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Native prey mortality increases but remains density-dependent following lionfish invasion

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ABSTRACT: Predators can regulate prey population dynamics, so the introduction of novel predators may alter predation-mediated regulatory mechanisms, potentially destabilizing prey populations. Compensatory density dependence is an essential condition for population regulation. Thus, understanding whether and how introduced predators alter the relationship between prey density and mortality can aid in predicting the ecological consequences of invasion. Here, we investigate the effects of invasive Indo-Pacific red lionfish *Pterois volitans* on density-dependent mortality patterns previously documented for a common native Atlantic prey species, the fairy basslet *Gramma loreto*. By repeating a pre-invasion density manipulation experiment, here in the context of predation by both native piscivores and lionfish, we provide a before-and-after comparison quantifying changes in prey mortality since the introduction of lionfish. Per capita loss of fairy basslet remained density-dependent in the presence of lionfish, but the overall magnitude of loss was higher compared to pre-invasion rates. In the presence of lionfish, 7 of 16 local basslet populations experienced greater than 50% loss over the 8 wk study duration, but there was no evidence of a difference in the slope of the density–mortality curve between pre- and post-introduction experiments. Thus, our experiment revealed a density-independent increase in per capita mortality rates since the start of the invasion. We conclude that local fairy basslet populations are facing an elevated risk of extirpation as a result of increased predation, and suggest that different predator foraging behavior and/or prey naïveté may explain the altered prey mortality patterns observed after the lionfish invasion.

KEY WORDS: Coral-reef fishes · Density dependence · Invasive predator · Population regulation · Predator–prey · *Pterois volitans*

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INTRODUCTION

Species introductions have been identified as one of the greatest threats to global biodiversity (Wilcove et al. 1998, Mack et al. 2000, Sax & Gaines 2008) and can drastically alter the population, community, and ecosystem-level properties of invaded systems (Gurevitch & Padilla 2004). Invasive predators in particular can have strong population-level impacts on native prey (Salo et al. 2007), effects that may be exacerbated by prey naïveté and/or novel hunting strategies of the invader that render anti-predator defenses ineffective (Sih et al. 2010). This lack of co-evolution between introduced predator and native prey can lead to rapid and severe declines in prey populations (Simberloff 1995). As predators may regulate prey population dynamics, it is important to determine whether novel predators alter predation-mediated regulatory mechanisms, potentially destabilizing prey regulation. An essential condition for population regulation is demographic density dependence where, as population density increases, the per capita loss rate (mortality and emigration) increases and/or the gain rate (birth and immigration) decreases (see review by Hixon et al. 2002). These compensatory processes can affect the bounded fluc-

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tuations and return tendency necessary for the long-term persistence of a population (Murdoch 1994). Thus, understanding how introduced predators can alter the relationship between prey density and mortality is important for predicting the possible ecological consequences of invasion.

In reef-associated fishes, local population sizes are often sensitive to changes in the rate of post-settlement mortality caused by predation (Almany & Webster 2006). In many cases, predation causes density-dependent mortality in early post-settlement reef fishes (see reviews by Hixon & Webster 2002, Osenberg et al. 2002, Hixon & Jones 2005, White et al. 2010). Density-dependent predation in fishes can be mediated by the interplay of multiple factors, including competition (Carr et al. 2002, Hixon & Jones 2005), availability of prey refuge (Forrester & Steele 2004), parasitism (Forrester & Finley 2006), behavioral responses by predators (Anderson 2001, Webster 2003), the synergistic effects of multiple suites of predators (Hixon & Carr 1997), the spatial scale of observation (White & Warner 2007), and the relative scales of predator and prey habitat use (White et al. 2010). Because the predatory response to prey density is mediated by the identity and behavior of predators, it is difficult to predict *a priori* how prey population dynamics may be altered by the introduction of an invasive predator, particularly when they share little or no evolutionary history.

Previous work comparing the functional responses of native and invasive freshwater and marine invertebrates in a laboratory setting demonstrated that the high predatory impact of non-native predators can be attributed, at least in part, to higher per predator consumption rates (Hooff & Bollens 2004, Bollache et al. 2008, Haddaway et al. 2012). Dick et al. (2014) demonstrated experimentally that invasive bloody red shrimp *Hemimysis anomala* had higher prey consumption rates than native analogues and that the invader displayed a potentially destabilizing Type II functional response. To our knowledge, however, no previous study has compared the density–mortality relationship in prey before and after a predatory invasion using paired field experiments. Here, we use such an approach to explore the predatory effects of a recent and especially harmful introduced predator, the Indo-Pacific red lionfish *Pterois volitans*.

Lionfish were first reported in the coastal waters of Florida in the 1980s (Morris & Whitfield 2009) and have spread rapidly throughout the tropical and subtropical western Atlantic and greater Caribbean regions (Whitfield et al. 2002, Schofield 2009, 2010). Within their invaded range, lionfish have reached much higher densities than those reported from the Indo-Pacific (Kulbicki et al. 2012). Their arrival in the region has precipitated large-scale declines in prey biomass (Green et al. 2012). Having high consumption rates and a generalist diet (Albins & Hixon 2008, Morris & Akins 2009, Côté & Maljković 2010), lionfish can drastically reduce recruitment of native species of reef fish (Albins & Hixon 2008, Albins 2013), and their direct and indirect effects on other species can substantially alter native reef fish communities (Albins & Hixon 2013, Côté et al. 2013).

A popular aquarium fish and common native species of the Caribbean that is now prey to invasive lionfish is the fairy basslet *Gramma loreto* (Morris & Akins 2009). Before the lionfish invasion, Webster (2003) demonstrated experimentally that local basslet populations in the Bahamas were regulated by temporal density-dependent mortality due to predation by small groupers and other native mesopredators. As predators provide a key mechanism of basslet population regulation, it is likely that fairy basslet dynamics would be sensitive to the addition of a novel predator. Therefore, to detect and quantify potential changes to the density-dependent processes observed after the invasion, we repeated an important component of Webster's fairy basslet density manipulation subsequent to the arrival of lionfish to the Bahamas. By replicating the original density manipulations at the same reef locations, now in the context of predation by both native piscivores and lionfish, we present an unprecedented before-and-after comparison of density-dependent mortality patterns, providing insight into a novel predator–prey interaction and its potential to disrupt prey regulation. Specifically, we tested (1) whether fairy basslet mortality rates have increased, (2) whether mortality in fairy basslet has remained density-dependent, and (3) whether and how density dependence (the intercept and slope of the density–mortality curve) has been altered following invasion by lionfish.

MATERIALS AND METHODS

Study species

The fairy basslet forms local populations of juveniles and adults, inhabiting the undersides of distinct reef ledges (Böhlke & Randall 1963, Böhlke & Chaplin 1994, Asoh 1996). Isolated aggregations are demographically open via pelagic larval dispersal, yet movement of juveniles and adults among reef ledges is sufficiently rare that they can be considered local

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populations (Webster 2003). These local populations form size-structured social hierarchies whereby larger individuals have first access to passing planktonic food by positioning themselves at the outermost opening of the ledge (Webster & Hixon 2000). Intraspecific aggression forces smaller fish to the backs of ledges (Webster 2004) where they are subject to greater mortality from native mesopredators such as graysby and coney groupers (*Cephalopholis cruentata* and *C. fulva*). This predation is density-dependent, contributing to between-generational regulation of local population sizes (Webster 2003).

Density manipulation: pre-invasion

Prior to the lionfish invasion, Webster (2003) conducted a controlled field experiment at 2 sites on natural reefs near Lee Stocking Island, Bahamas. This experiment involved 16 local fairy basslet populations initially ranging in size from 8 to 55 individuals. Basslet populations were paired by habitat similarity (ledge size, rugosity, substrate, etc.) with 1 from each of 8 pairs being randomly assigned to receive an increase in the density of new basslet recruits and the other being left as an unmanipulated control. Prior to density manipulations, all fish were tagged subcutaneously with elastomer pigment to differentiate these fish from subsequent settlers and to allow demographic rates (recruitment, mortality, immigration, and emigration) to be measured separately. Divers then enhanced basslet recruitment by transplanting new settlers (<2 cm total length) to populations >20 m from their natal reefs. Censuses commenced 24 h after recruit manipulations to allow transplanted fish to recover from any handling effects, and weekly censuses of each population continued for 50 d.

Density manipulations: post-invasion

From 4 July to 2 September 2011, we partially replicated the density manipulations described above, manipulating fairy basslet populations at the same time of year and on the same 16 reef ledges studied by Webster (2003). On ledges that received enhanced recruitment, we achieved densities nearly identical to the pre-invasion manipulations, with a mean of 13.82 fish m^{-2} and a range of 10.59 to 23.57 fish m^{-2} (compared to a pre-invasion mean of 13.35 fish m^{-2} and range of 10.93 to 22.17 fish m^{-2}). Unmanipulated population densities of fairy basslet were lower than those reported by Webster (2003), with a mean of 4.17 fish m^{-2} and a range of 2.02 to 7.41 fish m^{-2} (compared to a pre-invasion mean of 8.09 fish m^{-2} and range of 4.44 to 11.78 fish m^{-2}). This substantial decrease in population density is consistent with the findings of Green et al. (2012), who reported on average 65% declines in biomass across 42 native prey species contemporaneous with the ongoing invasion by lionfish.

We conducted a complete census of each basslet population weekly for 8 wk, recording the total number of fairy basslet individuals at each experimental ledge. Due to logistical constraints, we were unable to individually mark prior-resident fish before density manipulations, in contrast to the pre-introduction experiment. Therefore, we were unable to track separate demographic rates of each fairy basslet population. Rather, at the end of the 8 wk study period, we calculated net per capita loss of basslet for each population as the proportional change in abundance from the beginning to the end of the experiment. Loss (an aggregate measure of population change) differs from mortality in that it is inflated by emigration and reduced by natural settlement and immigration. Therefore, we re-analyzed Webster's pre-introduction data to calculate per capita loss in order to compare identical metrics of density dependence across experiments. It is likely that loss patterns reflect similar patterns of mortality for the following reasons: (1) previous work on these same populations showed that rates of emigration, while density-dependent, were negligible compared to mortality, such that loss of individuals from a population was overwhelmingly driven by post-settlement mortality (Webster 2003); and (2) both immigration and recruitment were shown to be independent of fairy basslet density, so patterns of loss would not be systematically biased by experimentally inflated recruitment.

Predator observations

Webster (2003) employed automated video monitoring on paired basslet populations to document density dependence in the presence of, and amount of time spent actively hunting by, native predators (i.e. an aggregative response). In the post-invasion experiment, we recorded the number, species, and total length of any predator within 2 m of the study ledges at the time of each weekly census. This approach allowed us to index the relative abundance of predators and to detect any aggregative response to local increases in prey density by lionfish or native predators.

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Statistical analysis

To quantify changes in fairy basslet mortality patterns since the introduction of lionfish, we employed a series of paired *t*-tests comparing per capita loss both between and within experiments. First, to test for the presence of density dependence in each experiment, we compared per capita loss rates between control and recruitment-enhanced populations using Webster's original population pairs. For this comparison, higher loss rates in the recruitment-enhanced populations would indicate (compensatory) density dependence. Second, to quantify changes in the mortality rates for each treatment group across experiments, we compared loss rates at identical ledges before and after the arrival of lionfish. Two-sided *t*-tests were employed throughout due to the potential for additive, compensatory, or synergistic effects of multiple predators. Because we sought simultaneous inferences regarding the resulting 4 (non-independent) pairwise comparisons, a Holm-Bonferroni correction was employed to account for the inflation of Type I error probability.

Natural variation in the unmanipulated densities of fairy populations created a continuous density gradient. Our recruitment enhancement extended this continuous range. Therefore, we used regression to quantify changes in the slope and/or intercept of the density–mortality curve using initial fairy basslet densities as a continuous predictor of per capita loss. We employed a linear mixed effects model (LMM) with 1 continuous and 1 categorical explanatory variable ('basslet density' and 'lionfish presence', respectively) as well as a 2-way interaction between those terms as fixed effects. A significant interaction between basslet density and lionfish presence would represent an alteration of the slope in the presence of lionfish (i.e. a change in the direction or intensity of density dependence). We also incorporated a 'ledge' term as a random effect in order to account for positive correlations in the response of identical basslet populations between experiments (non-independence).

We tested the significance of fixed effects using *F*-tests and estimated parameters of the final model using restricted maximum likelihood estimation (Zuur et al. 2009). Likelihood ratio tests (LRT) with a correction for 'testing-on-the-boundary' (Pinheiro & Bates 2000) indicated that inclusion of the random ledge term resulted in better fit than a fixed-effects only model (*L*-ratio = 16.35027, p = 0.010). A test for heterogeneity provided no evidence to reject the null hypothesis of equal variance (*L*-ratio = 1.529, p = 0.22) and visual inspection of the residuals indicated that all other assumptions of linear mixed effects model had been met.

We measured relative predator abundance by recording the number, species identity, and total length of each individual predator within 2 m of an experimental basslet population during the weekly censuses. These counts were then averaged across the total number of censuses to provide a metric of relative species encounter frequency throughout the post-invasion experiment. We acknowledge that individual predators are likely to have been counted in multiple censuses, and therefore cumulative counts do not provide a direct measure of absolute abundance of predators in the study area. Rather, time-of-census predator observations provide an index of relative predator abundance and species encounter rates.

To determine whether native predators and/or lionfish displayed aggregative responses toward higher densities of fairy basslet prey, we employed generalized linear mixed effects models (GLMM) with a logit link function, with the presence or absence of predators at the time of census as a function of the basslet population density (fixed effect). In order to account for repeated measures at the basslet population level, we included ledge as a random effect. LRT (*Z*-statistic) were used to test the significance of basslet density as a predictor of predator presence, (i.e. an aggregative response). 'Predator presence' was defined as one or more individual predators within 2 m of the focal fairy basslet population at the time of the census. For the small number of events (n = 9) where multiple native or multiple lionfish predators were observed on the same ledge during the same census, all individuals were recorded but for the purposes of the logistic regression, the response was collapsed to 'present.' GLMMs were employed separately for native predators and lionfish. All statistical analyses were conducted using R v.3.1.3 (R Core Team 2014) using the packages 'nlme' (Pinheiro et al. 2014) and 'lme4' (Bates et al. 2007) for analysis of mixed-effects models.

RESULTS

Predator observations

Potential predators were observed within 2 m of fairy basslet populations on 50 of 140 censuses. A total of 8 piscivore species from 4 families were observed (Table 1). During post-invasion censuses, lionfish were the second most commonly observed

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Table 1. Relative abundance of potential fairy basslet *Gramma loreto* predators as observed during weekly censuses. Total observations: the number of individuals of each species observed at experimental ledges over the course of the survey period; frequency: average number of individuals of each predator observed per basslet population per census. As individual predators may have been observed during multiple censuses, these values represent an index of relative predator abundance. Introduced lionfish (**bold**) were the second most frequently observed resident predator in the study area. Large, mobile predators (e.g. jacks) were not recorded

Rank	Scientific name	Common name	Family	Total observations	Frequency (obs. census^{-1})
1	*Cephalopholis fulva*	Coney grouper	Serranidae	26	0.186
2	***Pterois volitans***	**Red lionfish**	**Scorpaenidae**	**16**	**0.114**
3	*Cephalopholis cruentata*	Graysby grouper	Serranidae	12	0.086
4	*Epinephelus striatus*	Nassau grouper	Serranidae	12	0.086
5	*Aulostomus maculatus*	Trumpetfish	Aulostomidae	4	0.029
6	*Lutjanus apodus*	Schoolmaster snapper	Lutjanidae	3	0.021
7	*Liopropoma rubre*	Peppermint basslet	Serranidae	1	0.007
8	*Serranus tigrinus*	Harlequin Bass	Serranidae	1	0.007

predator species (observed at 16 censuses) while the most common native predators were coney grouper (26 censuses) and graysby grouper (12 censuses).

We found inconclusive evidence for aggregative behavior by native predators (GLMM LRT; $Z = 1.77$, $p = 0.076$) and by lionfish ($Z = -1.65$, $p = 0.098$). Notably, while these tests did not provide strong evidence, we observed opposing trends in aggregative behavior between native and non-native predators. On average, the odds of observing lionfish decreased with increasing basslet density, a multiplicative change of 0.80 for each individual m^{-2} increase in fairy basslet density (odds-ratio).

Density manipulation

After experimental manipulation of post-invasion populations, initial fairy basslet densities in recruitment-enhanced populations were not different from those of the pre-invasion experiment (2-sided paired *t*-test; $t = 1.302$, $df = 7$, $p = 0.23$). In contrast, unmanipulated fairy basslet populations had substantially lower initial densities in the post-invasion experiment ($t = -3.885$, $df = 7$, $p = 0.006$). Between experiments, mean basslet density decreased by 3.92 fish m^{-2}, an average decrease of 43.5%.

Per capita loss

Across experiments, per capita loss in fairy basslet over 8 wk ranged from −0.27 (negative value reflecting population growth over the study duration) to 0.91. Of the 16 populations in the post-invasion experiment, 7 experienced >50% loss over 8 wk, including 2 unmanipulated control populations. At one ledge, loss approached 100% during the study period despite a moderate initial basslet density. In contrast, prior to the lionfish invasion only 2 populations experienced per capita loss greater than 30%.

Across-experiment comparisons

Compared with pre-invasion rates, per capita loss was higher in 13 out of 16 basslet populations and in all 8 recruitment-enhanced populations (Fig. 1). In the post-introduction experiment, 5 of 8 unmanipulated control populations also experienced increased per capita loss despite lower initial basslet densities. Comparing control populations before versus after the lionfish invasion, we observed on average 1.7 times higher per capita loss in the presence of lionfish (Fig. 2); however, high variability in loss rates among populations meant that loss rates at basslet controls were not statistically different between experiments ($t = 0.35$, $p = 0.74$, $df = 7$). In contrast, recruitment-enhanced populations experienced higher per capita loss with lionfish present ($t = 5.87$, $p = 0.002$, $df = 7$). On average, per capita loss on manipulated ledges increased 1.6 times from 33.2 ± 7.61% (mean ± SE) pre-invasion to 53.8 ± 6.62% in the presence of lionfish.

Within-experiment comparisons

Re-analyzing Webster's pre-invasion data to calculate per capita loss did not qualitatively alter the

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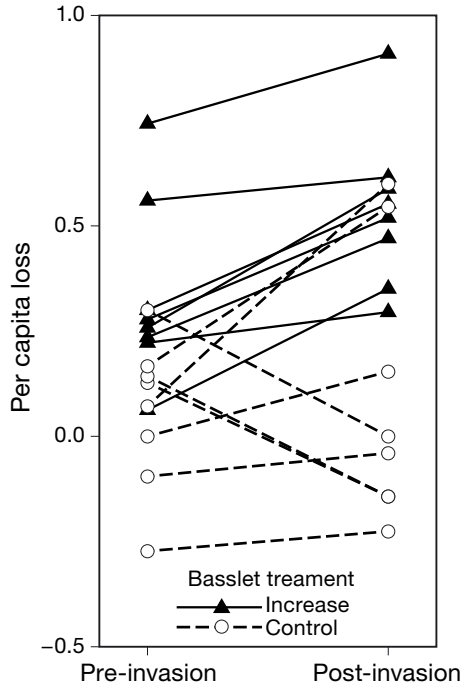

Fig. 1. Change in per capita loss of fairy basslet *Gramma loreto* over 8 wk in each local population across experiments, comparing pre-invasion loss rates to values observed in the same populations after the introduction of lionfish *Pterois volitans*. Of 16 populations, 13 experienced higher loss rates in the post-introduction experiment, including all 8 recruitment-enhanced populations ('increase'). Five of 8 unmanipulated populations ('control') also experienced increased per capita loss in the post-introduction experiment despite lower initial basslet densities

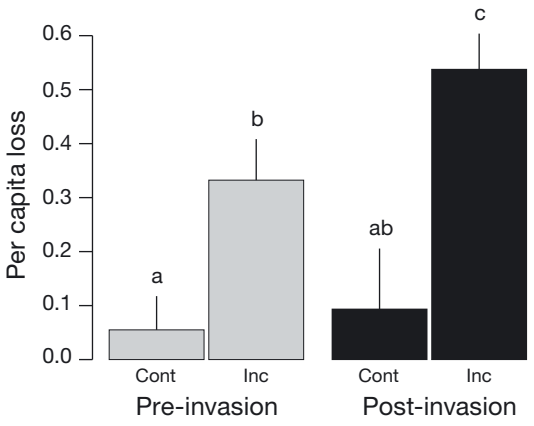

Fig. 2. Mean per capita loss of fairy basslet *Gramma loreto* over 8 wk during field experiments conducted before and after lionfish *Pterois volitans* invasion. Cont: average per capita loss in unmanipulated basslet populations (n = 8 populations experiment^{-1}); Inc: average per capita loss in populations that received enhanced recruitment (n = 8). Lowercase letter labels indicate significant differences (Holm-Bonferroni corrected paired *t*-tests; familywise threshold $p < 0.05$). Note: Error bars represent 1 SE of the group means and are not reflective of significance based on the paired-sample tests

results obtained by analyzing mortality; loss rates were higher in recruitment-enhanced populations compared to paired controls ($t = 2.953$, $p = 0.021$, df = 7), indicating density dependence in fairy basslet loss rates. Prior to the invasion, per capita loss was approximately 6 times higher at recruitment-enhanced populations versus pre-invasion controls (33.2 ± 7.6% vs. 5.50 ± 6.25%). Further, per capita loss remained strongly density-dependent in the post-invasion experiment, with recruitment-enhanced populations experiencing higher loss rates than paired control populations ($t = 5.81$, $p = 0.002$, df = 7). Loss at control populations averaged 9.35 ± 11.2% compared to 53.8 ± 6.62% at enhanced populations, a ratio nearly identical to the 6-fold increase between control and recruitment-enhanced populations observed in the pre-invasion experiment (Fig. 2). A time series of changes in basslet density and per capita loss over the course of the 8 wk post-invasion experiment is included in the Appendix.

Changes in density-dependent curve

The linear mixed-effects model selection procedure indicated significant terms for both 'basslet density' (LMM; $F = 11.3$, $p = 0.0047$, df = 14), and 'lionfish presence' ($F = 21.0$, $p < 0.001$, df = 14), confirming that per capita loss remained density-dependent (albeit at higher magnitude) in the post-invasion experiment. Per capita loss was positively correlated with initial prey density. On average, per capita loss increased 0.051 for each unit increase in fairy basslet density. After accounting for basslet density, loss rates increased by 0.142 compared to pre-invasion rates, a density-independent increase. However, we found no evidence to include an interaction term between 'experiment' and 'basslet density' factors ($F = 0.25$, $p = 0.62$, df = 13), suggesting no evidence that lionfish had altered the slope of density dependence in fairy basslet loss (Fig. 3). While a single basslet population was identified as highly influential due to the value of initial basslet density (see Fig. 3), its omission did not alter the conclusions of the analysis.

DISCUSSION

Since the introduction of Indo-Pacific red lionfish to Atlantic coral reefs, per capita loss rates of native prey fishes have increased greatly (Albins & Hixon 2008, Green et al. 2012, Albins 2013). Our field experiments, repeated before and after the arrival of this

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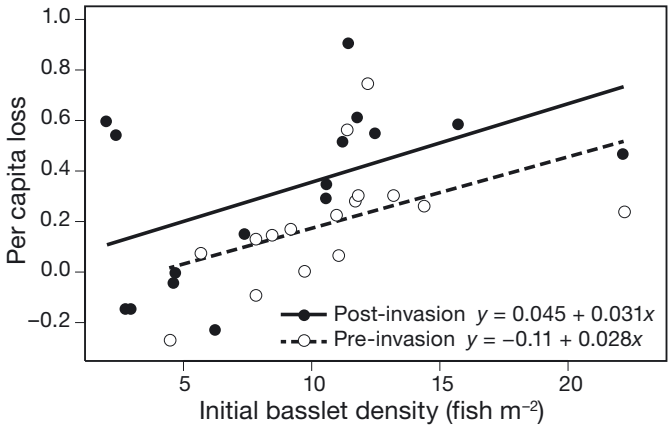

Fig. 3. Per capita loss of fairy basslet *Gramma loreto* as a linear function of initial density before and after lionfish *Pterois volitans* invasion. Post-invasion loss rates showed significantly higher intercept (*F*-test, $p < 0.001$) but no difference in slope ($p = 0.62$). Regression lines are fitted from GLMM

novel mesopredator, revealed a density-independent increase in loss of fairy basslet compared to a pre-invasion experiment, thereby providing circumstantial evidence that lionfish add substantially to post-settlement mortality of fairy basslet but have not (as yet) altered the direction or intensity of density-dependent loss.

Attributing mortality increase to lionfish

Although the marginal difference in loss between experiments cannot be attributed unambiguously to the invader because other sources of mortality may have changed before and after the lionfish invasion, additional evidence strongly implicates lionfish as a primary driver of increased post-settlement mortality. First, our predator observations at the time of the censuses indicated that lionfish were the second most abundant predatory fish on experimental reefs. Second, we observed that natural, unmanipulated densities of fairy basslet populations were nearly halved contemporaneously with the arrival and subsequent population explosion of lionfish. A 3 yr pre-invasion time series indicated that basslet population levels were tightly regulated and, after accounting for seasonal cycles, showed little year-to-year variability (Webster 2003). Therefore, an average reduction of 3.92 fish m^{-2} at the identical reefs over the same period falls well outside of the expected range of inter-annual variability. Additionally, our observed increase in prey mortality was corroborated by the results of previous lionfish manipulation experiments that recorded both drastic reductions in average abundances of small native fishes as well as declines in species richness in the presence of lionfish. In predator manipulations on natural and artificial patch reefs, Albins & Hixon (2008) observed 79% reductions in the recruitment of native prey caused by the presence of a single lionfish. A subsequent experiment (Albins 2013) demonstrated >90% reductions in native prey compared to predator-free controls, a predator effect 2.5 times greater than that of the native coney grouper. Our observed increase in prey loss also substantiates observations by Green et al. (2012), who demonstrated 65% reductions in the biomass of prey-sized fishes (including fairy basslet) at 9 locations in the Bahamas coinciding with a rapid increase in local lionfish abundance. Likewise, a comparison of the fish assemblage prior (2003 to 2006) and subsequent (2009) to the arrival of lionfish on mesophotic reefs (30 to 150 m) in the vicinity of our experimental reefs documented major reductions in abundance and species richness (Lesser & Slattery 2011). Thus, our observations, combined with previously published small-scale lionfish manipulation experiments and large-scale observations, implicate invasive lionfish as a dominant driver of changes in fairy basslet mortality.

Patterns of density dependence

While we observed an increase in loss on recruitment-enhanced populations compared to the identical populations in the pre-invasion experiment, there was no evidence that per capita loss differed statistically on unmanipulated basslet populations between experiments (Fig. 2). Among these populations, mean per capita loss increased nearly 70% in the presence of lionfish — despite the fact that initial prey densities were somewhat lower in the post-introduction experiment — but high variability in both experiments meant that this difference was not statistically significant. Two different factors may underlie this result. First, absolute population sizes on unmanipulated populations (rather than prey densities per se) tended to be small relative to the recruitment-enhanced populations. In a population with few individuals, a single predation event can substantially alter per capita loss. Thus, high variability in the per capita response among unmanipulated populations may be an artefact of their sensitivity to chance events. Secondly, the initial densities of these fairy basslet populations were much lower in the post-introduction experiment, which could bias loss rates downward. In order to replicate Webster's pre-lionfish manipulation as closely as possible, we allowed con-

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trol populations to remain at their observed densities, which were on average 43% lower than those observed pre-introduction (Fig. 3). As fairy basslet loss was strongly density-dependent in both experiments, lowered initial densities in the post-invasion experiment may have resulted in lower mortality than would have been observed had starting densities matched the pre-invasion experiment. Indeed, the 3 populations that experienced lower loss rates in the post-introduction experiment (Fig. 1, dashed lines with negative slope) were among those with the greatest decrease in initial prey density between experiments (ranked 1st, 3rd, and 4th), with an average 63% decrease in initial fairy basslet density.

Despite the potential bias of overall lower population sizes, we still observed a non-significant increase in per capita loss among fairy basslet control populations, an indication of the strength of lionfish direct effects. Indeed, the results of the linear model using initial prey density as a continuous predictor of basslet loss — thus accounting for differences among pre- and post-introduction population starting densities — indicated a change in intercept but not slope between experiments (Fig. 3). In essence, we found that while the overall magnitude of basslet loss was higher in the presence of the invader, the intensity of the density-dependent mortality curve was unchanged between experiments. Thus, we can conclude that mortality rates increased across the entire range of prey densities.

Potential behavioral mechanisms

A possible explanation for this cumulative density-independent increase in mortality rates is that lionfish themselves cause density-independent mortality that is simply added to the density-dependent mortality caused by native predators. Our experiment did not explicitly test the behavioral mechanisms underlying our results, yet based on our anecdotal observations as well as known behavioral and morphological differences between native predators and lionfish, several non-mutually exclusive hypotheses could explain these results.

First, differences in spatial patterns of foraging between native and introduced predators could lead to differential prey mortality patterns. Over the short span of an 8 wk field experiment, predators can cause density-dependent mortality only through (1) a Type III functional response, where individual (per predator) consumption rates are an accelerating function of increasing prey density (Holling 1959, Murdoch 1969); (2) an aggregative response, where predator density is associated with higher prey concentrations (Hassell & May 1974); or (3) a combination of both. Several studies of reef fishes have identified predator aggregation as a common behavioral mechanism leading to density dependence (Hixon & Carr 1997, Anderson 2001, Webster 2003, but see Overholtzer-McLeod 2006). In these same basslet populations prior to the invasion, data from automated time-lapse video cameras demonstrated that native predators were observed more frequently, and spent a greater proportion of their time actively hunting near recruitment-enhanced basslet populations compared to controls (Webster 2003). This aggregative response led to the density-dependent mortality observed in the pre-invasion experiment. In contrast, the current study demonstrated no such aggregative response by lionfish. In fact, lionfish presence was weakly associated with lower fairy basslet densities, a spatial distribution that would not, by itself, lead to density-dependent mortality.

It should be noted that we found inconclusive evidence of an aggregative response in either category of predators using time-of-census predator observations. Diver observations may be less powerful than remote video surveys for capturing unbiased counts due to altered fish behavior in the presence of divers (Lindfield et al. 2014). An additional caveat is that lionfish activity levels and foraging rates increase during the crepuscular period (Green et al. 2011, Cure et al. 2012); thus, diurnal time-of-census observations may not capture spatial patterns of foraging, including potential aggregative responses. While we would not recommend drawing any strong conclusions from our predator observations, we suggest that lionfish foraging behavior may be an important mechanism driving differential predator effects, and deserves further study.

The idea that different foraging behavior between native and invasive predators could lead to the observed prey mortality patterns is consistent with the scale-dependency advanced by White et al. (2010) for resolving contradictions in patterns of density-dependence observed in studies of reef fishes. While native ambush predators cause density-dependent mortality by aggregating at the scale of local basslet populations, lionfish foraging patterns may exceed the spatial scale at which basslet concentrations are clumped. Thus, lionfish predation could cause density-independent mortality at the spatial scales of our study because their foraging effort is distributed more or less evenly across a large area of continuous reef containing multiple local

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basslet populations. Future studies comparing how the spatial scale of foraging patterns differ between native and introduced predators may lead to insight into their effects on prey dynamics.

A second potential mechanism for resolving the observed mortality patterns involves prey refuge availability and predator recognition. In reef fishes, the relative availability of predator-free shelter space can mediate the per capita risk of predation, thereby altering the slope of density-dependent mortality (Forrester & Steele 2004). Anti-predator defense in fairy basslet is characterized by individuals fleeing into small refugia within the ledges that they inhabit (Webster & Hixon 2000). In Pacific damselfishes at high conspecific density, competition for limited refuge can result in correspondingly high per capita mortality as some individuals are unable to secure adequate shelter (Holbrook & Schmitt 2002). In contrast, low-density populations experience higher relative refuge availability and are subject lower per capita mortality rates. However, antipredator responses are only effective if predation threats are recognized as such. Lionfish are morphologically and behaviorally unique in their invaded range (Albins & Lyons 2012) and may not present similar predator cues to those provided by native piscivores (Albins & Hixon 2008). Indeed, evidence from a controlled lab study demonstrated that even sympatric Pacific damselfish *Chromis viridis* that were conditioned to *Pterois volitans* olfactory cues displayed reduced antipredator responses and increased mortality compared to trials with other scorpaenid and serranid predators (Lönnstedt & McCormick 2013). If fairy basslet are naïve to the threat of lionfish predation and fail to respond appropriately, then lionfish may consume a large proportion of prey even when shelter is abundant at low prey density. Anecdotally, we observed many native prey fishes, including fairy basslet, showing a reduced flight response to approaching lionfish compared to native predators. Thus, predator crypsis or lack of recognition may allow lionfish to exploit prey equally effectively across the range of prey densities — despite differences in relative shelter availability — resulting in the observed pattern of increased density-independent mortality.

Key assumptions

Attributing the observed density-independent increase to lionfish requires the nontrivial assumptions that (1) native predators have not increased fairy basslet consumption rates, and (2) lionfish foraging neither interferes with nor causes synergistic effects when combined with foraging by native predators. In evaluating the first assumption, an alternative explanation for our results is that the abundance and/or per capita consumption rates by native mesopredators have increased since the pre-invasion experiment leading to greater basslet post-settlement mortality. However, several lines of evidence argue against this scenario. First, while we cannot directly compare predator abundance, encounter, or consumption rates due to the different nature of the predator observations between the 2 experiments, regional trends in piscivore abundance over this time period offers circumstantial evidence. Observational data across the greater Caribbean from 1994 to 2008 indicate that the most commonly observed native mesopredators during the post-invasion experiment either show no temporal signal in abundance (*Cephalopholis fulva*, *C. cruentata*) or a significant decline (*Aulostomus maculatus*) (Stallings 2009). More recently, observations from another location in the Bahamas demonstrated substantial declines in the biomass of native mesopredators coinciding with increases in lionfish abundance (Green et al. 2012). In addition to density-mediated changes in predation rates, removal of large-bodied piscivores such as Nassau grouper *Epinephelus striatus* can lead to behavioral release of smaller mesopredators (Stallings 2008), potentially increasing prey mortality rates. However, large-bodied Nassau grouper were abundant on study reefs, with the greatest biomass and second greatest abundance of native piscivores observed during censuses. Also, while not enumerated during time-of-census surveys, large mobile predators such as reef sharks *Carcharhinus* spp. and large jacks (Family *Carangidae*) were commonly observed throughout the study area, further reducing the likelihood of behavioral-release of small groupers. It therefore appears unlikely that either increased abundance or behaviorally-mediated release of native mesopredators could alone account for the observed increase in basslet mortality.

In order to evaluate the potential for interactive effects of multiple predators (compensatory or synergistic predation), an alternative experimental design with lionfish presence/absence and native predator presence/absence manipulated orthogonally would be required. While we were prevented from employing a cross-factor predator manipulation due to the mobility of predators in continuous reef habitat, this design has been conducted on small patch reefs with *P. volitans* and *C. fulva* as the focal predators (Albins 2013). The results of this study were consistent with

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compensatory predation. However, it should be noted that predator interactions on small patch reefs may play out differently than on large, continuous reefs where predator movement may be more important and where habitat characteristics may play a different role. For example, we observed native predators most often lurking toward the back of reef ledges in our study, while lionfish more frequently foraged along the outer edge of the reef (authors' pers. obs.). Thus, predator avoidance in response to native predators could make fairy basslet more susceptible to predation by lionfish, and vice versa (synergistic predation, sensu Hixon & Carr 1997). As our study cannot directly discriminate between additive versus non-additive predation, future work should evaluate native and non-native predator interactions.

Implications for population regulation

This study provides evidence that an introduced predator can alter the density–mortality relationship derived from native interactions alone by raising per capita mortality rates across a range of prey densities. We found that since the introduction of lionfish, mortality rates increased substantially but remain density-dependent for local prey populations. The ultimate risk of such additional mortality is the extirpation or even extinction of native prey species. Subsequent field experiments with invasive lionfish have demonstrated cases of extirpation of local fairy basslet populations (K. E. Ingeman unpubl. data), as well as declines in local species richness compared to lionfish-removal reefs (Albins 2015).

The large-scale effects of local extirpation remain uncertain. Extinctions in marine systems where local populations may be continually recolonized by larval dispersal are relatively rare, and to date there are no known global marine fish extinctions (Dulvy et al. 2003). However, invasions by marine predatory fish are also rare, and lionfish may represent an unprecedented scenario whereby the wide geographic range, extreme population densities, and morphological and behavioral novelty contribute to extremely high predation rates (Albins & Hixon 2013, Côté et al. 2013). Our observation that loss in fairy basslet remained density-dependent in the presence of lionfish means a potential regulatory mechanism remains intact. Despite increased predation rates, prey populations could achieve (dynamic) stability over time, albeit at lower average densities. However, a density-dependent demographic rate is a necessary, but not sufficient condition of population regulation (Murdoch 1994), and determining the long-term viability of prey populations would require monitoring changes in recruitment rates as well. Further, from an invasion-wide perspective, there is no evidence that lionfish population densities have reached a maximum (Albins & Hixon 2013), and lionfish predation at the metapopulation scale may lead to regional declines, eventually swamping any compensatory dynamics at the level of local prey populations.

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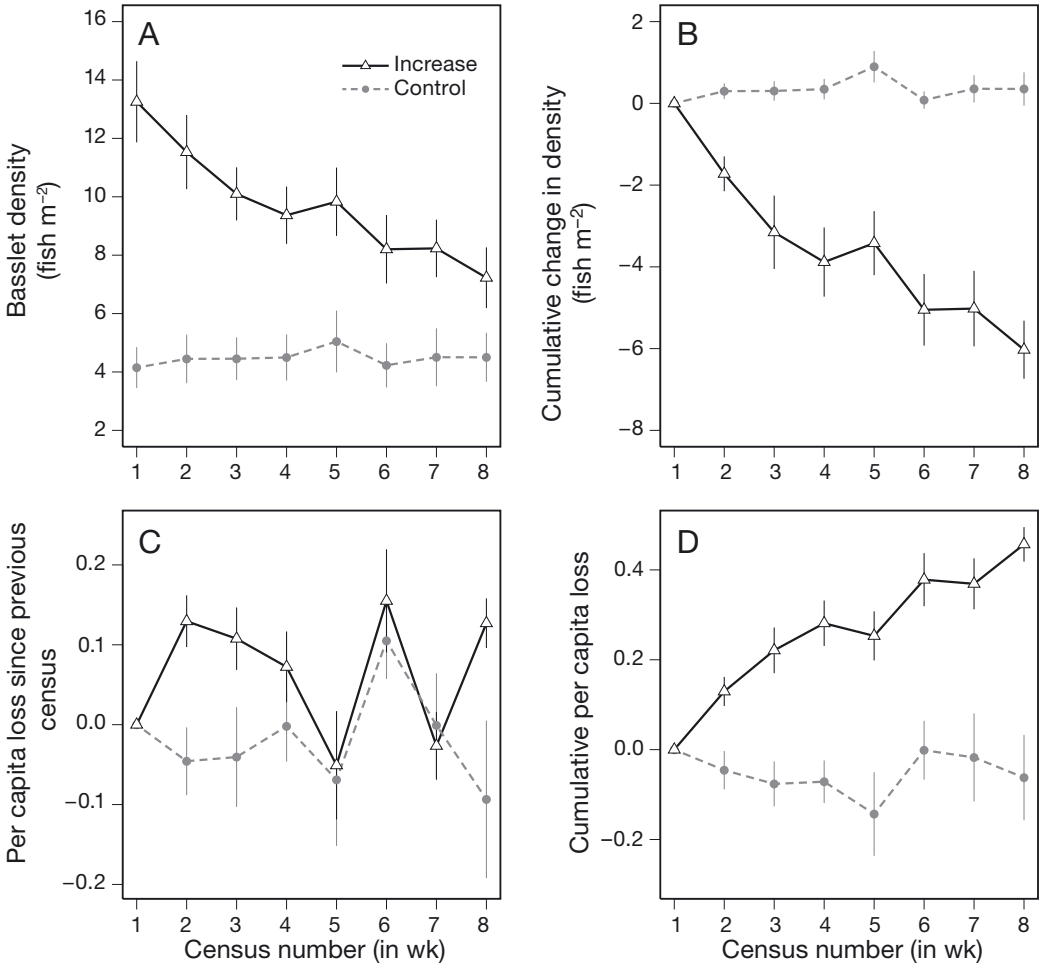
Appendix. Time series of fairy basslet (A) density, (B) cumulative change in density, (C) interval per capita loss (since previous census), and (D) cumulative per capita loss (since initial census) at both recruitment-enhanced and control populations. Comparing (A) and (C), it is evident that much of the loss in recruitment-enhanced populations was experienced early in the experiment when the absolute densities were highest. However, in the final censuses, after a short-term drop in per capita loss across both treatments (likely reflecting a natural recruitment pulse) loss is substantially higher in increase populations despite a moderate difference in prey density between treatments



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