301	0.172	0.081	
Small fish density	Intercept	10.18	0.598		NB [†]
	Macroalgal cover	-0.002	0.007	0.716	
	Environment	-0.401	0.325	0.217	
Medium fish density	Intercept	6.854	0.35		NB [†]
	Coral cover	0.002	0.02	0.929	
	Environment	0.09	0.29	0.762	
Medium fish density	Intercept	5.846	0.94		NB [†]
	Macroalgal cover	0.013	0.01	0.264	
	Environment	0.545	0.51	0.286	

*Model Standard Error

[†]Negative Binomial distribution

Table 2.2. Best statistical models for the effects of environment (leeward vs. windward) and fish densities (small and medium fishes, and large native predatory fishes) on lionfish abundance and the effects of environment and grouper biomass on lionfish biomass. Bolded values denote significant differences at $p < 0.05$.

Dependent variable	Independent variable	Coefficient Estimate	SE*	P	Distribution
Lionfish density	Intercept	3.891	0.284		ZINB [†]
	Small fish density [‡]	0.712	0.316	0.024	
	Environment	-1.488	0.571	0.009	
	Intercept (Zero-Inflation Model)	-1.634	0.719		
	Environment	2.419	1.058	0.022	
Lionfish density	Intercept (Count model)	2.872	0.33		ZINB [†]
	Medium fish density	<0.001	0.001	<0.001	
	Environment	-1.223	0.45	0.006	
	Intercept (Zero-Inflation Model)	-1.61	0.781		
	Environment	2.435	1.047	0.020	
Lionfish density	Intercept (Count model)	2.420	0.38		ZINB [†]
	Large predator density	0.028	0.008	<0.001	
	Environment	-0.721	0.51	0.158	
	Intercept (Zero-Inflation Model)	-1.650	0.802		
	Environment	2.441	1.066	0.022	
Lionfish density	Intercept (Count model)	1.544	1.81		ZINB [†]
	Species richness	0.077	0.06	0.22	
	Environment	-1.291	0.69	0.06	
	Intercept (Zero-Inflation Model)	-1.848	1.027		
	Environment	2.513	1.228	0.04	
Lionfish biomass	Intercept	2.833	0.51		Normal
	Grouper biomass	<0.001	0.001	0.465	
	Environment	-2.762	0.60	<0.001	

*Model Standard Error

[†]Zero Inflated Negative Binomial distribution

[‡]this variable was centered and scaled

Figure 2.1. Map of study sites in two islands in the Bahamas. Circles indicate leeward and triangles windward sites.

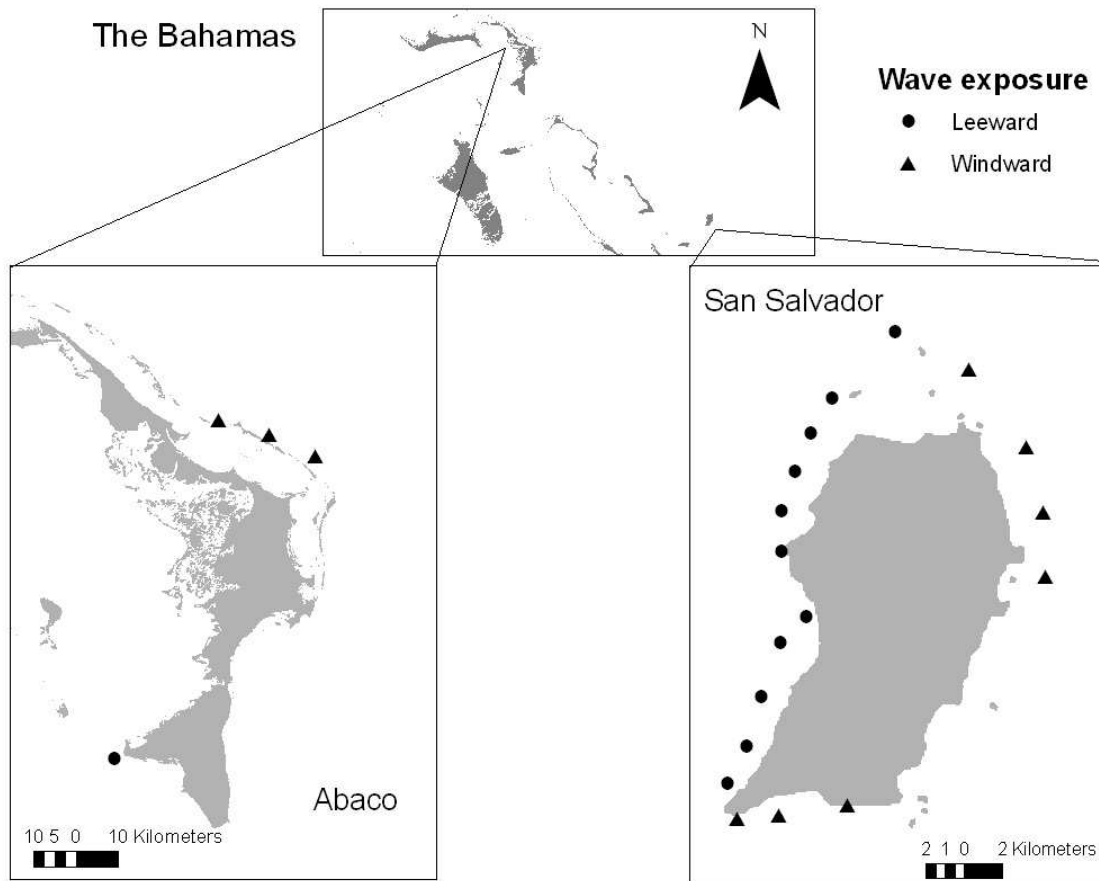


Figure 2.2. Boxplots of bottom velocity (m s^{-1}) averaged over summer months as a function of environment and site. Relationships of mean bottom velocity and environment (leeward and windward) at 22 sites in two Bahamian islands (listed in alphabetical order) for the summer months, defined as May, June, July, and August. The wave data used to calculate bottom velocity was collected hourly by oceanic buoys.

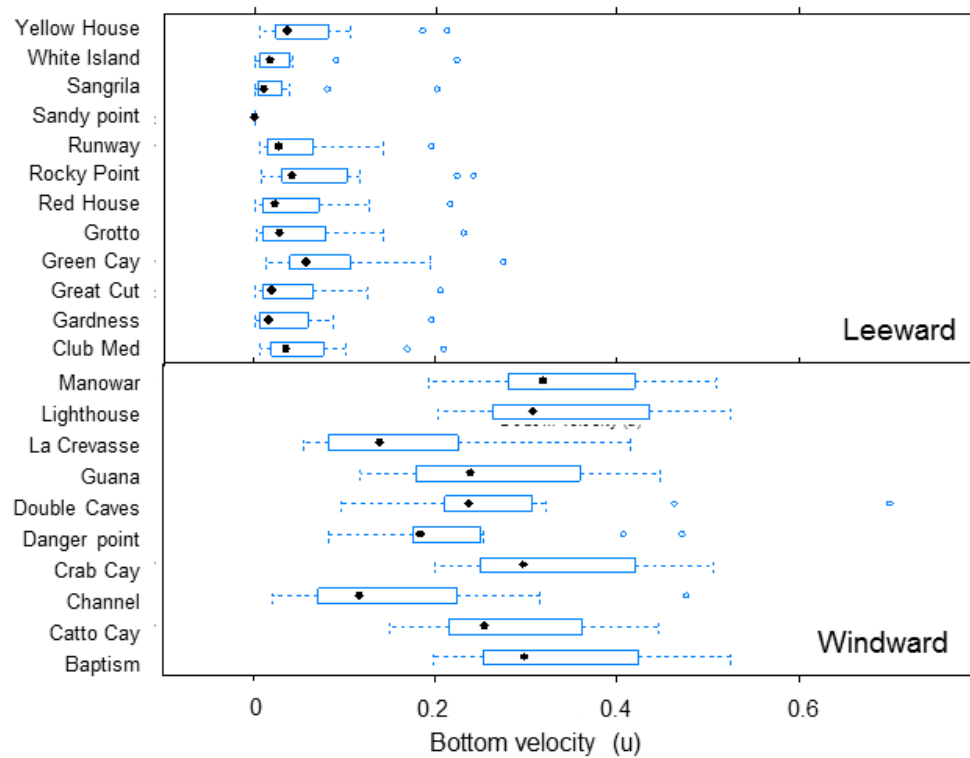


Figure 2.3. Effect of environment (lee- vs. windward wave exposure) on benthic habitat cover, fish density, or biomass. Relationships between the environment and (A) coral cover (%), (B) macroalgal cover (%), (C) fish species richness, (D) lionfish density (ha^{-1}), (E) lionfish biomass ($\text{g } 100\text{m}^{-2}$), (F) small fish density (ha^{-1}), (G) medium fish density (ha^{-1}), (H) density of large native predators (ha^{-1}), and (I) grouper biomass ($\text{g } 100\text{m}^{-2}$). p-values at <0.05 (represented by *) and at <0.0005 (represented by ***) indicate statistical significance.

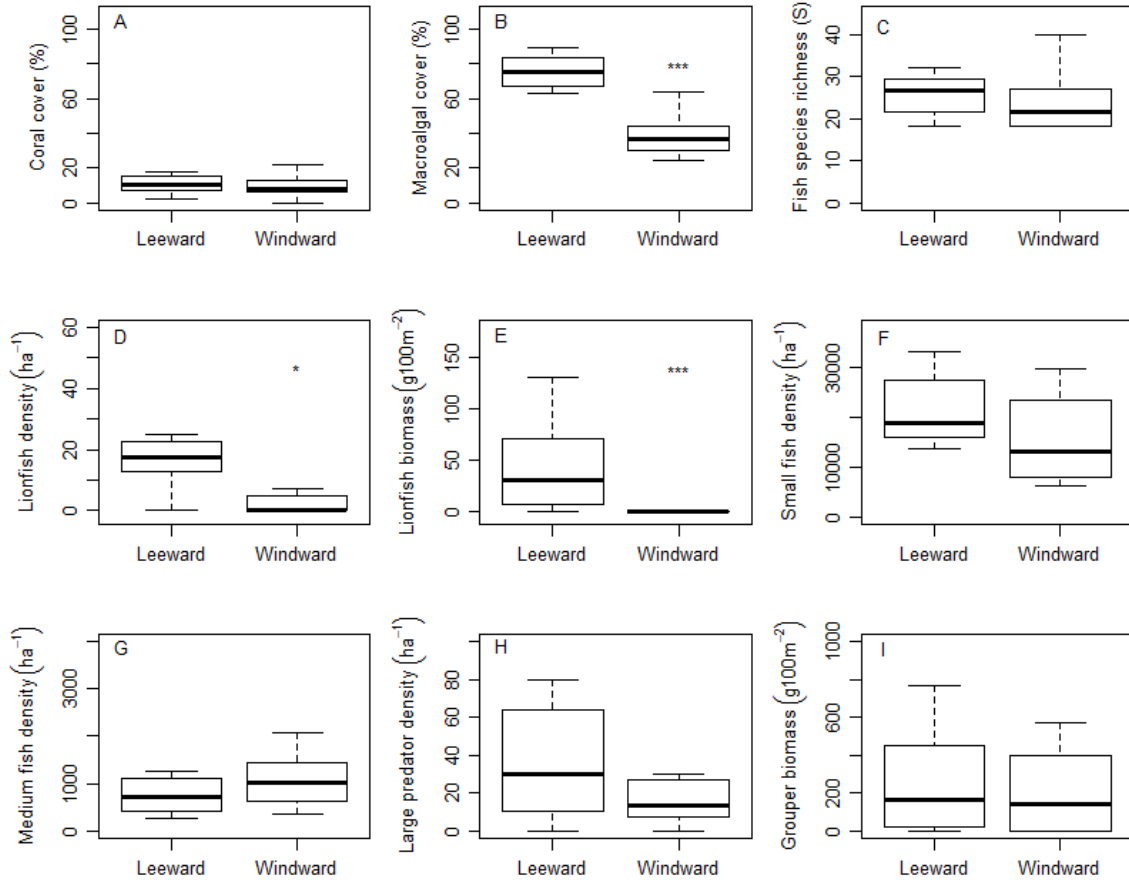


Figure 2.4. n-MDS plots depicting the composition of (A) small fish, (B) medium fish and (C) large predatory fish communities in lee-vs. windward environments. MDS ordination results depicting how community composition of small and medium fishes and large predatory fishes differs among the 22 sites between the environments (lee- vs. windward wave exposure). p-values from PERMANOVA at <0.05 indicate significant community differences between environments. 2D Stress is a measure of goodness of fit, with lower values indicating better fits.

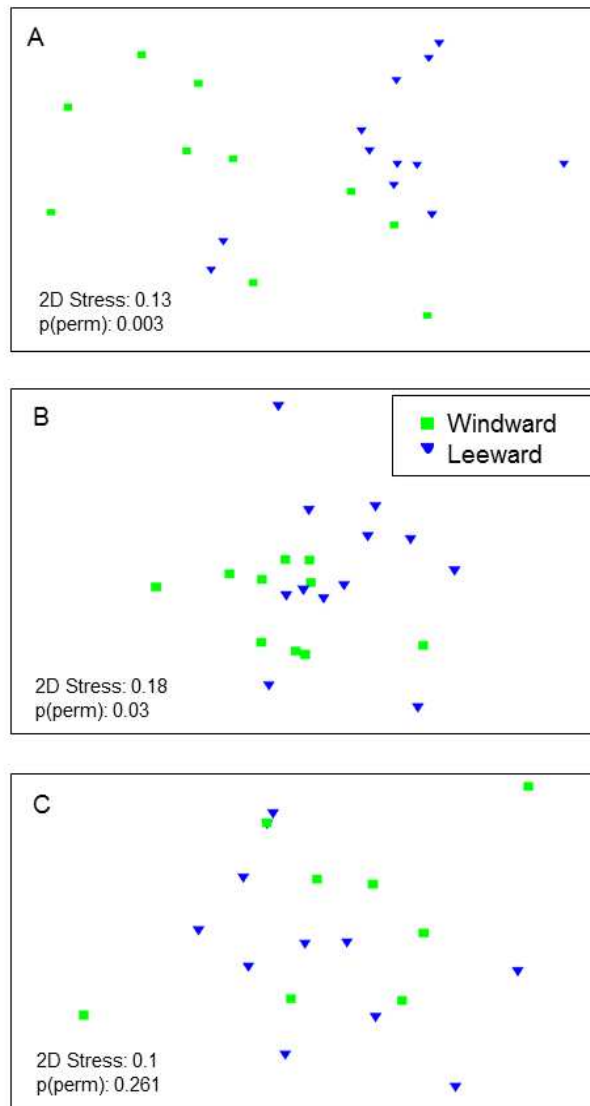
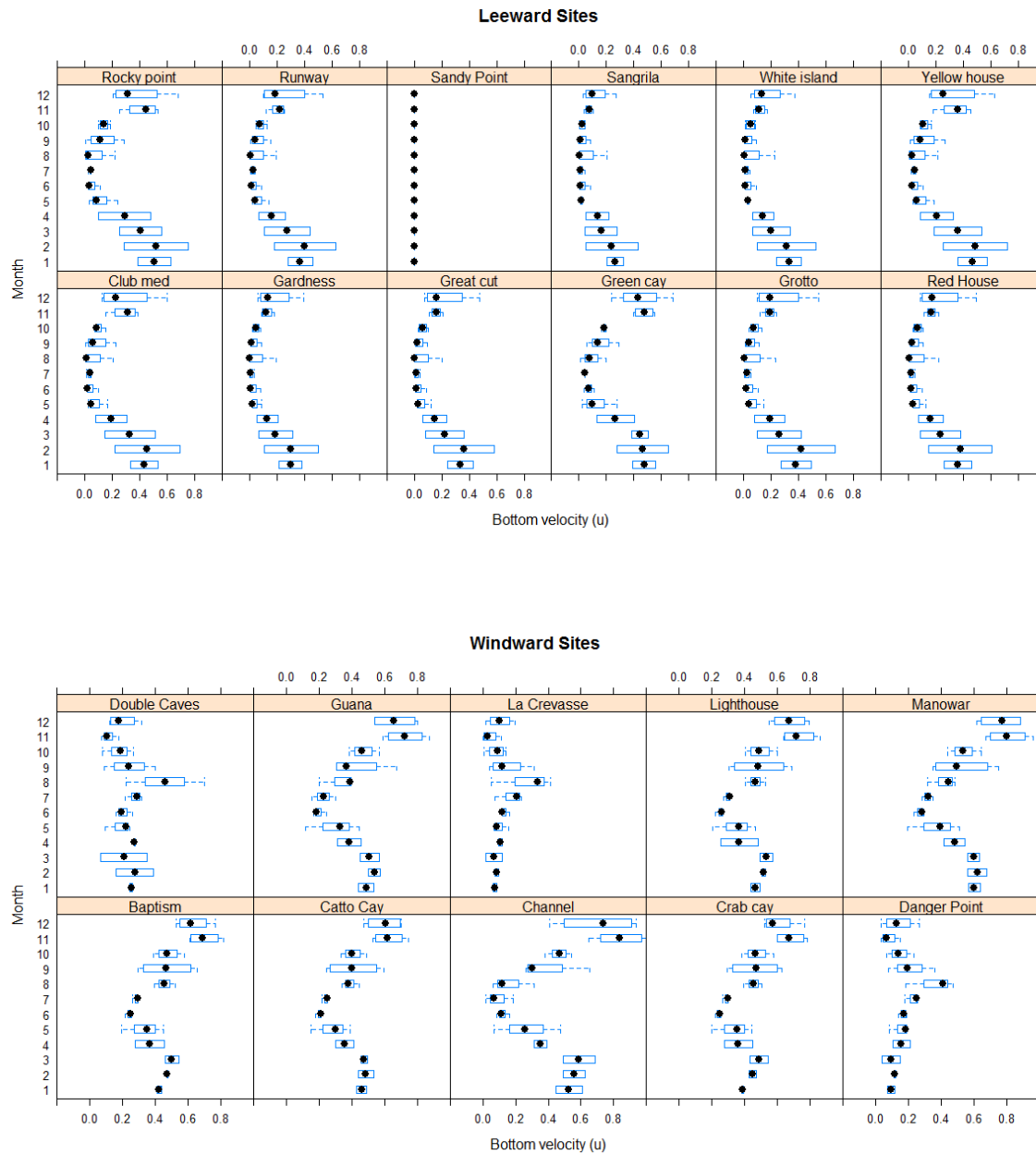


Table S2.1. List of the potential lionfish competitors and predators. These species comprised the large native predatory fishes categories. The two grouper species of which biomass was calculated have been bolded

Large predator species (>30cm total length)	
Common name	Scientific name
<i>Groupers</i>	
Rock Hind	<i>Epinephelus adscensionis</i>
Red hind	<i>Epinephelus guttatus</i>
Nassau grouper	<i>Epinephelus striatus</i>
Tiger grouper	<i>Mycteroperca tigris</i>
<i>Snappers</i>	
Mutton snapper	<i>Lutjanus analis</i>
Schoolmaster snapper	<i>Lutjanus apodus</i>
Cubera snapper	<i>Lutjanus cyanopterus</i>
Dog snapper	<i>Lutjanus jocu</i>
Yellowtail snapper	<i>Ocyurus chrysurus</i>
<i>Sharks</i>	
Nurse shark	<i>Ginglymostoma cirratum</i>
Blacktip shark	<i>Carcharhinus limbatus</i>
Reef shark	<i>Carcharhinus perezii</i>
<i>Other fishes</i>	
Great Barracuda	<i>Sphyraena barracuda</i>

Figure S2.1. Boxplots of bottom velocity (m s^{-1}) as a function of environment and site. Relationships of bottom velocity and month at 12 leeward sites (top) and 10 windward sites (bottom) in two Bahamian islands. The wave data used to calculate bottom velocity was collected hourly by oceanic buoys.



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CHAPTER 3

Comparison of anti-predator responses to lionfish in native and invasive range by small reef-fish

ABSTRACT

Prey detecting predators is an essential component of anti-predator behavior since the avoidance of predators increases the chances of prey survival. The prey naiveté hypothesis predicts inappropriate anti-predator behavior by prey lacking co-evolutionary history with the predator. We examined whether potential prey fish in the Caribbean are naïve to lionfish (*Pterois volitans*), an invasive predator from the Pacific by comparing small fish behavior within the invaded range with small fish behavior in the Pacific, where lionfish are native. We experimentally quantified two metrics of anti-predator behavior, closest approach distance and frequency of predator inspections, to lionfish in three families of fish in the Caribbean and Pacific. Findings from field experiments indicate that the three families of small fish maintained greater distances from lionfish in the Pacific than in the Caribbean. In addition, small fish inspected lionfish more often in the Caribbean than in the Pacific, indicating that lionfish in the Caribbean is perceived as a lower-risk species than in the Pacific. Field observations of free-ranging lionfish and small fish also revealed that two fish families in the Caribbean maintained greater distances from lionfish in the Pacific than in the Caribbean. These results suggest that prey fish in the Caribbean engage in more risky behavior in the presence of lionfish, indicating prey naiveté where lionfish are novel. Prey

naiveté may play a role in the successful and rapidly expanding lionfish invasion of the Western Atlantic.

INTRODUCTION

Invasions by predators represent a major threat to biodiversity as they often induce acute declines in the populations of indigenous prey (Salo et al. 2007, Dorkas et al. 2012).

Native prey species poses adaptive morphological and behavioral defenses to local predators acquired over the course of co-evolution (Freeman and Herron 2007). These behavioral defensive traits may be nonfunctional against novel predators if prey fail to recognize the newly introduced species as a predator. For example, tadpoles of the Iberian green frog (*Rana perezi*) detect chemical cues from native predatory dragonfly nymphs (*Anax imperator*) and respond by reducing their activity and developing a defensive morphology against this native predator. However, these defensive responses are not activated against a novel predator, the invasive predator red crawfish (*Procambarus clarkii*), reducing tadpole survival in the presence of the novel predator as compared to native nymphs (Gomez-Mestre and Diaz-Paniagua 2011). Such inappropriate anti-predator behaviors by native prey towards novel predators has been termed prey naiveté and has been suspected to be a relevant factor explaining the devastating effects of some invasive predators when introduced in areas outside their native ranges (Fritts and Rodda 1998).

Red lionfish (*Pterois volitans*), a predator native to the tropical Pacific region, was first sighted in 1985 off Florida and in recent years have quickly spread over most of the Caribbean Basin and parts of the Western Atlantic (Whitfield et al. 2007, Schofield 2010). Lionfish maximum densities in the invaded range are fifteen times higher than in

their native range (Kulbicki 2012). On reefs in the Bahamas, lionfish imposed strong negative impacts on native fish by reducing fish recruitment and biomass (Albins and Hixon 2008, Green et al. 2012). The direct and indirect effects of lionfish on marine ecosystems is of great concern for conservation and is now considered one of the top ten most threatening emerging environmental issues in the world (Sutherland et al. 2010). An ecological mechanism that may partially explain the lionfish invasion success of the Caribbean is the inability of prey fish to recognize lionfish as a predator. A recent study performed in the Bahamas found that white grunts maintained shorter distances to lionfish than to native Caribbean predators (Anton et al. 2013). Moreover, field observations of other prey fish from three different families also maintained larger distance to novel predators than to invasive lionfish in the Caribbean (Anton et al. 2013). However, the response of prey fish to lionfish in the Pacific remains unknown, and such information is needed to understand the magnitude of prey naiveté in the Caribbean. Quantitative comparison of the ecology of invasive species in their native and invaded range provides crucial information to understand the ecology of the invasive species and its impacts (Kulbicki et al. 2012, Moroney and Rundel 2013). The purposes of this study were to 1) determine from field experiments the behavioral responses of potential prey fish to lionfish in their native range as compared to potential prey fish behavior to lionfish in the invaded range, and 2) compare potential prey fish behavior to lionfish in the native and invaded regions using field observations of free-ranging lionfish and potential prey fish. In the experiments, we examined the responses of three families of potential prey fish (Pomacentridae, Scaridae, and Labridae) to lionfish using two metrics of predator avoidance (closest approach distance and frequency of predator inspections)

and then, compared the responses between the Caribbean and in the Pacific. In addition, field observations of free-ranging fish quantified avoidance behaviors to lionfish in two families of potential prey in both the Caribbean and the Pacific to assess the generality of our experimental results.

METHODS

We ran identical field experiments during the period of June to August 2010 in Marsh Harbor, Abaco, Bahamas (26° 33' 14" N, 77° 02' 02" W) in the invaded range and Agana Bay, Guam, USA (13° 28' 45" N, 144° 44' 37" E) in the native range of lionfish. In both locations we deployed a cage (80 x 18 x 18 cm) that was divided by 1 mm mesh to separate a lionfish from three individual potential prey fishes in the adjacent separate compartment. The lionfish compartment was 35cm long and the small fish compartment was 45 cm long. This cage size was chosen to ensure close proximity of the predator to the potential prey, yet allow enough space for the small fish to swim around in the cage. The prey side of the cage was marked with pins every 5 cm that we used as a reference to quantify distance. The lionfish was unable to consume the prey but the small fish could use visual and chemical cues to identify lionfish. The experiments were performed in 1-3 m deep sandy areas containing sparse seagrass: *Thalassia testudinum* in Abaco and *Thalassia hemprichii* in Guam. We collected lionfish and prey from nearby locations using small aquarium nets. Prey fish in the Bahamas were collected from a population that had been syntopic with lionfish for about five years because lionfish were first observed in Abaco in 2005 (Schofield 2010).

Within field cages, we compared metrics of predator avoidance by prey fish towards lionfish between native and invasive regions. The experiment had three factors. The first

factor was location being Guam within the native range of lionfish in the Pacific Ocean and Abaco within the invaded range in the Caribbean Sea. The second factor was prey fish family with three levels: Scaridae family with *Sparisoma aurofrenatum* in the invaded range and the *Scarus* family in the native range, Pomacentridae family with *Abudefduf saxatilis* in the Caribbean and *Chromis viridis* in the Pacific, and the Labridae family with *Thalassoma bifasciatum* in the Caribbean and *Thalassoma quinquevittatum* in the Pacific. *Sparisoma aurofrenatum*, *Abudefduf saxatilis*, *Chromis viridis* have been reported prey of lionfish in the Caribbean (Morris and Akins 2009, Green et al. 2011, Layman and Allgeier 2012). The lionfish diet in the Pacific, like in the invaded range, is composed mainly by crustaceans and fishes (Anton *unpublished results*) but the specific families or species have not yet been determined and from now on prey fish will be referred to as potential prey fish. The third factor was lionfish presence/absence that included 2 levels: lionfish presence in the cage and no lionfish as control treatment. We used different newly collected lionfish and potential prey fishes in each replicate (one per day). Neither lionfish nor prey species size by family (measured as total length; TL) varied significantly across location (Caribbean/Pacific), as indicated by independent t-test analysis ($p=0.744$ and $n=7$ for lionfish, $p\text{-value}=0.143$ and $n=7$ for Pomacentridae, $p=0.053$ and $n=7$ for Scaridae, and $p=0.067$ and $n=4$ for Labridae), and therefore, neither factor was included as a covariate in subsequent analyses.

Videos were taken of the cage and its fishes using an underwater Sony Digital Handycam DCR-PC101 MiniDV camera (Sony Corporation of America, New York, USA). Videos were taken for three min per species of prey fish in each trial. After starting the recording, researchers left the area to minimize any possible disruption of natural fish behavior.

Lionfish and the three small fish were given one min to acclimate to the cage before the video recording began. Fixed images were extracted from each video every ten sec for the first two min for a total of 12 images per treatment to document the behavior of potential prey fish.

Prey avoidance was quantified using two metrics (Table 1). Approach distance to a predator is considered one reasonable measure of predator avoidance (Arai et al. 2007). The closest approach distance was the minimum distance to the lionfish that any of the three potential prey individuals expressed during the trial. We hypothesized that the shorter the predator-prey distance, the harder for prey to avoid the predator in an attack (Dugatkin and Godin 1992a, Mahjoub et al. 2008). In order to be able to compare closest approach distance in the control cages and the cages containing lionfish, we measured the minimum distance that any of the prey approached the 1 mm mesh cage divider. This measurement was similar to distance between the small fish and lionfish because they were positioned near the cage divider with their mouth often near it (AA pers. obs.). However, to ensure that distance between small fish and lionfish and small fish and divider were similar, we additionally measured distances from small fish to the mouth of the lionfish in every trial and compared these measurements in a t-test. We found no significant differences between the minimum distance from small fish to lionfish mouth and the distance from the small fish to the cage divider ($p=0.118$, $n=36$), and from now on distance between small fish and cage divider will be referred to as distance to predator. A total of three measurements of predator-prey distance were measured per image, which resulted in 36 measurements per replicate. The shortest of these distances was selected as the closest approach distance. Images from videos were extracted using

Adobe Premiere Elements 10 (Adobe Systems Incorporated, San Jose, USA) and distances between prey, predator and cage divider were calculated using ImageJ (Abramoff et al. 2004).

The second metric to quantify lionfish avoidance behavior was the number of predator inspections, which was the number of times one or more prey fish cautiously approached the lionfish, inspected it, and then quickly retreated (Rehage et al. 2005). Number of predator inspections was calculated by watching each 3 minute video. We hypothesized that more inspections would indicate that a fish is less fearful of the predator (Dugatkin and Godin 1992b). For both experiments, each replicate was run on a separate day between 11:00-15:00 h (n=7 per experiment, with the exception of the family Labridae, which had only 4 replicates in the Pacific).

Field observations of free-ranging fish

To assess behavioral responses of free-swimming lionfish and small fish, fishes were observed in shallow reefs in Guam and Abaco. Lionfish were chosen as encountered while slowly snorkeling over shallow reefs at 1-10 m depth on Abaco (the Bight 26° 20' 43" N, 77° 01' 21" W and Sandy Point 25° 59' 51" N, 77° 24' 12" W) and Guam (Pago Bay 13° 25' 36" N, 144° 47' 56" E, Tumon Bay 13° 30' 43" N, 144° 48' 07" E, Togcha Bay 13° 21' 42" N, 144° 46' 48" E and Bile Bay 13° 16' 1" N, 144° 40' 3" E) from 9:00-16:00 h. After a few minutes observing the lionfish and smaller fish in its vicinity to ensure that they appeared unaffected by the presence of the snorkeler, the distance between individual lionfish and individual small fish around them was quantified through spot-check observations conducted by one observer. Per individual lionfish, several

observations were conducted for three min. The snorkeler was always at least 2.5 m away from all fishes observed, a distance previously considered reasonable to assess lionfish behavior in the field (Cure et al. 2012, Anton et al. 2013). For each lionfish, the observer haphazardly selected one small fish <5 cm TL, within 60 cm of and approaching the lionfish, observed its movements, and recorded the distance at which it turned away from or stopped approaching the lionfish as an estimate of closest approach distance, analogous to our experimental protocol. The distance between the mouth of the small fish (potential prey) and the mouth of the lionfish was visually estimated for each individual potential prey and lionfish with the help of an underwater slate. Distance from potential prey to lionfish was categorized to one of eight classes (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-60 cm). We observed 19 lionfish in Abaco and 12 in Guam. Thirteen and seven prey species were observed around lionfish in Abaco and Guam respectively (Table S3.1). Within the three min period, 7 individual prey fish on average were sequentially observed per focal lionfish. The observer remained as still as possible to minimize any influence on fish behavior. All small fish were identified to the species level.

Statistical analyses

In field experiments, to determine statistical differences in closest approach distance a randomized blocked design was used with invasive/native range, presence/absence of lionfish, and potential prey family as fixed factors and prey ID as random factor. A block design was needed because the same set of three potential prey fish was sequentially exposed to an empty cage and then to a lionfish. Predator inspections were not assessed

in the control cages because there was no fish to observe and the potential prey did not perform predator inspections. A fixed effects model was used to assess statistical significance of any of the differences in the number of times prey fish inspected lionfish using native and invaded range and potential prey fish family as fixed factors. Closest approach distance was fitted a normal distribution because it was a continuous variable and predator inspection was best fitted a negative binomial distribution, as indicated by AIC and the examination of model residuals. For both metrics of predator avoidance, closest approach distance to lionfish and number of inspections, a stepwise model simplification approach was used to find the best model (Crawley 2007). When a fixed factor was not significant, as determined by likelihood ratio test and Akaike's Information Criterion (AIC), it was excluded from the model following the principle of parsimony (Crawley 2007).

To examine how closely a small fish approached lionfish in the two geographical locations while free-ranging in the field, a randomized blocked design was used. Only individual observations from potential prey species that were observed interacting with lionfish in the native and invaded ranges at least 3 times were included in the statistical analyses (Table S1). The mixed-effects model for the field observations included lionfish total length (TL), potential prey family (Pomacentridae/Labridae), and location (Caribbean/Pacific) as fixed factors with all interactions and lionfish ID as a random factor because multiple observations were conducted on each individual lionfish. Minimum distance between the potential prey fish and lionfish was the dependent variable, which was fitted to a normal distribution. We again followed stepwise model simplification approach to find the best model to explain distance to lionfish (Crawley

2007). From the best model's regression table of coefficients, we identified any significant differences in the dependent variables. All statistical tests were performed with R version 2.15.2 (R project for Statistical Computing, <http://www.r-project.org>) using the nlme (Pinheiro et al. 2012) and the MASS (Venables and Ripley 2002) packages. Statistical significance was characterized by p-values less than 0.05.

RESULTS

Field experiment

The mean closest approach distance by small fishes from lionfish was 43% shorter in the Caribbean than in the Pacific and around 50% shorter in the absence of lionfish than in its presence (Figure 3.1). We found no significant effect of prey family or interaction between prey family and location and prey family and lionfish presence/absence; and these factors were excluded from the final model. A significant interaction between location and lionfish presence/absence was found and resulted from the closest approach distance by potential prey being greater in the Pacific than the Caribbean (Figure 3.1). We found an effect of location and small family on the number of times the small fish inspected lionfish and their effects did not exhibited a significant interaction. Twice as many fish inspections occurred in the Caribbean per unit of time than in the Pacific. The average rate of predator inspections was similar between Pomacentridae and Scaridae but was around four times larger for the Labridae family (Figure 3.2).

Field observations on free-ranging fishes

Location affected the distance potential prey fish maintained from lionfish (Figure 3.3, Table 3.2). Potential prey fish revealed a 27% closer approach distances to lionfish in the Caribbean than in the Pacific (Figure 3.3). We found no effect of fish family and the interaction between biogeographic region and fish family was not significant (Figure 3.3).

DISCUSSION

Our experiments suggest that small individuals of potential prey fish from three families (Pomacentridae, Labriade and Scaridae) of fish exhibit prey naiveté to lionfish in the invaded range. Potential prey in the Pacific alter their behavior in presence of lionfish by maintaining a larger distance from the side of the cage containing the lionfish than from the empty cage whereas no behavioral change was detected when potential prey fish of the same families were exposed to lionfish in the Caribbean (Figure 3.1). Our field observations of free-ranging lionfish further support limited anti-predator behavior of potential prey fish to lionfish by two fish families, Pomacentridae and Labridae in the Caribbean, since potential prey fish made closer minimum approaches to lionfish in the Caribbean than in the Pacific (Figure 3.3). Coral reef fish are generally able to visually recognize their predators and display behavioral anti-predator responses (McCormick and Manassa 2008) and they can also differentiate predators from non-threatening species (Coates 1980). Our observations agree with previous sets of experimental and observational data on prey fish from the Caribbean that maintained greater minimum distances from native predators than from invasive lionfish (Anton et al. 2013). The closest approach distance metric presumes that shorter separation distance from a

predator implies higher risk of predation for small prey fish (Arai et al. 2007, Takahara and Yamaoka 2009, Kelley and Magurran 2003), since capture success of predators has been suggested to be greater the closer the predator are to the prey (Dugatkin and Godin 1992a). For instance, the feeding success of the larvae of European whitefish (*Coregonus lavaretus*) on zooplanktonic prey increases the closer the larvae are from their prey before the strike (Mahjoub et al 2008). Our results suggest that prey fish in the Caribbean, by approaching more closely to a novel but dangerous predator than co-evolved small fish approach that same predator in its native range in the Pacific, are engaging in a risky behavior that strongly suggests prey naiveté to lionfish.

Visual inspections of potential predators allow prey to assess predator identity and motivation (Lima and Dill 1990). We found that small prey fish in the Caribbean tended to inspect lionfish more often than they do in the Pacific. Although we found differences across fish families in the number of predator inspections performed on lionfish per unit of time independent of the prey fish family, the rate that potential prey fish inspected lionfish was consistently greater in the Caribbean than in the Atlantic. The understanding predator inspections on invasive species is limited. Predator inspections have been hypothesized to occur less often when prey are naïve to a novel predator, however, this hypothesis has not been yet supported by robust results (Kelley and Marrugan 2003, Rehage et al. 2009). On the other hand, predator inspections represent high-risk approaches to the predator (Smith and Belk 2001) and have been shown to be more frequent when prey inspect less dangerous targets (Dugatkin and Godin 1992b). In our study small prey fish consistently inspected lionfish more often in the Caribbean than in the Pacific. These results suggest that lionfish were perceived as a low-risk species by

prey in the Caribbean, providing further support of the conclusion that prey are naïve to lionfish in the invaded range.

The lack of appropriate anti-predator behavior to lionfish can be a consequence of the lack of exposure to lionfish in the Caribbean on an evolutionary time scale. This failure to recognize lionfish as predator to the same degree as small fish in the native range respond to lionfish presence supports the prey naiveté hypothesis (Cox and Lima 2006): when facing a novel source of predation risk, anti-predator behavior may not always be elicited (Banks and Dickman 2007). The prey naiveté hypothesis (Cox and Lima 2006) suggests that predator archetypes, that is a set of predator species that have similar morphological adaptations and/or use similar behavior to capture prey, might be determined at the family level. Although the lionfish family Scorpaenidae is represented in the Caribbean by five species, they differ from lionfish in obvious morphological traits, thus representing a potentially different predator archetype (Cox and Lima 2006). Unlike any native species of Caribbean Scorpaenidae, lionfish have conspicuous white and red stripes that could function as aposomatic coloration (Hofreiter and Schoneberg 2010) and/or disruptive-cryptic coloration (Albins and Hixon 2011). Regardless of the function of lionfish coloration and pattern, it may represent a novel trait among predatory fish in the Caribbean that could limit the capacity of prey to recognize lionfish as a predation risk. Moreover, invasive lionfish have large and feathery pectoral fins that are absent in other species of Scorpaenidae in the Caribbean. These morphological traits make the visual appearance of lionfish different from the Scorpaenidae species in the Caribbean but further investigation is needed to determine the specific morphological traits that drive predator recognition by prey fish in the Caribbean.

Invasive predators can exert especially large impacts on native prey populations (Dorcas et al. 2012) and are more dangerous than native predators to prey populations since they can have twice the effect on prey than native predators (Salo et al. 2011). On reefs in the Bahamas, lionfish have been shown to have significant impact on reef-fish populations (Albins and Hixon 2008, Green et al. 2012, Albins 2013). Prey naiveté has been suspected to be a key mechanism in the invasion success and proliferation of non-native predator (Fritts and Rodda 1998) but has been rigorously tested in only a few cases (Barrio et al. 2010, Kuehne and Olden 2012). This paper suggests that prey naiveté towards invasive lionfish in the Caribbean may contribute to the severity of impacts of the lionfish invasion of the Western Atlantic.

Table 3.1. Metrics of predator avoidance recorded in the field experiment to assess predator recognition and prey responses to lionfish.

Prey avoidance behavior	Defined as	Method	Units
Closest approach distance	Closest distance between the prey and the predator	Measurements from images from videos	mm
Predator inspections	Cautious approaches by one or a few members of the school toward the predator while being visually fixated on it followed by a quick retreat	Counts from continuous examination of videos	number of inspections per 3 min

Table 3.2. Summary of the best regression model for metrics of anti-predator response of potential prey fish to lionfish in field experiments and observations in Guam (Pacific Ocean) and Abaco Island (Caribbean Basin) . Non-significant predictors were excluded from each model and are not reported here (see Methods-Statistical analysis). Bolding indicates significant p-values.

Metrics of predator avoidance			
Field experiments			
Closest approach distance			
Predictors	DF	F value	p
Location (Caribbean/Pacific)	35	9.86	0.003
Lionfish (Presence/Absence)	28	9.61	0.004
Location*Lionfish interaction	28	6.10	0.019
Predator inspections			
Predictors	DF	z value	p
Location (Caribbean/Pacific)	34		<0.001
Prey Family	32		0.007
Pomacentridae vs Scaridae		0.64	0.517
Pomacentridae vs Labridae		2.83	0.004
Scaridae vs Labridae		3.14	0.001
Field observations			
Closest approach distance			
Predictors	DF	F value	p
Location (Caribbean/Pacific)	21	12.86	0.001

Figure 3.1. Box plot of the closest approach distance by potential prey in the presence and absence of lionfish within their native range in Guam (Pacific Ocean) and the invaded range in the Abaco Island (Caribbean Basin) from field experiments. Tan bars and dark grey bars represent absence and presence of lionfish respectively. Circles denote mean values, lines are median values, the box represents the middle 50% of scores with the upper and lower quantiles representing 75% and 25% of the scores respectively, and the upper and lower whiskers represent scores outside the middle 50%. Statistical results are reported in Table 3.2.

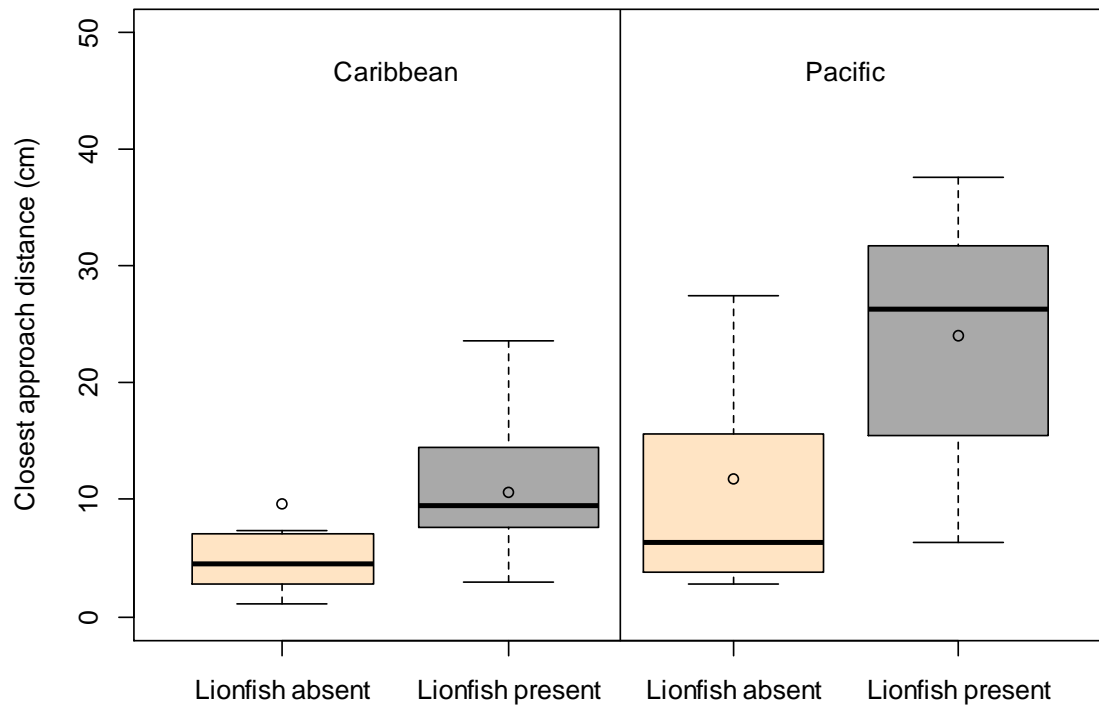


Figure 3.2. Box plot of the number of lionfish inspections per 3 min observation period by members of three families of prey fish in Guam (Pacific Ocean represented in dark grey bar) and Abaco Island (Caribbean Basin represented in tan bar) from field experiments. Circles denote mean values, lines are median values, the box represents the middle 50% of scores with the upper and lower quantiles representing 75% and 25% of the scores respectively, and the upper and lower whiskers represent scores outside the middle 50%. Statistical results are reported in Table 3.2.

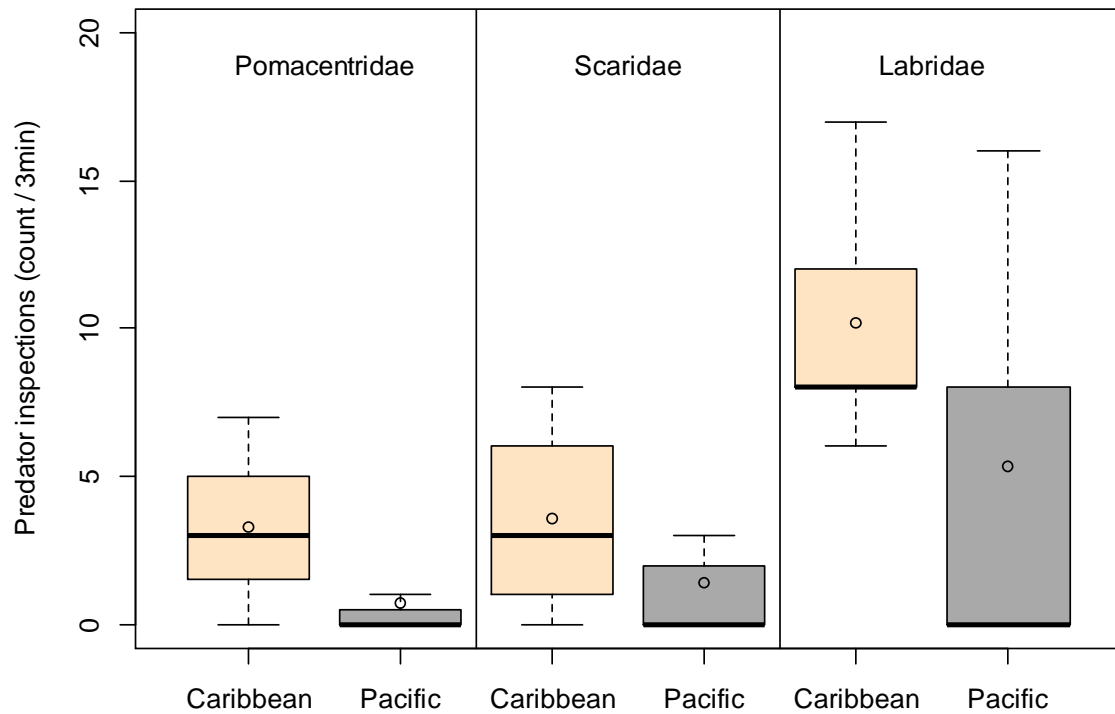


Figure 3.3. Box plot of the closest approach distance to lionfish by members of two families of potential prey fish in Guam (Pacific Ocean represented in dark grey bar) and Abaco Island (Caribbean Basin represented in pale tan bar) during field observations. Circles denote mean values, lines are median values, the box represents the middle 50% of scores with the upper and lower quantiles representing 75% and 25% of the scores respectively, and the upper and lower whiskers represent scores outside the middle 50%. Statistical results are reported in Table 3.2.

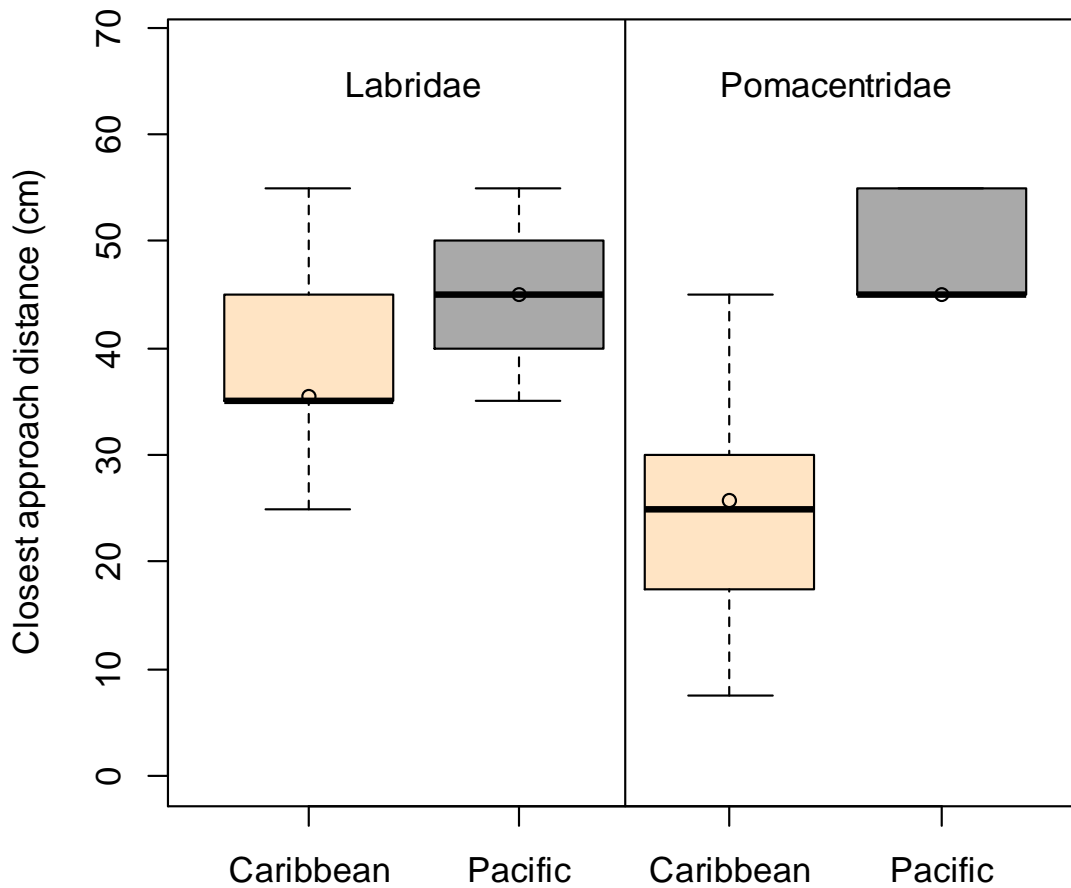


Table 3.S1. List of all prey fish that approached free-ranging lionfish while observed in the field in Guam (Pacific Ocean) and Abaco Island (Caribbean Basin). Small fish species included in the statistical analysis are bolded.

Observed species common name	Observed species scientific name	Family
Abaco (Caribbean; lionfish invaded range)		
Beaugregory damselfish	<i>Stegastes leucostictus</i>	Pomacentridae
Longfin damselfish	<i>Stegastes diencaeus</i>	Pomacentridae
Sergeant major	<i>Abudefduf saxatilis</i>	Pomacentridae
Blue tang	<i>Acanthurus coeruleus</i>	Acanthuridae
Princess parrotfish	<i>Scarus taeniopterus</i>	Scaridae
Red band parrotfish	<i>Sparisoma aurofrenatum</i>	Scaridae
French grunt	<i>Haemulon flavolineatum</i>	Haemulidae
Tomtate	<i>Haemulon aurolineatum</i>	Haemulidae
White grunt	<i>Haemulon plumieri</i>	Haemulidae
Slipery dick wrasse	<i>Halichoeres bivittatus</i>	Labridae
Yellowhead wrasse	<i>Halichoeres garnoti</i>	Labridae
Bluehead wrasse	<i>Halichoeres bifasciatum</i>	Labridae
Caribbean sharpnose puffer	<i>Acanthigaster rostrata</i>	Tetraodontidae
Guam (Pacific; lionfish native range)		
Spotted toby	<i>Canthigaster solandri</i>	Tetraodontidae
Green chromis	<i>Chromis viridis</i>	Pomacentridae
Pale-tail chromis	<i>Chromis xanthura</i>	Pomacentridae
Blue devil	<i>Chrysiptera cyanea</i>	Pomacentridae
Tracey's demoiselle	<i>Chrysiptera traceyi</i>	Pomacentridae
Blue-steak cleaner wrasse	<i>Labroides dimidiatus</i>	Labridae
Ocellate damselfish	<i>Pomacentrus vaiuli</i>	Pomacentridae

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CHAPTER 4

Effects of biotic and abiotic factors on the early lionfish invasion of Belizean coral reefs and its impacts

ABSTRACT

Lionfish (*Pterois volitans*), a venomous predator from the Indo-Pacific, are the first marine fish to successfully invade the Caribbean. The direct and indirect effects of lionfish on marine ecosystems in the Caribbean are of great concern for conservation. Quantification of fish community abundance and composition before and after (2009 and 2011) lionfish were first detected on 15 coral reef sites in the Belizean Mesoamerican Barrier Reef permitted an assessment of: (1) the factors influencing their early invasion success, including marine protection status and wave exposure of the reefs; and (2) the short-term impact of the invasion on reef-fish communities. Lionfish abundance (density and biomass) was negatively related to large grouper biomass, wave exposure, and marine protection from reef fishing. Because these three predictors were correlated with each other, we could not determine each independent effect on lionfish abundance, however, insights from our most northern sites suggests that high-energy of exposed environments might be the dominant determinant of the lionfish density pattern in Belize. We also found a negative effect of lionfish abundance on the change in small reef-fish abundance and community composition: these effects were mainly driven by a single abundant species, sharpnose puffer (*Canthigaster rostrata*). Our results on lionfish impacts are novel in two ways: first, they show changes in the fish community abundance and composition only 2 years after first lionfish detection on coral

reefs and second they show that the impacts of lionfish on reef-fish communities are detectable at large spatial scales.

INTRODUCTION

Invasive species are a global problem and negatively affect the ecology of native communities. The impact of the invasive species depends on the susceptibility of recipient communities to invasion and proliferation of invasive species. The invasive species must be able to physiologically tolerate the physical environment: harsh environments such as deserts have been shown to be the least invaded worldwide (Lonsdale 1999), perhaps because the suite of non-native species pre-adapted to those extreme environmental conditions is limited. Alternatively, when environmental conditions are tolerable, biotic resistance, which stems from community diversity (Stachowicz et al. 1999) or from the effects of strong local enemies (deRivera et al. 2005), may alone inhibit local invasion success (Elton 1958). For instance, the native blue crab (*Callinectes sapidus*) provides biotic resistance against invasion by green crabs (*Carcinus maenas*) through direct predation in eastern North America (deRivera et al. 2005).

If the native community and its physical habitat are unable to provide enough biotic and /or environmental resistance to newly arrived exotic species, ecological effects on the recipient community can be devastating. Damaging invasions have resulted from exotic predators depleting local prey populations (Salo et al. 2007). For instance, the accidental introduction of the brown tree snake (*Boiga irregularis*) on the island of Guam has resulted in the extinction of numerous species of birds, mammals, and reptiles (Fritts and Rodda 1998). Likewise, the introduction of the Burmese python, native to Asia, in South Florida has driven

populations of multiple species to the brink of local extirpation (Dorcas et al. 2012). While introductions of predatory freshwater fishes like the Nile perch (*Lates niloticus*) in Lake Victoria had devastating effects on native communities (Witte et al. 1992), the impacts of marine invasive fish are largely unknown. Red lionfish (*Pterois volitans*), a native invasive predator from the Indo-Pacific has spread throughout marine ecosystems in the Caribbean in less than a decade (Schofield 2010). Lionfish have the potential to alter reef-fish communities dramatically. For example, using artificial experimental patch reefs, Albins and Hixon (2008) found that invasive lionfish can cause up to 80% reductions in the recruitment of native Caribbean fishes. Similarly, lionfish consumed 65% of the biomass of known prey fishes around a small island in the Bahamas (Green et al. 2012). However, it is unknown if similar impacts occur over larger scales (>100 kms) on natural coral reefs.

In order to rigorously attribute changes in communities to ecological perturbations, such as species invasions, data are best collected before and after the impact (Anton et al. 2009, Geraldi et al. 2009). By comparing pre- and post-invasion community information, changes in community composition and structure after the invasion can be assessed. We performed reef-fish surveys at 15 sites located along the Belizean Mesoamerican Barrier Reef (Fig. 4.1), the second largest barrier reef in the world, to answer two independent ecological questions related to the lionfish invasion of Caribbean coral reefs. First, we tested whether potentially important physical and biological factors influencing the early stages of the lionfish invasion. We operationally defined early invasion success as either lionfish density or biomass at initial stages of the invasion. Because biological factors related to fish abundance and composition were assessed before the invasion, we evaluated how these factors affect

lionfish invasion success. Second, sites were surveyed before and two years after the lionfish invasion to assess the short-term impact of this exotic predator on the reef-fish community.

METHODS

Field Sampling

We conducted field surveys at 15 coral reefs in the Belizean Mesoamerican Barrier Reef in May-June 2009 and in May 2011 (Fig. 4.1). The reefs had a spur and groove formation and we surveyed 5 no-take zones (hereafter referred to as marine parks) and 10 control sites (where fishing is allowed) at depths from 12-17 m. Five lionfish sightings were reported in Belize between December 2008 and May 2009 at locations different from our 15 study sites (Schofield 2010), but we did not encounter a lionfish in our surveys in the summer of 2009. Replicate (3-6) transects 50 m long were deployed on the spurs to survey fish abundances at each site. Transects were run parallel to the spur and groove formation and conducted between 9:00-16:00 h. On each transect, 2 divers working together but on different sections along the transect followed a sequence of sampling protocols. We quantified lionfish and large (> 30 cm in total length, TL) native predatory fish abundances by species (listed in Table S1) and estimated TL of each individual within a 500 m^2 ($50 \times 10 \text{ m}$) area along the transect. Care was taken to include searches for lionfish within cryptic habitats to avoid underestimating their density (Green et al. 2013). We counted potentially suitable prey fishes less than 5 cm total length (TL), termed small fishes, in a 15 m^2 ($15 \times 1 \text{ m}$) area. In addition, we counted potentially suitable prey fishes of 5-10 cm TL, termed medium fishes, in a 60 m^2 ($30 \times 2 \text{ m}$) area. Large native predatory fishes were surveyed in 2011. To compare our findings with

previous correlations between grouper and lionfish (Mumby et al. 2011), densities of each species were transformed to biomass and then their biomass was summed by taxon. The density of individuals larger than 30 cm TL for four grouper species (see Table S4.1) were converted to mass using allometric scaling relationships with body length from Marks and Klomp (2003). Lionfish lengths in cm were converted to biomass (g) using allometric scaling parameters $a=0.00492$ and $b=3.31016$ (Anton et al. 2013).

We estimated wave exposure using a map of the Belizean Barrier Reef originally created by Chollett and Mumby (2012) that represents the wave exposure (in J m^{-3}) calculated using fetch and both wind speed and wind direction obtained from QuikSCAT satellite scatterometer data from 1999 to 2008 (within a range of wave exposure of 0.3 - 0.9 J m^{-3}). We georeferenced that map using ArcGIS and overlaid our study sites on top (Fig. S4.1). We then visually estimated the wave exposure for each of our study sites and categorized them into one of three classes: low, medium, or high wave exposure.

Statistical analyses

We assessed the effect of year (2009 and 2011) on lionfish abundance (density and biomass) to document the arrival and increase of lionfish abundance in Belizean coral reefs. To determine potentially important physical and biological factors influencing the early success of the lionfish invasion, we employed generalized linear fixed effects models to assess the independent effects of wave exposure, protection of the marine parks, and large grouper biomass on the two lionfish abundance variables (lionfish density and biomass). Lionfish density and biomass (dependent variables) were best fitted by a zero-inflated negative binomial (ZINB) distribution because these response variables contained more zeros than

expected by chance based on a negative binomial distribution (Zuur et al. 2009). Large predatory fish density, large grouper biomass, small fish density, and medium fish density are biotic variables that can potentially influence lionfish density through bottom-up (food provision) and/or top-down (predation) effects. In order to elucidate the effects of wave exposure and protection from fishing on these biological variables (large predatory fish density, large grouper biomass, small fish density, and medium fish density), we employed similar generalized fixed effects models where these biotic factors were included in each model as dependent variables. Small and medium fish densities, large grouper biomass and large predator density were best fitted by normal distributions in generalized linear models as determined by Akaike's Information Criterion (AIC) and visual observation of the model residuals.

To determine the early impacts (< 2 years) of lionfish on the fish community, we performed two types of analyses. First, using two independent generalized linear models we examined the relationship between lionfish abundance in 2011 (as independent variable) and the change in small and medium fish abundance between 2009 and 2011 (each as dependent variables).

The most abundant small fish species on the Belizean reefs in 2009 and 2011 was the sharpnose puffer (*Canthigaster rostrata*) and its abundance could have an effect on small fish abundance change across years. Hence, we performed an additional generalized linear model to examine the relationship between lionfish abundance in 2011 and the change in the small fish abundance (excluding sharpnose puffer) between 2009 and 2011.

Second, we used n-MDS ordination plots in combination with PERMANOVA analyses to display, quantify, and test statistical significance of changes on patterns of fish community composition at the study sites (Kruskal 1964). The effect of lionfish abundance (in 2011) on

reef-fish fish community composition change from 2009 to 2011 was tested using PERMANOVA (Anderson 2005) and illustrated by n-MDS plots. We subtracted fish species abundance in 2011 from the abundance in 2009 to calculate change in each fish species within the small and medium fish communities. To determine the relative impacts of the common vs. rare species within each reef-fish community, PERMANOVAs were performed with raw data and three types of transformations (square root, fourth root, and $\log(X+1)$): PERMANOVA analysis with raw data is primarily influenced by common species, square root and fourth root transformation reduces the influence of common species and $\log(X+1)$ transformation is more equally influenced by both common and rare species. The small and medium fish community change (between 2009 and 2011) data sets were then converted to a resemblance matrix to depict for the compositional dissimilarity between pairs of sites using Bray-Curtis transformation (Clarke and Gorley 2006). PERMANOVAs were run for each fish category (small and medium fish community) as a continuous dependent variable, lionfish density in 2011 as categorical (lionfish presence/absence) independent variable, and site as a categorical random factor, using 999 unrestricted permutations. In addition, if the results from the PERMANOVA were significant, we used a similarity of percentage (SIMPER) analysis to determine which species within the fish community were driving the Bray-Curtis dissimilarity across study sites taking into account lionfish density. For the SIMPER analysis, lionfish density in 2011 was classified by category: present vs. absent. SIMPER analysis was performed to identify the most sensitive fish species to lionfish presence. A full description of SIMPER analysis is provided in Clarke (1993). To georeference the Chollet and Mumby (2012) map, we used ArcGIS 10. n-MDS plots, PERMANOVAs, and SIMPER analyses were performed with PRIMER 6, whereas the other

statistical analyses were conducted using R version 2.15.2 (R project for Statistical Computing, <http://www.r-project.org>) with MASS (Venables and Ripley 2002) and pscl (Zeileis et al. 2008) packages.

RESULTS

Lionfish abundance increased from 2009 to 2011 on the Belizean Barrier reef (Fig. 2).

Lionfish density and biomass were zero in 2009 and ranged from 0 to 60 individuals ha^{-1} (average of 19 individuals per ha^{-1}) and from 0 to 210 g 100 m^{-2} (average of 60 g per 100 m^{-2}) respectively in 2011 (Fig. 4.2). Lionfish density and biomass in 2011 were affected by wave exposure (Fig. 4.3), such that high wave exposure environments had 2.8 lionfish ha^{-1} and low and medium wave exposure environments had on average ~ 25 lionfish ha^{-1} .

Similarly, lionfish abundances were higher outside of the marine parks: the average lionfish density and biomass were on average 6 and 9 times higher outside of the parks than within the marine parks (Fig. 4.3). Large predator densities were statistically similar across the three wave exposure categories and two levels of marine protections (Fig. 4.3). The biomass of a subset of large groupers (the four bolded species in Table S4.1) was not detectably related to wave exposure, but the average biomass of large groupers was more than 7 times higher inside the marine parks than in the control sites where fishing is allowed (Fig. 4.3). Lionfish abundance (density and biomass) was negatively associated with biomass of large groupers (Fig. 4.4).

Densities of small and medium fishes in 2009 (before lionfish arrival to the reefs) were similar across wave exposure environments and marine protection when data from all sites were pooled (Fig. 4.5). In contrast, when we examined changes in small fish abundances

from 2009 to 2011 site by site, there was a significant association between lionfish abundance and change in small fish abundance (Fig. 4.6). A similar analysis excluding sharpnose puffer data failed to demonstrate a significant effect of lionfish abundance in 2011 on the difference in small fish abundance between 2009 and 2011 (Fig. S4.2). The association between lionfish abundance and change in medium fish abundance between 2009 and 2011 failed to show a significant effect (Fig. 4.6).

Multivariate n-MDS ordinations and associated PERMANOVAs assessed whether the small and medium fish communities changes between 2009 and 2011 related to lionfish presence/absence. The PERMANOVA statistical analyses indicate that the small fish community change was affected by lionfish presence (Fig. 4.7) for three out of the four transformations used in the PERMANOVAs (Table 1). The SIMPER analysis of the small fish community revealed that eight species of reef-fish (Table S4.2) contributed to ~90% of the dissimilarity in the small fish community between 2009 and 2011. The SIMPER analysis revealed that the sharpnose puffer (*Canthigaster rostrata*) accounted for almost half of the community dissimilarity and that the difference in sharpnose puffer abundance between 2009 and 2001 was negatively correlated to lionfish abundance in 2011 (Table S4.2). To test for the influence of sharpnose puffer on the change in the small reef-fish community dissimilarity, we performed PERMANOVA analysis of the reef-fish community without sharpnose puffer. The results of the PERMANOVA show that the change in small reef-fish community was not statistically dissimilar where lionfish were present as compared to where lionfish were absent in 2011 (Table S4.3). These results were also evident in the n-MDS plot (Fig. S4.3). Finally, the change in the community composition of medium fish between 2009 and 2011 was unrelated to lionfish abundance presence/absence (Fig. 4.7).

DISCUSSION

Lionfish abundance (density and biomass) was negatively associated with biomass of large groupers and the presence of marine parks. These patterns agree with those of Mumby et al. (2011), who found lower lionfish biomass within the Exuma Cays Land and Sea Park (ECLSP) than in neighboring fished sites. They concluded that the lower densities of lionfish within the park were related to the higher abundance of large groupers, presumably by depressing lionfish density through predation (Mumby et al. 2011). We similarly found that marine parks had 1/9th of the lionfish biomass ($\sim 9 \text{ g } 100 \text{ m}^{-2}$) found in fished sites ($85 \text{ g } 100 \text{ m}^{-2}$). Mumby et al. (2011) concluded that a biomass of large groupers equal to $800 \text{ g } 100 \text{ m}^{-2}$ was sufficient to suppress lionfish biomass by 50%. We documented an average large grouper biomass of $722 \text{ g } 100 \text{ m}^{-2}$ within the five marine parks in Belize, which based on Mumby's et al. (2011) computations and interpretation should be sufficient to depress lionfish abundance. However, an alternative, and maybe complementary, explanation is that lionfish are effectively removed within the marine reserves by park managers and organized lionfish removals like those led by the Reef Environmental Education Foundation across the Caribbean (REEF, Akins 2012). Efforts to harvest lionfish by divers can result in over 1,400 lionfish collected in one day during derby-style events (Morris and Whitfield 2009). In 2011, REEF divers removed more than 500 lionfish at two Belizean atolls in 6 days (Akins, *personal communication*). None of these removals were performed within the marine parks included in this study; however, if these efforts are routinely performed within Belizean marine parks, it could potentially explain the low lionfish abundances that are documented

within the parks. Hence, reduced lionfish density in marine parks may result from predation from large groupers and/or from lionfish removals.

Wave exposure also had an influence on lionfish abundance (density and biomass) on coral reefs in Belize. Our results agree with a previous study that revealed that wave exposure may inhibit early invasion success of lionfish (Anton et al. 2013). Anton et al. (2013) showed that leeward sites in two islands in the Bahamas had an 11-fold higher density and nearly a 100-fold greater biomass of lionfish than windward sites. Our new Belize study indicates a similar trend where low wave exposure sites had a 10-fold higher density and 17-fold greater biomass of lionfish than sites exposed to high-energy conditions. Other species of fish have previously been shown to be suppressed in abundance by wave exposure (Schmiing et al. 2013) perhaps because the energetic cost of swimming represents a significant physiological barrier to fitness (Bellwood et al. 2002). Lionfish are semi-demersal (live and feed near the bottom), usually resting in crevices or swimming slowly, hovering over the reef (AA pers. obs.). In addition, lionfish hunting strategies such as corralling prey with their pectoral fins are quite sophisticated (Allen and Eschmeyer 1973, Albins and Lyons 2012) and appear to require low wave exposure. Our observations concur with the interpretation by others (Anton et al. 2013) that energetic environments may impose natural resistance to the lionfish invasion, but further process-oriented, mechanistic investigation of the underlying mechanisms determining the effect of water velocity on lionfish feeding behaviour is necessary.

Unfortunately, the three factors (wave exposure, large grouper biomass, and marine protection) that were negatively associated with lionfish abundance were correlated with each other: the five marine parks surveyed harbored a larger biomass of large groupers and

had medium to high wave exposure compared to the ten fished sites. This same pattern of covariance among factors might also apply to the sites surveyed by Mumby et al. (2011): Examination of a map of the Bahamas reveals that the marine park in the Exuma Cays (ECLSP) may be more exposed to high-energy conditions than the control fished sites because of their exposure to oceanic swell and winds. We gain insight into the effects of both wave exposure and large grouper biomass on lionfish abundance from our four most northern sites (two marine park and two control sites), all of which had high wave exposure (Fig. S4.1). The two marine park sites had high large grouper biomass and the two control sites had zero large grouper biomass; however, all four sites had between zero and very low lionfish density, suggesting that high-energy of exposed environments is the dominant determinant of the lionfish density pattern in Belize, not large groupers might ultimately inhibit the establish. Further investigation parsing the effects of wave exposure, marine park protection, lionfish removals, and predation of lionfish by large groupers is still required to understand biotic and environmental resistance to lionfish.

Abundances of small and medium fishes, which are potential prey for lionfish, were not influenced by the intensity of wave exposure in our Belize study, results that agree with previous studies in the Bahamas (Anton et al. 2013). Similarly, small and medium fish abundance did not change with degree of marine protection. These results are surprising because lower abundances of smaller fish would be expected inside of the marine parks than in fished sites (Nagelkerken et al. 2012) because of higher predation rates on prey fish within marine protected areas as compared to fished areas (Micheli et al. 2004, Stallings 2008). We did detect a decrease in the abundance and a change in the composition of the small fish community between 2009 and 2011 related to increasing lionfish density (Fig. 4.7), but these

changes were mainly driven by one fish species, the sharpnose puffer (*Canthigaster rostrata*), which abundance changes were negatively correlated to lionfish abundance. This species of puffer has been identified as lionfish prey in the Caribbean (Morris and Akins 2009) and a negative effect of lionfish on *Canthigaster rostrata* recruitment was detected in an experiment on artificial reefs in the Bahamas (Albins and Hixon 2008). Direct predation is the likely cause of the decline in abundance of this species in the reefs where lionfish became abundant in 2011, however, other factors might have affected the abundance of the sharpnose puffer and further investigation is necessary.

Our results agree with previous studies showing rapid and abrupt impacts of lionfish on the abundances of native small and medium fish 4-6 years after lionfish are first detected (Green et al. 2012). However, our results are novel in two ways: first, they show changes in the fish community abundance and composition only 2 years after first lionfish detection on coral reefs in the Belizean Mesoamerican Reef. Second, the impact of lionfish on reef-fish communities was assessed at a large spatial scale (e.g. hundreds of kilometers) on the second largest barrier reef of the world. Hence, the effects of the lionfish invasion on reef-fish communities are also detectable at large spatial scales and might be already affecting the entire Caribbean region.

Table 4.1. Results of the PERMANOVA analyses of the small and medium fish communities.

Small Fish Community (0-5cm TL)						
Transformation of data	Factor	df	SS	MS	Pseudo-F	P (permanova)
None (Raw)	Lionfish presence/absence	1	21.463	21.463	4.382	0.006
	Residual	13	63.674	4.898		
	Total	14	85.138			
Square root	Lionfish presence/absence	1	5.254	5.254	3.515	0.006
	Residual	13	19.433	1.495		
	Total	14	24.687			
Fourth root	Lionfish presence/absence	1	1.474	1.474	2.677	0.025
	Residual	13	7.159	0.551		
	Total	14	8.633			
Log(X+1)	Lionfish presence/absence	1	0.443	0.443	1.459	0.267
	Residual	13	3.949	0.304		
	Total	14	4.392			
Medium Fish Community (5-10cm TL)						
Transformation of data	Factor	df	SS	MS	Pseudo-F	P (permanova)
None (Raw)	Lionfish presence/absence	1	2.024	2.024	0.495	0.937
	Residual	13	53.154	4.089		
	Total	14	55.177			
Square root	Lionfish presence/absence	1	0.590	0.590	0.440	0.939
	Residual	13	17.437	1.341		
	Total	14	18.027			
Fourth root	Lionfish presence/absence	1	0.146	0.146	0.318	0.952
	Residual	13	5.969	0.459		
	Total	14	6.115			
Log(X+1)	Lionfish presence/absence	1	0.049	0.049	0.211	0.960
	Residual	13	3.007	0.231		
	Total	14	3.056			

Figure 4.1. Map of the 15 study sites along the Belize Barrier reef surveyed in 2009 and 2011. Triangles denote no-take zones (marine parks) and circles denote fished sites.



Figure 4. 2. Box plots of the effect of year on lionfish abundance (density and biomass). Bars and triangles indicate the median and mean per treatment respectively. The box represents the middle 50% of scores with the upper and lower quantiles representing 75% and 25% of the scores respectively, and the upper and lower whiskers represent scores outside the middle 50%. Outliers were omitted. Differing lower case letters denote significant differences across treatments obtained from the regression table of coefficients associated to the generalized fixed effects models.

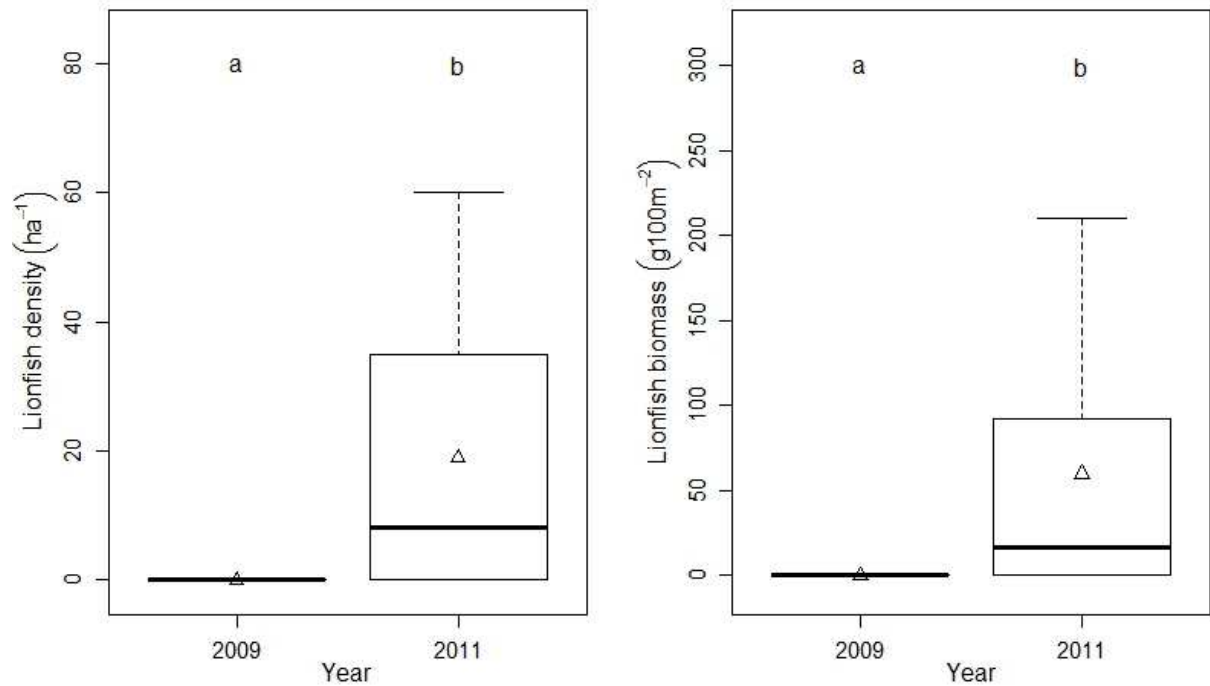


Figure 4.3. Box plots of the effects of wave exposure (right column) and category of marine protection (left column) on lionfish density, lionfish biomass, large grouper biomass (of the four bolded species in Table S1) and large predator density. Bars and triangles indicate the median and mean per treatment respectively. See detailed explanation of box plot representation on the legend in Fig. 2. Outliers were omitted. NTZ denotes no-take zone. Differing lower case letters denote significant differences across treatments obtained from the regression table of coefficients associated to the generalized fixed effects models.

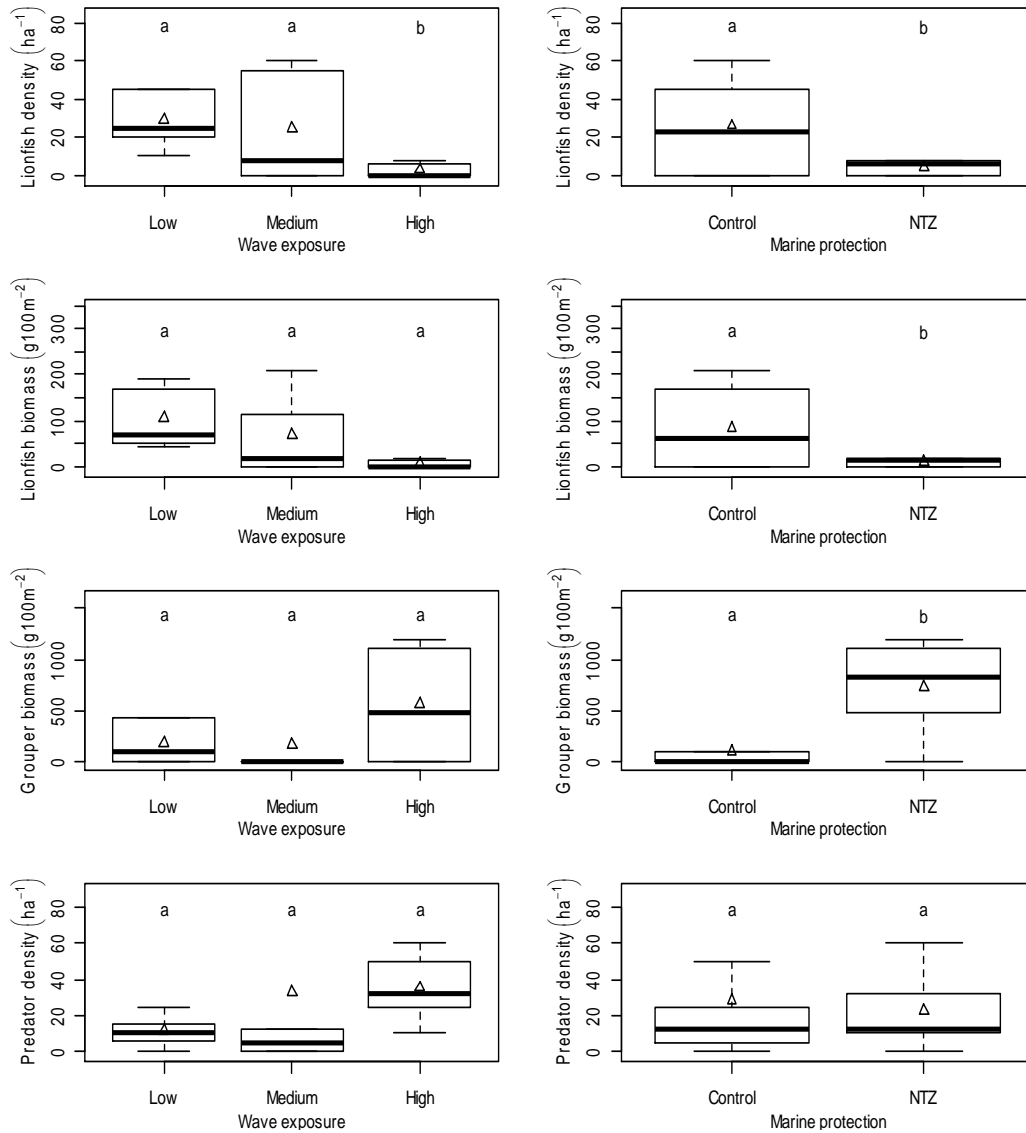


Figure 4.4. Scatter plot of large grouper biomass compared with density and biomass of lionfish. Dashed line indicates significant results from generalized linear models at p-values < 0.05.

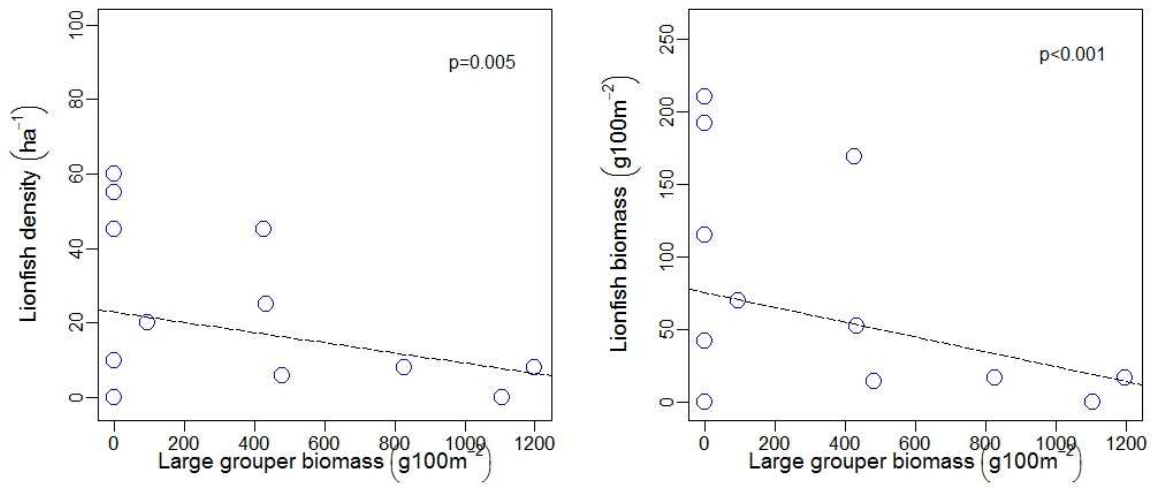


Figure 4.5. Box plots of the effects of wave exposure and marine protection on small and medium fish densities. Bars and triangles indicate the median and mean per treatment respectively. Outliers were omitted. NTZ denotes no-take zone. Differing lower case letters denote significant difference across treatments obtained from the regression table of coefficients associated to the generalized fixed effects models.

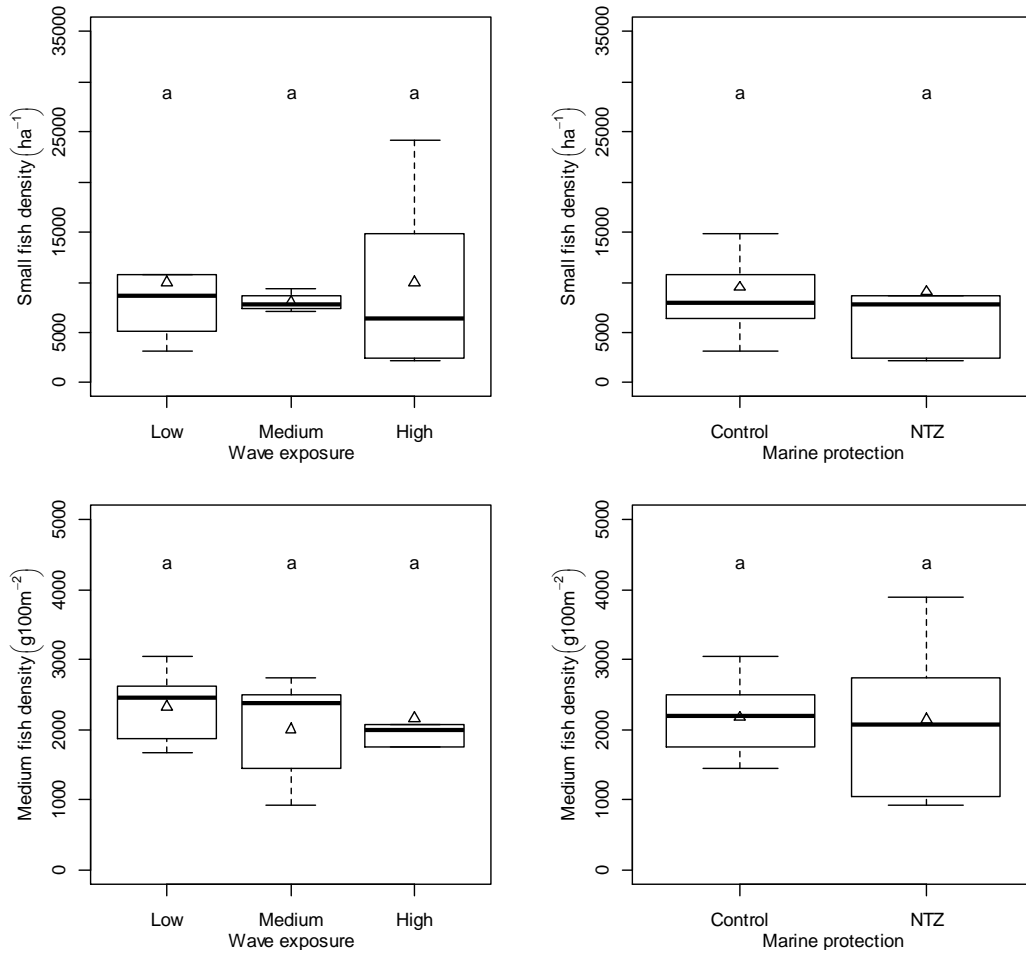


Figure 4.6. The change in average abundance of small and medium fish from 2009 to 2011 for each site compared to lionfish density. Y-values above and below the origin line indicate positive and negative changes in fish abundance on each study site. Dashed line indicates significant results from generalized linear models at p-values < 0.05.

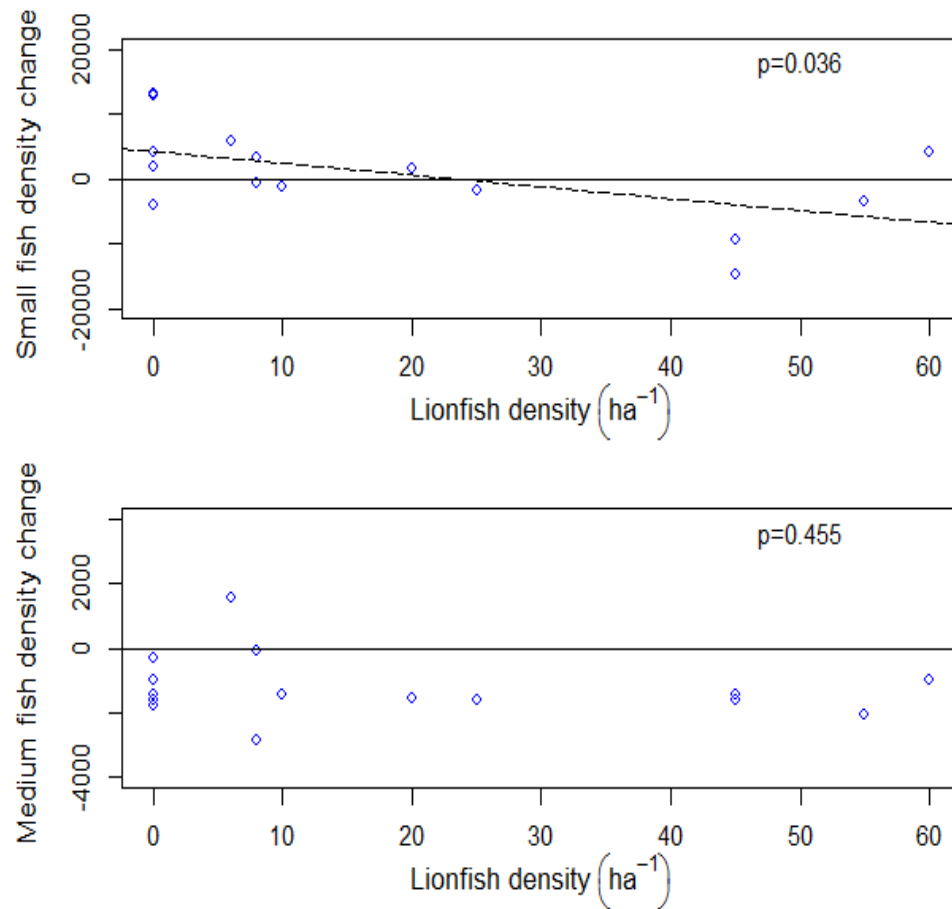
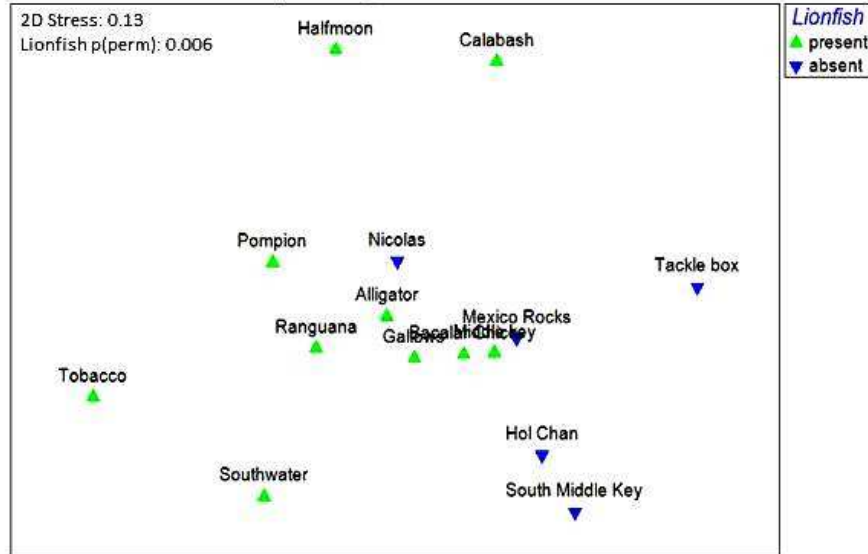


Figure 4.7. n-MDS plots depicting the composition of small and medium fish communities in 2009 and 2011. The size of the bubbles indicates lionfish abundance. Stress values lower than 0.2 reflect a good agreement between the distances depicted in the graph and the fish community dissimilarities among sites. The axis on n-MDS plots serve as an arbitrary coordination system (Kruskal and Wish 1978). p-values were obtained from the PERMANOVA analysis.

Small fish community change



Medium fish community change

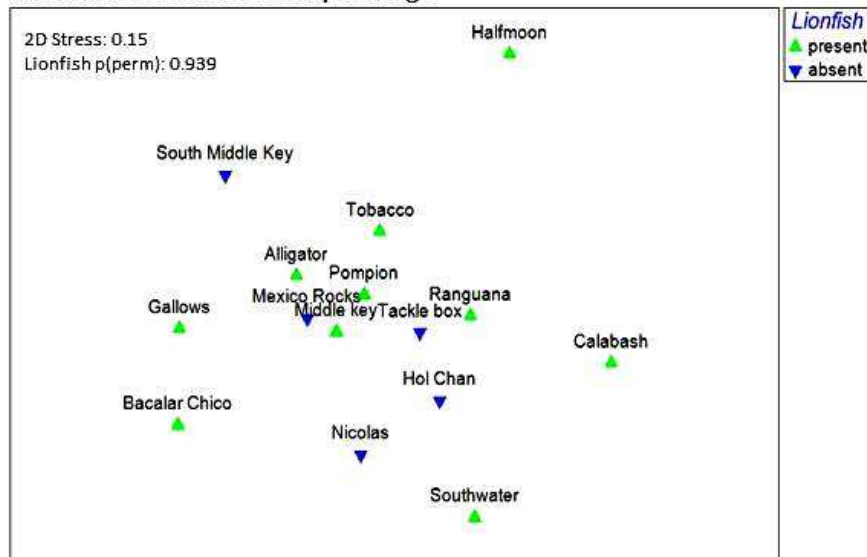


Table 4.S1. List of the potential lionfish predators. These species comprised the large native predatory fish category. The subset of grouper species of which biomass was calculated have been bolded.

Large predator species (>30cm total length)	
Common name	Scientific name
<i>Groupers</i>	
Red hind	<i>Epinephelus guttatus</i>
Nassau grouper	<i>Epinephelus striatus</i>
Black grouper	<i>Mycteroperca bonaci</i>
Tiger grouper	<i>Mycteroperca tigris</i>
Yellowfin grouper	<i>Mycteroperca venenosa</i>
<i>Snappers</i>	
Mutton snapper	<i>Lutjanus analis</i>
Schoolmaster snapper	<i>Lutjanus apodus</i>
Dog snapper	<i>Lutjanus jocu</i>
Yellowtail snapper	<i>Ocyurus chrysurus</i>
<i>Sharks</i>	
Nurse shark	<i>Ginglymostoma cirratum</i>
<i>Other fishes</i>	
Tarpon	<i>Megalops atlanticus</i>
Cero	<i>Scomberomorus regalis</i>

Table 4.S2. Results of the SIMPER analysis for dissimilarity in the species that drove the small fish community change between 2009 and 2011. Present and absent categories refer to the 2 categories of lionfish abundance in 2011 used in the SIMPER analysis. Reef-fish species listed in order of contribution to community dissimilarity.

Average dissimilarity = 1.84

Lionfish abundance	Absent	Present					
Fish Species	Abundance	Abundance	Change	Dissimilarity	Dissimilarity (SD)	Contribution (%)	Cumulative (%)
<i>Canthigaster rostrata</i>	119.46	80.77	-	0.8	1.3	42.6	42.6
<i>Chromis cyanea</i>	89.26	91.46	+	0.3	1.2	16.2	58.8
<i>Stegastes partitus</i>	102.47	95.48	-	0.2	1.5	10.6	69.3
<i>Clepticus parrae</i>	95.74	101.27	+	0.1	0.6	5.6	74.9
<i>Thalassoma bifasciatum</i>	91.22	94.58	+	0.1	1.2	5.3	80.2
<i>Halichoeres garnoti</i>	101.26	97.15	+	0.1	1.5	5.0	85.2
<i>Scarus iseri</i>	93.64	93.90	+	0.1	1.0	2.9	88.1
<i>Sparisoma aurofrenatum</i>	95.21	95.20	-	0.1	1.0	2.7	90.8

Table 4.S3. Results of the PERMANOVA analysis of the small fish communities after removing the most abundant fish species (*Canthigaster rostrata*).

Small Fish Community (0-5cm TL)						
Transformation of data	Factor	df	SS	MS	Pseudo-F	P (permanova)
Raw	Lionfish presence/absence	1	11.03	11.03	1.02	0.382
	Residual	13	140.22	10.79		
	Total	14	151.25			
Square root	Lionfish presence/absence	1	2.69	2.69	0.68	0.579
	Residual	13	51.19	3.94		
	Total	14	53.87			
Fourth root	Lionfish presence/absence	1	0.88	0.88	0.45	0.731
	Residual	13	25.55	1.97		
	Total	14	26.43			
Log(X+1)	Lionfish presence/absence	1	0.48	0.48	0.36	0.822
	Residual	13	17.17	1.32		
	Total	14	17.65			

Figure 4.S1. Map of the average wave exposure (logarithm of wave exposure in J m^{-3}) for the Belize Barrier Reef obtained from Chollett and Mumby (2012). Chollett and Mumby (2012) estimated wave exposure using simple physical models based on the configuration of the coast line and regional meteorological conditions such as in Denny and Gaylord (2010). Our survey sites are labeled in white. Triangles denote no-take zones (marine parks) and circles denote control sites where fishing is not permitted.

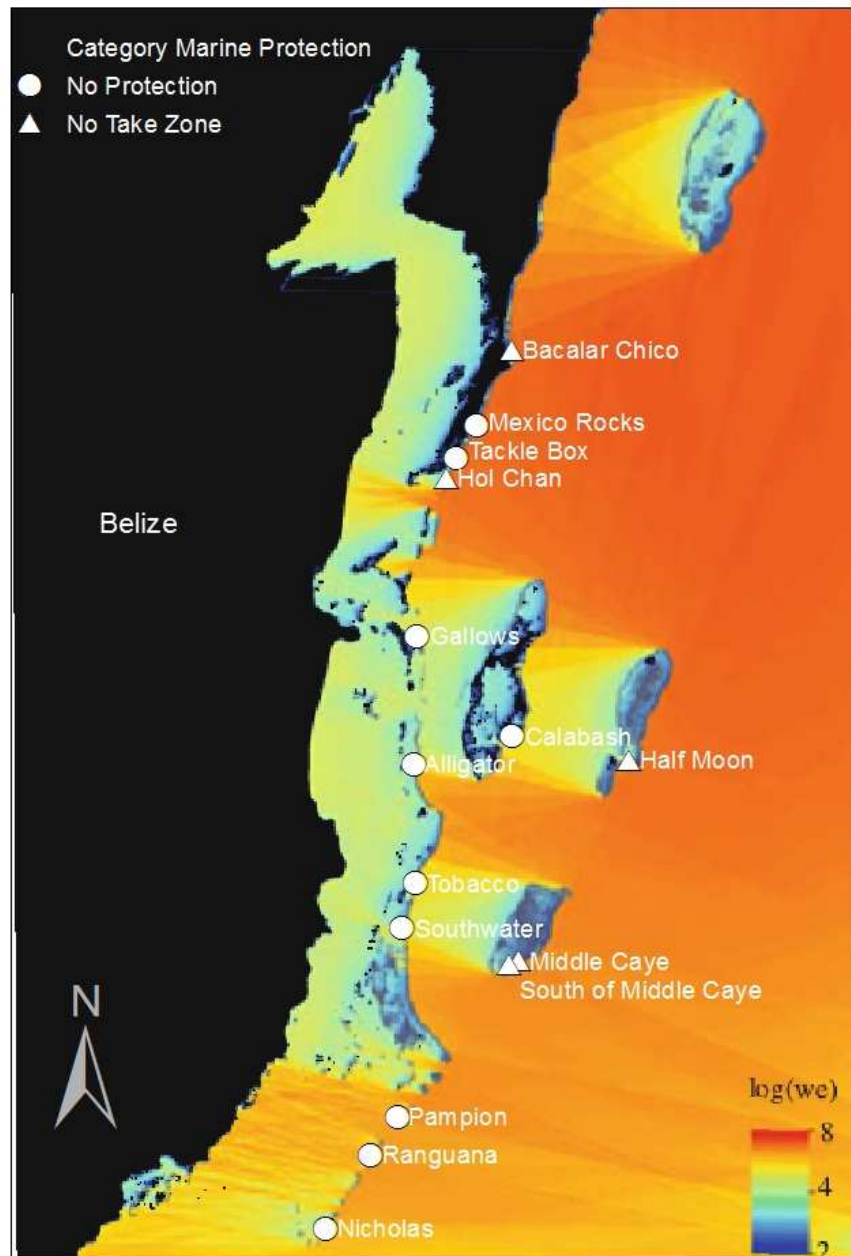


Figure 4.S2. The change in average abundance of small fish (excluding the most abundant species, *Canthigaster rostrata*) from 2009 to 2011 for each site compared to lionfish density. Y-values above and below the origin line indicate positive and negative changes, respectively in fish abundance on each study site.

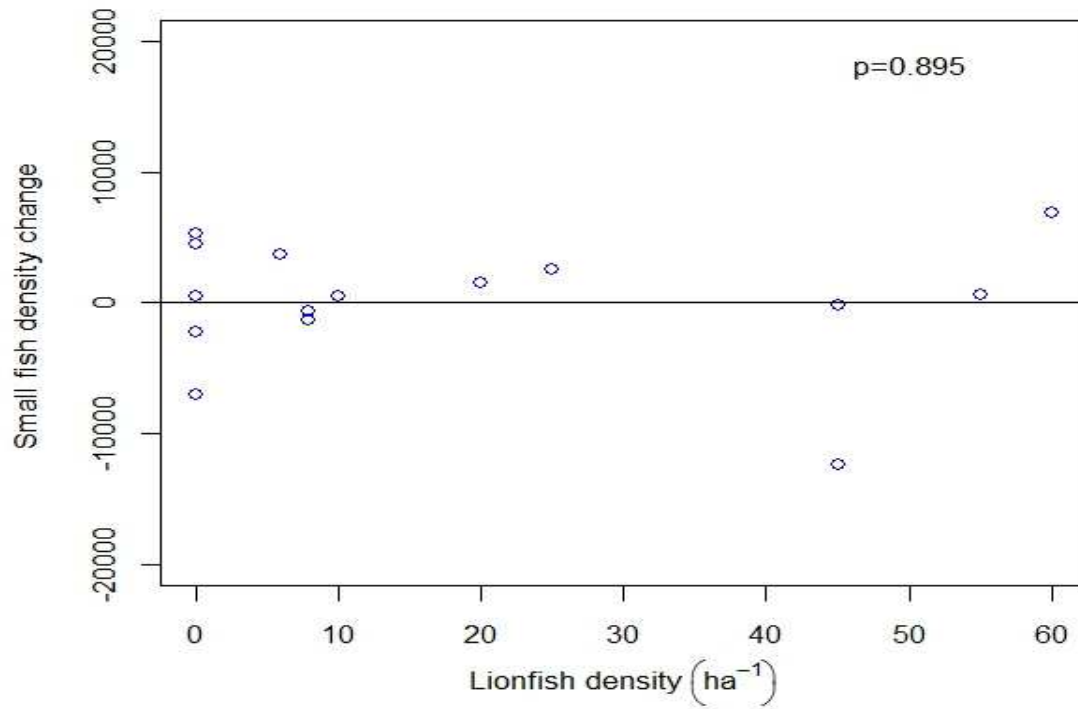
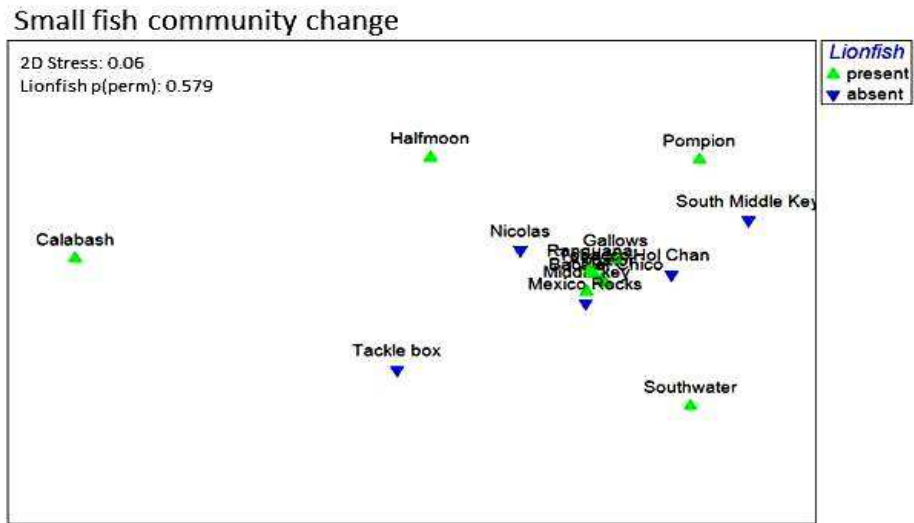


Figure 4.S3. n-MDS plots depicting the composition of the change in the small fish community (excluding the most abundant species, *Canthigaster rostrata*) between 2009 and 2011 and lionfish abundance (presence/absence) in 2011. The size of the bubbles indicates lionfish abundance. Stress values lower than 0.2 reflect a good agreement between the distances depicted in the graph and the fish community dissimilarities among sites. The axis on n-MDS plots serve as an arbitrary coordination system (Kruskal and Wish 1978). p-values were obtained from the PERMANOVA analysis.



APPENDICES

Appendix 2.1. Potential predation/competition between moray eels and invasive lionfish in the Caribbean.

On July 2010, a lionfish (250mm TL) was collected from a wreck in the Bahamas and was kept in a fish trap overnight. The next morning a spotted moray eel (*Gymnothorax moringa*) was found inside of the fish cage. The lionfish was dead and had a severe wound on the dorsal part of the body. We paired lionfish with several Caribbean moray species inside cages for a week. Two spotted moray and one green moray (*G. funebris*) were collected and kept in individual fish traps. Then we added a healthy lionfish to each fish trap. In a week, the lionfish that shared the cage with the green moray had three wounds (presumably from bites). The lionfish in cages with the three largest eels were always at a safe distance from the head of the eels. Once, a spotted moray eel was observed to attack a lionfish by biting it.

Appendix 2.2. Length-weight a and b parameters for lionfish

The biomass of individual lionfish (*Pterois volitans*) was calculated using the allometric length-weight conversion (Binohlan and Pauly, Fishbase):

$$W = a \cdot L^b$$

- W: weight (g)
- a and b: constant parameters
- L: Length (mm)

In 2010, 137 lionfish (*Pterois volitans*) were captured in Abaco (The Bahamas). Their length and weight were quantified and length-weight a and b parameters were calculated.

The a and b parameters for lionfish from Abaco were:

$$\begin{aligned} a &= 0.004922288 \\ b &= 3.310168 \end{aligned}$$

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