

Behavioral response of native Atlantic territorial three spot damselfish (*Stegastes planifrons*) toward invasive Pacific red lionfish (*Pterois volitans*)

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Abstract The Pacific red lionfish (*Pterois volitans*) has been recognized as a top conservation issue in the world due to its ability as an invasive predator to greatly reduce recruitment of native reef fishes, and with potential long-term ecosystem-level effects. This study tested for territorial aggression of native three spot damselfish (*Stegastes planifrons*) toward invasive lionfish as a possible source of biotic resistance that may provide prey refugia for coral-reef fish recruits. Throughout July and August 2011, I conducted a field experiment in the Bahamas and the Cayman Islands using a model-bottle design specifically developed for examining three spot damselfish behavior. I compared the behavioral response of 40 damselfish to the presence of invasive lionfish and three native fishes presented individually in clear bottles, as well as to an empty bottle control. Despite lionfish having invaded these islands in different years, damselfish response did not differ between the Bahamas and the Cayman Islands. Overall, damselfish response toward invasive lionfish was not significantly different from the minimal response toward the empty bottle control. In contrast, damselfish actively responded to all native fishes, with species-specific behaviors and levels of aggression that depended on the

ecological relationships between damselfish and intruding fishes. Differences in the seafloor rugosity of damselfish territories among study sites also appeared to influence damselfish response. The lack of damselfish response towards lionfish demonstrates that territories are unlikely to serve as native prey refugia, and may indicate lack of recognition of this invasive predator compared to native predators.

Keywords Invasive lionfish · Biotic resistance · Territorial aggression · Three spot damselfish · Behavior · Habitat

Introduction

Biological invasions can have severe ecological consequences on native ecosystems and associated socioeconomic repercussions by reducing biodiversity, altering community structure, and disrupting ecosystem function (Ruiz et al. 1997; Bax et al. 2003). As the number of marine invasions increases at an accelerating rate (Cohen and Carlton 1998; Rilov and Crooks 2008), eradication strategies for established invasive species remain poorly developed, and so far, largely ineffective (Thresher and Kuris 2004). Therefore, it is important to identify and understand any natural mechanisms of biotic resistance that effectively limit local populations of invasive species.

The range expansion of an invasive marine fish has never progressed as rapidly as that of the Pacific red lionfish (*Pterois volitans*) throughout the coral reefs of

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the tropical western Atlantic and Caribbean (Schofield 2009, 2010). Invasive lionfish occur in high densities (Green and Côté 2009; Kulbicki et al. 2012), with recent evidence suggesting that maximum densities have yet to be reached (Benkwitt 2013). These highly efficient predators (Côté and Maljković 2010) can greatly reduce recruitment of native reef fishes (Albins and Hixon 2008; Green et al. 2012; Albins 2013), including the juveniles of species known to be important for both reef resilience and local fisheries (Morris and Akins 2009; Muñoz et al. 2011; Layman and Allgeier 2012; Valdez-Moreno et al. 2012; Côté et al. 2013a). With concerns for potential long-term effects on invaded reefs, both direct and indirect (Albins and Hixon 2013; Côté et al. 2013b), the lionfish invasion has been recognized as one of the top conservation issues in the world (Sutherland et al. 2010).

Lionfish management efforts have been restricted to local control via removals on targeted shallow reefs (Morris and Whitfield 2009; Akins 2012; Frazer et al. 2012; Albins and Hixon 2013), even though the geographic distribution of this invader includes a broad variety of habitats (Whitfield et al. 2007; Barbour et al. 2010; Lesser and Slattery 2011; Claydon et al. 2012; Jud and Layman 2012) and an extensive depth range reaching over 300 m (Gilmore pers. comm.). Various models have indicated that manual removal efforts must remain intense and necessitates long-term commitment to effectively reduce adult lionfish densities, which further limits control mostly to small, localized areas (Morris et al. 2011; Arias-González et al. 2011; Barbour et al. 2011). Green et al. (2013) demonstrated that maintaining lionfish at targeted densities on small patch reefs can be achieved with monthly lionfish removals, which were effective at mitigating lionfish predation effects on native fish communities. In order to ameliorate the effects of invasive lionfish at a broader scale, however, feasible sources of biotic resistance by native Atlantic species must be identified and promoted.

Native communities can provide biotic resistance (sensu Elton 1958), thereby preventing establishment and spread of an invasive species via predation, competition, parasites, and/or disease (Sakai et al. 2001; Levine et al. 2004). Unfortunately, there have been no reports of lionfish disease in either the invaded nor native waters, and invasive lionfish exhibit low infection levels of endo- and ecto-parasites (Morris et al. 2009; Tuttle et al. unpubl. data). Albins (2013) found evidence of an ecologically similar native predator unable to

effectively limit invasive lionfish via competition. There is currently substantial debate regarding the extent to which native groupers control the abundance of invasive lionfish (Mumby et al. 2011, 2013; Bruno 2013; Bruno et al. 2013; Hackerott et al. 2013; Valdivia et al. 2014), yet there has been only one report of large native groupers containing lionfish in their stomachs (Maljković et al. 2008). The only study to date indicating that native grouper may limit invasive lionfish suggests that native Nassau grouper (*Epinephelus striatus*) displace lionfish foraging off of small patch reefs (Pusack 2013). Unfortunately, Nassau grouper are currently listed as an endangered fish by IUCN (Albins et al. 2009), and like most large predators, is severely overfished regionally (Sadovy and Eklund 1999; Stallings 2009). Native predators, including sharks, groupers, and moray eels, have been observed consuming injured and/or dead lionfish that had been speared (Jud et al. 2011; Pimiento et al. 2012; Kindinger pers. obs.), and Diller et al. (2014) conditioned large predators to consume tethered lionfish. However, all of these instances were limited in that they required human interaction.

I investigated another possible source of biotic resistance to the invasion: interspecific aggression by the native three spot damselfish (*Stegastes planifrons*). In general, inter specific aggression has typically been tested in the context of explaining behavioral mechanisms underlying an invader's success, and few studies have tested specifically for interspecific aggression of a native species as an effective source of biotic resistance against an invader. Blight et al. (2010) found a dominant native ant in the Mediterranean capable of decreasing invasion success of Argentine ants through aggressive interactions. Native ant assemblages in Northwest Patagonia were documented attacking an invasive wasp competing for food sources by aggressively biting the wasp's legs (Masciocchi et al. 2009). Eastern mosquito fish native to Florida's freshwater habitats attacked and killed two non-indigenous fishes in a mesocosm experiment (Thompson et al. 2012). Observations of invasive lionfish and stomach content analyses reveal lionfish hunt and consume several species of native damselfishes (Morris and Akins 2009; Green et al. 2011; Curé et al. 2012; Layman and Allgeier 2012; Valdez-Moreno et al. 2012; Côté et al. 2013a, b). Despite having seen invasive lionfish hunting three spot damselfish (Kindinger pers. obs.), there is yet to be any reports of this damselfish as lionfish prey.

Studies throughout their range have shown three spot damselfish to be abundant and highly aggressive toward both conspecifics and heterospecifics (including potential predators) (Myrberg and Thresher 1974; Robertson et al. 1976; Kaufman 1977; Williams 1978, 1980; Knowlton et al. 1990), and this species is the competitive dominant within its ecological guild (Robertson 1996). In addition, the three spot damselfish effectively excludes certain species from their guarded territories (Kaufman 1977; Williams 1980; Knowlton et al. 1990). As a species of farming damselfishes (Family Pomacentridae, genus *Stegastes*), aggression by three spot damselfish fosters the growth of filamentous turfs of algae which serve as a source of food, shelter from predators, and a nesting site (Myrberg and Thresher 1974; Robertson et al. 1976; Thresher 1976). Importantly, an ecologically similar damselfish in the Pacific has been shown to indirectly provide prey refugia for small recruit fishes by excluding predators from their territories (Green 1992). If this phenomenon occurs in the Atlantic, then territorial damselfish could perhaps provide refugia from invasive lionfish predation for native reef fish recruits until they reach relatively invulnerable body sizes.

In order to assess the potential of three spot damselfish providing refugia, I used a model-bottle study design whereby invasive lionfish and native fishes were presented in clear bottles to three spot damselfish at set distances from damselfish territories (Myrberg and Thresher 1974). This method allows for the comparison of three spot damselfish behavior towards various intruding fishes in order to address my main objective of determining at what level—if at all—three spot damselfish are aggressive towards a novel predator. I addressed the following questions: (1) Do three spot damselfish exhibit a behavioral response when invasive lionfish are present? (2) How do three spot damselfish respond to the presence of invasive lionfish compared to the empty bottle control? (3) Do three spot damselfish differ in their response toward invasive lionfish and three native fish species, especially an ecologically-similar predatory grouper?

Methods

Area of study and microhabitat assessment

I conducted this study during July–August 2011, observing the behavioral response of 40 three spot damselfish:

20 in the Bahamas and 20 in the Cayman Islands. In addition to enhancing the generality of the study, I chose to observe damselfish in these two locations because of their difference in timing of the lionfish invasion: lionfish were first sighted in the Bahamas in 2004 and in the Cayman Islands in 2008 (Schofield 2009). In the Bahamas, I studied damselfish at three sites in the shallow waters (sites were <4 m deep) of the Great Bahama Bank in the vicinity of Lee Stocking Island, which is part of the Exuma Cays. Study sites consisted of patch reefs composed of small coral heads and larger coral bommies surrounded by sand and seagrass beds. About 380 miles Southwest of Lee Stocking Island, I observed damselfish behavior off of Little Cayman Island at three deeper sites (6–12 m deep) located along the northern side of the island, just inshore of the Bloody Bay Wall. This area is characterized by continuous stretches of reef that includes coral heads of various sizes and large coral formations.

The benthic territories maintained year-round by three spot damselfish are less than 1 m² and are easily identified by the algal gardens covering reef substrata that the damselfish cultivate (Brawley and Adey 1977). The underlying substrata of damselfish territories differed at sites both within and between the Bahamas and Cayman Islands. Since the type of habitat could potentially affect damselfish response by influencing an individual's ability to defend its territory, I characterized the microhabitat of each damselfish territory by recording the following four habitat categories: (1) low-relief dead coral rubble (mostly *Acropora cervicornis*), (2) low-relief continuous reef, (3) high-relief large coral bommies, and (4) high-relief continuous reef. Low-relief habitats lacked vertical structure, whereas high-relief habitats consisted of vertical structure >1 m high, which could potentially interfere with the ability of damselfish to detect intruders.

Experimental treatments and fish capture

Each three spot damselfish was exposed to a series of treatments consisting of a single individual of (1) invasive lionfish, or the following native fishes, all of which are commonly found on reefs near three spot damselfish territories and are chased at varying degrees by damselfish (Thresher 1976; Robertson 1984): (2) herbivorous ocean surgeonfish (*Acanthurus bahianus*), a potential food competitor; (3) white grunt (*Haemulon plumierii*), a potential egg predator; and (4) coney grouper

(*Cephalopholis fulva*), a mesopredator ecologically similar to lionfish and at larger sizes is a potential predator of three spot damselfish. At both study regions, I captured 2–3 individuals per fish species, which were rotated daily for experimental use based on each individual's appearance, apparent condition, and behavior. All fish were caught underwater from non-study sites using hand nets and the fish anesthetic quinaldine when needed. Body size of individual fish, ranging from 10 to 18 cm TL, was restricted to allow for ease of movement in bottles during the experiment. At these sizes, both lionfish and coney grouper were sufficiently large to pose a threat to small recruit fishes inhabiting damselfish territories (Albins 2013). Fish were maintained in flow-through aquarium tanks both prior to and between daily observational trials.

Model-bottle experiment

Using a model-bottle study design (Myrberg and Thresher 1974), I presented individual fish in weighted, clear-plastic gallon bottles to haphazardly located adult damselfish (7–11 cm total length [TL]) in order to measure the relative behavioral responses exhibited by each focal damselfish. Bottle lids were replaced with secured mesh screening to allow for flow of both water and any fish chemical cues. An empty bottle was used as a control treatment. I introduced each treatment in random order to individual damselfish territories. All fishes inside bottles were either resting or hovering upon introduction.

To measure damselfish aggression per treatment, each bottle was sequentially placed at 100, 50, and 0 cm away from the center of each territory (Fig. 1). At each increment, I observed damselfish behavior from a distance of 3 m for 2 min, counting the number of times the focal damselfish made physical contact with the bottle (attack rate) and tallying which aggressive behaviors each damselfish displayed: (1) contact with the mouth while hovering in place directly next to the bottle (nip); (2) contact with the caudal fin while hovering in place directly next to the bottle (butt); (3) starting from a distance, swimming with force directly towards the bottle, making contact with mouth, and then quickly swimming away from the bottle (charge); and, (4) repeatedly charging the bottle multiple times (continuous attack). These categories encompass three spot damselfish behavior known to effectively exclude intruders (Thresher 1976). I also looked for avoidance behavior

by damselfish, such as entering refuge sites within their territories (Helfman 1989).

I then returned the bottle to the closest distance to the territory at which the damselfish had previously made no physical contact with the bottle, then gradually moved the bottle closer to the center of the territory until the damselfish approached the bottle and made physical contact. If the damselfish had previously attacked the bottle at 100 cm away from the territory, I placed the bottle at 150 cm where all damselfish ceased attacking the bottle, and gradually moved the bottle closer to the territory from there. This method provided a measurement of the “maximum distance of attack” (sensu Myrberg and Thresher 1974) per treatment.

Statistical analyses

All assumptions of normality and homogeneity of variance were not met based on residual analyses, and transformations failed to normalize the data. To test for a difference in damselfish response between the Bahamas and the Cayman Islands, I used nonparametric Wilcoxon signed-rank tests for repeated measurements of the attack rate and maximum distance of attack. Binary counts of whether individual damselfish attacked each treatment or not when placed inside damselfish territories (distance of 0 cm) were also compared between the two study regions using McNemar's test. Results from all tests revealed that damselfish response did not significantly differ between regions for all three response variables measured: (1) attack rate (Wilcoxon signed-rank test, $n=40$, $V=5,857$, $P=0.1121$); (2) maximum distance of attack (Wilcoxon signed-rank test, $n=40$, $V=1,702$, $P=0.0902$); and, (3) number of damselfish that attacked (McNemar's test, $n=40$, $\chi^2=0.2273$, $P=0.6336$). Damselfish from both locations were thus combined during all subsequent statistical analyses.

With the exception of the surgeonfish treatment, there was no discernable difference in damselfish attack rates among treatments until bottles were placed directly in the center of damselfish territories (Online Resource 1), so I compared the number of damselfish that attacked and the attack rate in response to each treatment measured only at this 0 cm distance. The proportion of damselfish (out of 40 individuals) that attacked each treatment was compared using a nonparametric Cochran's Q test. I rank-transformed the attack rate and maximum distance of attack, and then tested for differences in response among treatments and among

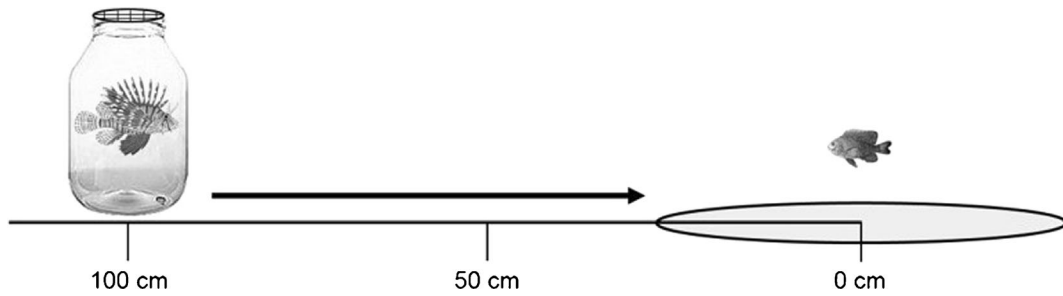


Fig. 1 Model-bottle study design. Shaded ellipse represents the damselfish’s guarded territory, with the focal damselfish positioned above. Bottles in each treatment (*invasive lionfish* treatment pictured here) were sequentially placed at 100, 50, and 0 cm away from the center of the territory, and damselfish attack rate and

behavior was observed for 2 min at each increment. Then, the model bottle was moved to a distance where the focal damselfish had not responded, and then gradually moved closer to the territory until the damselfish made physical contact (i.e. maximum distance of attack). Images courtesy of FAO.

microhabitats of damselfish territories by performing one-way repeated measures analyses of variance (ANOVAs). Results from these ANOVAs were compared with the results from Friedman tests, and were found to provide consistent conclusions. Therefore, I report only results from the one-way repeated measures ANOVA, because this provides a more robust analysis with greater statistical power compared to the Friedman test (Zimmerman and Zumbo 1993).

In addition, I performed post-hoc pair wise comparisons of all three response variables among treatments and among microhabitats (when applicable) with Wilcoxon signed-rank tests. Reported p-values from these multiple comparisons were corrected using Holm’s adjustment method, which does not assume independence of groups when controlling the family-

wise error rate (Holm 1979). All statistical tests were conducted using the statistical software *R* version 3.0.0 (*R* Development Core Team 2013) with the associated packages, *car* (Fox et al. 2009), *nlme* (Pinheiro et al. 2014), and *RVAideMemoire* (Hervé 2014).

Results

Treatment had a significant effect on all damselfish response variables: (1) proportion of damselfish that attacked (Fig. 2, Cochran’s Q test, $n=40$, $Q_4=72.7917$, $P<0.0001$); (2) attack rate (Fig. 3, repeated measures ANOVA, $n=40$, $F_4=89.1661$, $P>0.0001$); and (3) maximum distance of attack (Fig. 3, repeated measures ANOVA, $n=40$, $F_4=68.3478$, $P<0.0001$). Post-hoc pair

Fig. 2 Proportion of damselfish ($n=40$) that attacked each model-bottle treatment when placed in the center of the damselfish territory (0 cm distance). Proportions with different letters are significantly different (pair wise Wilcoxon signed-rank tests with Holm’s correction method, $P<0.05$).



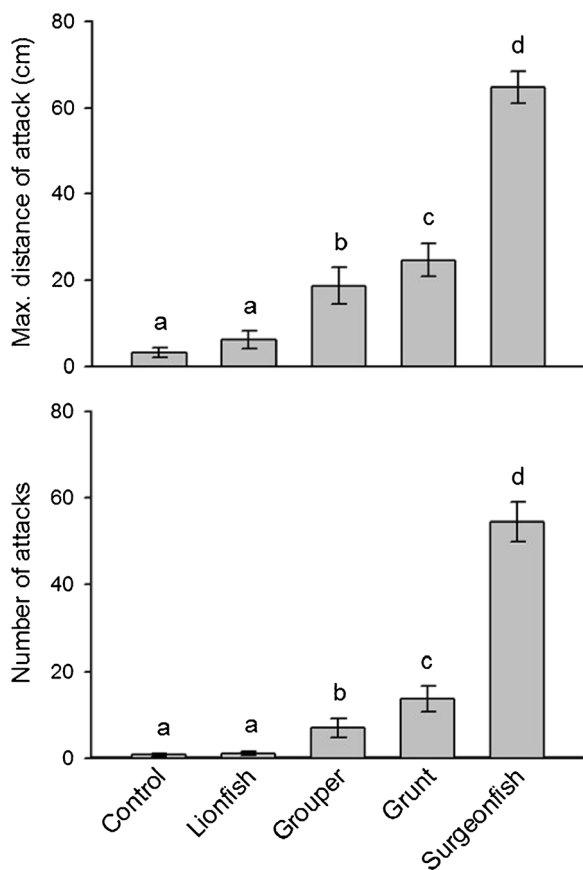


Fig. 3 Mean damselfish response (\pm SEM) to each model-bottle treatment ($n=40$ damselfish) measured as the maximum distance of attack and the attack rates when treatments were placed in the center of damselfish territories. Response levels with different letters are significantly different (pair wise Wilcoxon signed-rank tests with Holm's correction method, $P<0.05$).

wise Wilcoxon signed-rank tests of all three variables revealed that damselfish response did not significantly differ between the empty-bottle control and invasive lionfish treatment (proportion of damselfish, $P=1.0000$; attack rate, $P=0.7296$; maximum distance of attack, $P=0.2814$), which were significantly lower levels of damselfish response than those exhibited towards the native fish treatments ($P<0.05$), including the ecologically-similar coney grouper. Damselfish response towards the native fishes remained consistent among all three variables, with a general trend of significantly increasing levels of response (pair wise Wilcoxon signed-rank tests, $P<0.05$) towards the grouper, grunt, and surgeonfish treatments, respectively (Figs. 2 and 3).

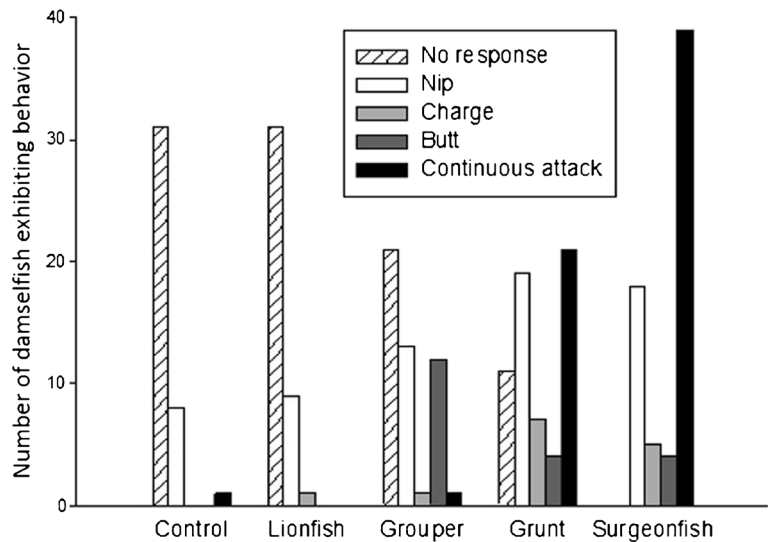
Only nine out of the 40 three spot damselfish attacked the empty-bottle control, and similarly, only

10 damselfish attacked the invasive lionfish treatment (Fig. 2). Five of these damselfish attacked every treatment, which suggests these may have been individuals with higher levels of aggression. When bottles were placed directly in the center of territories, damselfish never made physical contact with the empty-bottle control nor invasive lionfish treatment more than ten times within the two-min observation period. Individual damselfish attacked the grouper, grunt, and surgeonfish treatments as many as 45, 59, and 100 times, respectively, all within two minutes. The furthest distance damselfish attacked the empty-bottle control was 30 cm away from their territories, whereas the maximum distance damselfish attacked invasive lionfish was 50 cm. In contrast, damselfish attacked all native fishes at distances over 50 cm, with damselfish attacking the coney grouper up to 70 cm away. The greatest distance an individual damselfish attacked any fish was 110 cm away from its territory in response to a surgeonfish.

Damselfish displayed only two aggressive behaviors in response to the empty-bottle control and the invasive lionfish treatment, but responded to the native fish treatments with all four aggressive behaviors (Figs. 4 and 5). The majority of damselfish showed no response to the empty-bottle control and the invasive lionfish treatment, and the individuals that did respond used mostly nips, with only one instance each of a damselfish continuously attacking the empty bottle control and charging the invasive lionfish treatment. This nip behavior was commonly observed in response to all fishes, whereas damselfish continuously attacked only the grunt and the surgeonfish, and most often butted the coney grouper. Avoidance behavior by damselfish was not observed during any experimental trials.

In the Bahamas, the microhabitat of observed damselfish territories consisted of low-relief dead coral rubble ($n=11$) and high-relief large coral bommies ($n=9$), whereas in the Cayman Islands, damselfish territories were observed on low- and high-relief continuous reef ($n=7$ and 13, respectively). The type of microhabitat where damselfish territories were located had a moderate effect on overall damselfish response (repeated measures ANOVAs: attack rate, $n=40$, $F_3=3.3797$, $P=0.0286$; maximum distance of attack, $n=40$, $F_3=3.1672$, $P=0.036$). Damselfish on high-relief continuous reef attacked treatments both at distances closer to their territories and with lower attack rates than damselfish observed on low-relief dead coral rubble (Fig 5; pair wise Wilcoxon signed-rank tests: attack rate, $P=0.035$;

Fig. 4 Number of damselfish exhibiting each behavior observed in response to the five model-bottle treatments ($n=40$ damselfish). In order of increasing level of aggression, behaviors consisted of no response, nip, charge, butt, and continuous attack (see Methods for further description).



maximum distance of attack, $P=0.042$), and this pattern in damselfish response remained consistent across all native fish treatments (Online Resource 2).

Discussion

One likely explanation for a native species lacking an effective behavioral response to an invasive predator is prey naïveté (Diamond and Case 1986; Cox and Lima 2006; Freeman and Byers 2006). Such naïveté may result in a native species failing to recognize predation threats, having an inappropriate anti-predator response, or having an appropriate response, but one that is ineffective (Banks and Dickman 2007). In this study, three spot damselfish showed minimal behavioral response to the presence of invasive lionfish that was most similar to the response elicited by an inanimate object, the empty bottle. Damselfish responded to all native fishes with species-specific levels of aggression that were consistent with previous behavioral studies of this species (Myrberg and Thresher 1974; Thresher 1976). In particular, the level of aggression and variety of aggressive behaviors displayed by damselfish toward the native predator (coney grouper) were consistently greater than those elicited by invasive lionfish, which suggests that damselfish are unable to recognize lionfish as a potential predator.

Helfman (1989) determined that three spot damselfish respond to predators with a variety of avoidance behaviors, and the orientation and size of predators

affect the level of threat perceived by individual damselfish. The coney grouper and lionfish used in this study were never oriented in any hunting postures inside the bottles. In addition, the sizes of coney grouper were never large enough to effectively consume adult damselfish, which could explain the lack of damselfish avoidance behaviors in response to this native predator. Regardless, damselfish still responded with aggression towards coney grouper. Similarly-sized coney and graysby (*Cephalopholis cruentatus*) groupers were often observed swimming through the underlying habitats of damselfish territories, especially in areas of dead coral rubble, so perhaps these small groupers pose a competitive threat to damselfish shelter.

While the “nip” behavior seemed to be a general response by three spot damselfish, they used a “butting” behavior toward coney grouper more so than towards any other species, whereby they made physical contact with the bottle using only the caudal fin. In contrast, damselfish continuously attacked the native grunt and surgeonfish, which resulted in damselfish remaining in close contact with the bottle while performing continuous series of head-first charges. Perhaps damselfish are relatively cautious when warding off a potential predator by limiting physical contact with the predator and resorting to attacks with the caudal fin rather than the head.

Invasive lionfish are capable of consuming prey as large as about 50 % of their body length (Morris and Akins 2009), so some of the lionfish used in this study were large enough to consume some of the observed

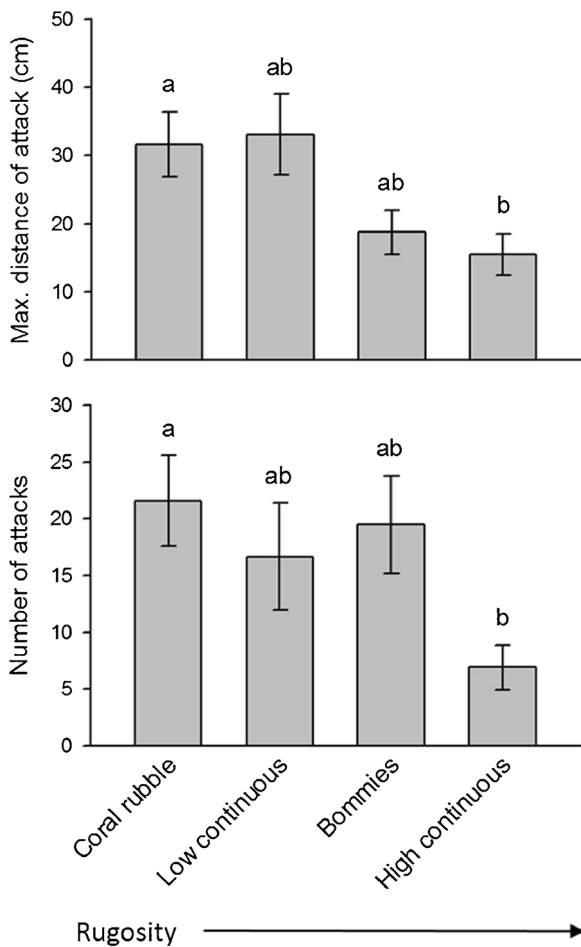


Fig. 5 Mean damselfish response (\pm SEM) in each of four microhabitats in which damselfish territories occurred (left-to-right in order of increasing rugosity): dead coral rubble, low-relief continuous reef, coral bommies, and high-relief continuous reef. Low-relief habitats lacked vertical structure and high-relief habitats consisted of vertical structure >1 m high. Response levels with different letters are significantly different (pair wise Wilcoxon signed-rank tests with Holm's correction method, $P < 0.05$).

three spot damselfish. Despite the potential benefit for damselfish to perceive lionfish as a predatory threat, no avoidance behaviors were displayed, and in contrast to the coney grouper, damselfish never used the butting behavior towards lionfish. Lionfish hunting within and around damselfish territories could also be perceived as a general intrusion to damselfish, such as when three spot damselfish attack intruding divers (Helfman 1989; Kindinger pers. obs.), yet damselfish consistently exhibited minimal response toward lionfish.

The cue similarity hypothesis highlights the importance of comparing cues between native and non-native predators, and understanding whether prey use general

versus specific cues while assessing risk during detection and recognition of predators (Sih et al. 2010). Both the appearance and behavior of lionfish are unique compared to native predators in the Atlantic. Lionfish have cryptic coloration and striped patterning, with elongated fin rays that have been postulated to enhance mimicry and/or camouflage (Albins and Hixon 2013). The hunting behaviors of lionfish are described as a slow stalking of prey and use of fanlike pectoral fins for herding prey (e.g. Randall 2005; Green et al. 2011; Curé et al. 2012), as well as a unique blowing behavior, whereby lionfish aim jets of water at prey, which apparently increases the probability of head-first capture (Albins and Lyons 2012). It seems plausible that invasive lionfish and native predators provide dissimilar cues to potential prey.

Native species can learn to recognize novel predators (Payne et al. 2004; Carlsson et al. 2009) and there is evidence of some fishes having learned predator recognition and antipredator response (Kelley and Magurran 2003). Marsh-Hunkin et al. (2013) reported that native gobies in the Bahamas recognize and respond to invasive lionfish cues. This discrepancy in findings with three spot damselfish may be explained by potential differences in the relative use of general versus species-specific cues between Atlantic gobies and damselfishes. General cues include chemicals (Sih et al. 2010), as well as the visual cue of any moving organism or object that is larger than a minimum size threshold (Dill 1974; Sih 1986).

Native prey are also expected to shift towards adaptive antipredator behavior either through evolutionary time (Losos et al. 2006; Strauss et al. 2006) or through ecological time within a generation (Berger et al. 2001; Caro 2005). Even though invasive lionfish have been established in the Bahamas years longer than populations in the Cayman Islands (Schofield 2009), I found no evidence of a shift in three spot damselfish behavior towards this invasive predator. Since lionfish have been observed hunting three spot damselfish (Kindinger pers. obs.), perhaps with continued overlap in distribution over larger temporal scales three spot damselfish will eventually respond to lionfish. However, further study is needed to enhance our understanding of lionfish recognition by prey fishes in general, and to help elucidate how the behavior and ecology of native fishes will eventually change in response to the lionfish invasion.

Three spot damselfish response varied depending on the microhabitat in which their territories occurred. If

there is potential for this damselfish to provide prey refugia from any predators, it may be that this phenomenon is context-dependent on local habitat characteristics. In this study, reef formations that damselfish used as substrate for their algal gardens on high-relief continuous reef consisted mostly of tall (>1 m) isolated structure, which generated a vertical component into the total guarded area and often appeared to be a visual obstruction for detecting intruders. As a result, it required relatively more time for some damselfish to encounter fish in model bottles placed near their territories. All other types of observed microhabitat consisted of damselfish territories with vantage points containing fewer visual obstructions, especially territories located on dead coral rubble, which had the lowest rugosity.

Three spot damselfish behavior had not been assessed since the invasion of lionfish throughout the Atlantic Ocean. Here, I have determined that damselfish behavior in response to intruding native fishes corroborates the original patterns described by previous model-bottle studies (Myrberg and Thresher 1974; Thresher 1976). Based on the low level of behavioral response towards invasive lionfish, it is unlikely that the three spot damselfish is effectively excluding lionfish, and thus is not providing any sort of refuge from invasive lionfish predation for native recruit fishes. Importantly, if damselfish are effectively providing refugia for native fishes by excluding native predators from their territories, but fail to deter invasive lionfish, then lionfish could potentially exploit damselfish territories as possible sources of prey. Understanding these potential interactions can provide insight into the relative ability of native communities to provide biotic resistance, and may inform predictions of the long-term effects of an invasive marine predator on native community structure.

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