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Invasive lionfish *Pterois volitans* reduce the density but not the genetic diversity of a native reef fish

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ABSTRACT: The Indo-Pacific red lionfish *Pterois volitans* has spread throughout the western Atlantic causing declines in biomass and diversity of native species at local reefs; worst-case scenarios predict species extinctions and ecosystem phase shifts. While reductions in reef fish population density and recruitment are evident, it is not known whether lionfish are reducing genetic diversity of native species, a major driver of extinction in natural populations. A before-after control-impact experiment was used to determine whether lionfish removals cause an increase in density of native species and genetic diversity in one species, the bicolor damselfish *Stegastes partitus*. We found that removing lionfish significantly augmented the density of several reef fish species. However, while allelic frequencies in bicolor damselfish recruits changed after removals, genetic diversity did not increase substantially despite a 3-fold increase in recruit density. Responses to lionfish removal differed among native species; rare species with small population sizes may be more susceptible to recruitment failure and diversity loss as a result of lionfish predation than widespread species with large populations.

KEY WORDS: Lionfish · Invasive predator effects · Genetic diversity · Native prey · Targeted removals

INTRODUCTION

Biological invasions are among the greatest threats to biodiversity, costing billions of dollars annually in damages to infrastructure and decimating local populations (Pimentel et al. 2005). Invasive predators reduce local prey through direct interactions but also affect non-prey species through competition for prey or trophic cascades (Johnson et al. 2009). Changes to community structure and trophic organization associated with invasions are of major concern for the structure and function of native communities and ecosystems and the services they provide. Negative effects of invasive species on native ecosystems have been described; however, the potential effect of invasive species on native genetic diversity has received less attention (Parker et al. 1999, Vilà et al. 2011).

Loss of genetic diversity in populations is troubling, because it hinders the ability for populations to respond to environmental changes and stressors (Booy et al. 2000). In a meta-analysis, Spielman et al. (2004) reported that out of 170 pairs of taxa, 77% of endangered or threatened species showed an average of 35% lower genetic diversity (i.e. heterozygosity) than comparable non-endangered or threatened species in the same taxonomic group. Frankham (1995) named biological invasions along with habi-

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tat loss as a primary factor that causes extinction for genetic reasons; biological invasions can lead to increased inbreeding depression, accumulation of deleterious mutations, and loss of diversity in native populations due to genetic drift.

Invasive predators can have 2 main effects on genetic populations, viz. selection and genetic drift. Selection occurs when predators remove prey non-randomly from a population, where prey with a certain trait are more or less likely to be removed. Selection is an ongoing process and begins as soon as prey removal begins. The strength of selection and size of the prey population will determine the rate at which observable changes in gene frequencies and diversity appear in the prey population. Even rapid evolutionary changes are expected to take years to decades to manifest themselves in the gene frequencies of the populations (Thompson 1998). Additionally, selection is expected to act only to change gene frequencies of the specific genes under selection, leaving unaffected or unlinked genes unaltered. Drift, on the other hand, is the random removal of genotypes from the population (Hartl & Clark 2007). Drift is also an ongoing process in every population and at low levels; in large genetic populations, it will not significantly change genetic diversity of a population. However, in situations where predation is severe and the predator removes a large proportion of the population creating a genetic bottleneck, predation can act as a strong drift effect and cause genetic diversity reductions. In the case of drift, changes in gene frequencies are expected to happen across the genome (Hartl & Clark 2007). When such predation bottlenecks occur on a recruitment cohort (e.g. Doherty et al. 2004), the effect on gene frequencies and genetic diversity can occur over short periods of time (days to months) as the cohort of recruits passes through the predation gauntlet (Larson & Julian 1999).

To our knowledge, only 2 studies have investigated the effect of invasive predators on genetic diversity or patterns of genetic differentiation of native prey species (Gasc et al. 2010, Iwai & Shoda-Kagaya 2012). Iwai & Shoda-Kagaya (2012) concluded that predation by invasive mongoose has driven genetic differentiation among populations of the Japanese Otton frog *Babina subaspera*. Gasc et al. (2010) found a decrease in genetic diversity (i.e. heterozygosity and allelic richness) of brown anole lizards *Anolis sagreii* in the Bahamas after the invasion of a rat predator. The impact of invasive predators on genetic diversity of native species may be a more widespread phenomenon than is currently appreciated; more studies are needed to assess this.

The invasive lionfish *Pterois volitans* was first observed in the western Atlantic in southern Florida in the mid-1980s and has since spread as far north as New York (USA) and as far south as Brazil (Ferreira et al. 2015). In some places, such as the Bahamas, the lionfish is among the more abundant fish species (Morris & Whitfield 2009), reaching densities of 450 ind. ha^{-1}, more than an order of magnitude higher than in its native range (Kulbicki et al. 2012). The lionfish exhibits various traits that contribute to its success: it grows and matures quickly (Morris & Whitfield 2009), and it is a voracious, generalist predator that consumes over 70 species of small reef fish as well as shrimps and other invertebrates (Morris & Akins 2009).

Some prey species in the invaded range appear to be highly susceptible to lionfish predation. For example, Kindinger (2015) found that the native Caribbean damselfish *Stegastes planifrons* fails to recognize the lionfish as a predator and does not respond to lionfish with evasive behavior. In studies of small patch reef sites, lionfish have been shown to reduce the abundance of newly recruited reef fishes by up to 90% (Albins & Hixon 2008) and reduce prey species richness up to 2.4 times more than a native predator (Albins 2013). Lionfish have been predicted, in the worst-case scenarios, to cause reef fish extinctions (Albins & Hixon 2013). This novel predator's voracious appetite, coupled with the naïveté of native prey species, could effectively reduce prey population sizes sufficiently to lower population genetic diversity as other invasive predators have been shown to do (Gasc et al. 2010), exacerbating extinction risk for these species. However, whether lionfish are affecting the genetic diversity of native species is currently unknown.

Here, we experimentally determined the effect of lionfish on the densities of several reef fishes and on the genetic diversity of a common Caribbean native, the bicolor damselfish *S. partitus*. We hypothesized that if lionfish are sharply reducing the abundance of recruits of this (and other) species, then they may create a genetic bottleneck within recruitment cohorts and, therefore, reduce the genetic diversity of those cohorts as they pass through the predation gauntlet. We use a before-after control-impact experiment to determine whether the removal of lionfish can result in increased density and diversity of the bicolor damselfish in Panama. We also compared genetic diversity of bicolor damselfish populations pre- and post-lionfish invasion in Panama.

MATERIALS AND METHODS

Study location

We implemented a before-after control-impact design to study the effects of lionfish removal on the density and genetic diversity of native reef fishes (Fig. 1). We established 1 control (C) and 1 treatment site (T1) in October 2013, and a second treatment site (T2) in May 2014 for additional replication of the study of genetic diversity only. All sites contained lionfish at the time of first survey. Lionfish were removed from the treatment sites during the course of the experiment; the densities of lionfish were not manipulated at the control site. We established these sites at Tiger Rock, located northeast of the Bocas del Toro province of the Republic of Panama (Fig. 1A). The treatment and control sites were chosen based on their close proximity (~1–2 km) to one another and their similar biological structure (see below for description) to standardize naturally occurring differences in currents, recruitment, and substrate class over the archipelago. The habitat is a chain of discrete calcium carbonate reef formations. The 2 western-most sites (C: 9° 13′ 15.94″ N, 81° 56′ 46.95″ W; and T1: 9° 13′ 06.47″ N, 81° 56′ 27.09″ W) are ~100 m long on their seaward sides where surveys were conducted, with a circumference of ~500 m. The reefs extend to near 35 m deep, and each island is separated by ~1 km of sand habitat from the nearest reef. The third site (T2: 9° 12′ 47.77″ N, 81° 55′ 34.44″ W) is ~300 m long on its seaward side and 1 km in circumference, 30 m deep, and 1 km away from the next adjacent reef. All sites experienced a strong northward-flowing current heading out to sea. The benthic reef community is sponge-dominated and home to many reef fish, crinoids, sharks, and, since 2009, invasive lionfish (Schofield 2010).

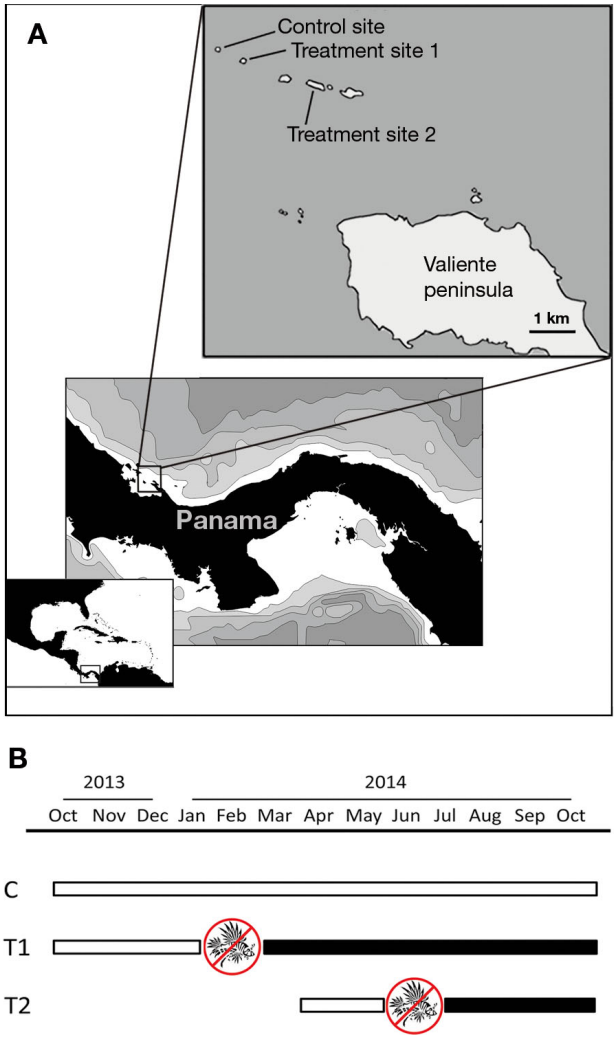

Fig. 1. (A) Study region in the Republic of Panama. Bottom left inset shows the northwestern Atlantic; upper inset shows the study sites at Tiger Rock in relation to the Valiente Peninsula. (B) Timeline for each of 3 sites: Control (C) and Treatments 1 and 2 (T1, T2). White bars: time period during which lionfish densities were unmanipulated; black bars: post-lionfish removal periods

Lionfish surveys and removals

To gauge the efficacy of lionfish removals, we surveyed lionfish densities approximately monthly (weather permitting) at each site with a lionfish-focused survey approach using 4 transects (each 3 m × 30 m) at each site. Divers would swim in a sinusoidal pattern along the transect, roving ~1.5 m on either side of the transect tape. Transects were conducted at depths of 10, 12, 15, and 18 m, parallel to the reef crest. We collected baseline ('before') data at sites C and T1 for 3 mo prior to the experimental removal of lionfish starting in October 2013. At T2, we surveyed baseline lionfish density in May and June 2014. The lionfish removal began in February 2014 at T1 and in June 2014 at T2. Surveys continued until October 2014 (Fig. 1B).

At T1 and T2, lionfish removals occurred in 1 bout with continued monitoring and 'clean-up' of any lionfish found after the major removal effort in February 2014 and June 2014, respectively (Fig. 1B). Divers on SCUBA harvested lionfish using pole spears until none were visible. Lionfish were removed first from the transect area, starting at the deepest part of the wall and proceeding shallower on the seaward (north-facing) sides. To prevent migration of lionfish into the transect area, we removed lionfish from buffer areas adjacent to the study site located to the

western, eastern, and southern sides of T1 (500 m × 35 m buffer area) and T2 (1 km × 30 m buffer area). After the initial removals, any lionfish observed in the treatment sites were removed to maintain low densities throughout the course of the experiment.

Reef fish surveys

To assess whether lionfish removals caused an increase in the density of 2 size classes of reef fishes (recruits and adults), we surveyed densities at all sites before and after lionfish removals on an approximately monthly basis (weather permitting). New recruits of each species were defined by size class, ≤2.5 cm total length (TL). We chose this size class because individuals of this size are typically less than 1 mo old (Hogan 2007). Counting ~1 mo old recruits ensures independence of our recruit densities between monthly surveys. 'Adults' were defined as all individuals >2.5 cm, which also includes non-reproductive, juvenile individuals, but for brevity, we call this size class 'adults.' Sizes were estimated by surveyors on SCUBA to the nearest 0.5 cm size class. Surveyors calibrated size estimations prior to the beginning of the experiment by estimating fish sizes underwater and then catching the same fish and measuring them. Recruit surveys were conducted at each site along 1 m × 30 m transects (n = 4) per site; 1 transect was surveyed parallel to the reef crest at each of 4 depths (10, 12, 15, and 18 m) within each site. Adult densities were estimated using 3 m × 30 m transects to account for the larger territory of adult fishes. A PVC T-bar was implemented to ensure that transect widths were consistent. As with the lionfish density surveys (see above), we collected baseline data on the densities of native species at C and T1 for 3 mo starting in October 2013 prior to lionfish removal in February 2014; at T2, baseline surveys were conducted in May and June 2014 prior to lionfish removal in late June 2014. Surveys continued until October 2014 (Fig. 1B).

The densities of 3 different species were recorded: bicolor damselfish *Stegastes partitus*, yellowhead wrasse *Halichoeres garnoti*, and bluehead wrasse *Thalassoma bifasciatum*. We chose these species for their ease of identification, their common occurrence in the reef community, and their known susceptibility to lionfish predation (Morris & Akins 2009).

We used a model selection approach based on the deviance information criterion (DIC) (Spiegelhalter et al. 2002) to determine what, if any, response native species had to the removal of lionfish. We fit 6 models to the data for each species and size-class:

$$D_{j,k} = \alpha + \varepsilon_{j,k} \quad (1)$$

$$D_{j,k} = \alpha_k + \varepsilon_{j,k} \quad (2)$$

$$D_{j,k} = \alpha + \beta I_j + \varepsilon_{j,k} \quad (3)$$

$$D_{j,k} = \alpha_k + \beta_k I_j + \varepsilon_{j,k} \quad (4)$$

$$D_{j,k} = \alpha + \beta I_j t_j + \varepsilon_{j,k} \quad (5)$$

$$D_{j,k} = \alpha_k + \beta_k I_j t_j + \varepsilon_{j,k} \quad (6)$$

where $j = 1, 2, \ldots, 11$ indexes the measurement dates; $k = 1, 2, 3, 4$ indexes the measurement depths; $D_{j,k}$ is the difference in native fish densities between the treatment and control site for measurement j at depth k; t_j is the difference in days between the date of observation j and the date of removal of lionfish; I_j is an indicator variable for the removal of lionfish from the treatment transects for measurement j; $I_j = 0$ before first removal, 1 afterwards; $\varepsilon_{j,k}$ represents a random error term, described below; and α and β are also described below. Modeling the difference in densities between the treatment and control transects should remove the influence of any annual/seasonal trend in the populations under study.

Model (1) is the null model: the removal of lionfish from the treatment transect had no effect on the difference in density of native species at any depth or time: α, the difference in density due to treatment, remains the same throughout the experiment. Model (3) suggests a step effect: removal of lionfish from the treatment transect resulted in a single constant change in the difference in densities of native species. Here, α becomes the difference in density before the intervention, while β represents the constant change in density after the removal. Model (5) suggests that the difference in densities of native species changed linearly starting with the date of removal of lionfish. α remains the difference in density before the intervention, while β now represents the rate of change in density after the removal. Finally, Models (2), (4), and (6) are similar to models (1), (3), and (5), respectively, except that the size of any effect was allowed to differ by depth k. Thus, the single α and β from Models (1), (3), and (5) are replaced by different α_k and β_k for each depth k. Our primary parameters of interest are the β values, since they estimate the change in density differences that occurs due to lionfish removal.

To account for potential lack of statistical independence by time and/or depth, we estimated for each model a correlation matrix $\mathbf{V}$ for $\varepsilon_{j,k}$ using a separable exponential formula that decomposes the relationship between 2 observations into a product of exponential functions of their distance apart in time and space (Mitchell & Gumpertz 2003):

$$\mathrm{corr}(\varepsilon_{j_1,k_1}, \varepsilon_{j_2,k_2}) = \phi_t^{|t_{j_1} - t_{j_2}|} \times \phi_d^{|d_{k_1} - d_{k_2}|} \quad (7)$$

where ϕ_t and ϕ_d represent temporal and spatial correlations, respectively. Thus, the error terms $\varepsilon_{j,k}$ were modeled as $\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \boldsymbol{\Sigma})$ where $\boldsymbol{\Sigma} = \mathbf{S} \times \mathbf{V} \times \mathbf{S}$, and $\mathbf{S}$ was a diagonal matrix of standard deviations σ_k for the observations. σ_k was assumed to be constant by depth k for each model.

We fit Models (1) to (6) using Markov chain Monte Carlo (MCMC) methods. Using $N(x,y)$ to represent a normal distribution with mean x and variance y, and Unif(x,y) to represent a uniform distribution (Unif) bounded between x and y, here is a list of our parameters and their (uninformative) priors:

- $\alpha \sim N(0,100^2)$ and $\beta \sim N(0,100^2)$; the same priors were used for models where α and β varied individually by depth
- $\sigma_k \sim \mathrm{Unif}(\frac{1}{b}, b)$ for each depth k, with $(b-1) \sim \mathrm{Exp}(0.001)$; by using partial pooling of the standard deviations, we guard against overfitting the data (Gelman et al. 2014)
- $\phi_t \sim \mathrm{Unif}(0,1)$ and $\phi_d \sim \mathrm{Unif}(0,1)$

Convergence of the MCMC chains was monitored using the Gelman-Rubin diagnostic (Gelman & Rubin 1992) and visual inspection of the traceplots of the chains for the parameters α and β. The Raftery-Lewis diagnostic (Raftery & Lewis 1992) was used to monitor precision of quantile estimates. Models were compared using Gelman's estimate of DIC (Spiegelhalter et al. 2002, Gelman et al. 2014), with smaller DIC indicating a better model, and models having $\Delta\mathrm{DIC} < 4$ considered to be of similar quality. Where multiple models had similar DIC values, the most parsimonious model was chosen.

Models were fit using the statistical software JAGS version 4.1.0 (Plummer 2003) and R version 3.2.5 (R Core Team 2016), including R packages coda version 0.18-1 (Plummer et al. 2006), rjags version 4.4 (Plummer 2016), and R2jags version 0.5-7 (Su & Yajima 2015).

Genetic diversity

We chose the bicolor damselfish as a model species for this study because of its ubiquity on Caribbean reefs, ease of capture, documented interactions with lionfish (Morris & Akins 2009), and the availability of molecular genetic markers. Additionally, previous genetic data are available for this species from Panama for comparison to our results (Salas et al. 2010). To determine the effect of lionfish predation on the genetic diversity of bicolor damselfish, we opportunistically collected ~50 individuals of the recruit size class (≤2.5 cm) from all sites at depths from 10 to 18 m before and after lionfish removal; there was no systematic difference in the depths of collection among sites. We focused on the recruit size class because lionfish preferentially target smaller individuals (Green & Cote 2014). Also, we independently sampled each recruit cohort by focusing on fish of a certain size, whereas adults are an amalgam of multiple separate genetic cohorts. Pre-removal genetic samples were taken in January 2014 for T1 and C and in June 2014 for T2. Post-removal genetic samples were taken in October 2014 for all sites. Divers collected samples using a clove oil mixture (9:1; 70% isopropanol:pure clove oil) and hand nets. Fish were collected within the bounds of each site (~360 m^2) but not specifically from the transect areas where surveys were conducted. Each fish was euthanized humanely, measured for TL, and a fin clip was taken for DNA analysis and stored in 95% ethanol. Samples were brought back to the lab at Texas A&M University – Corpus Christi for genomic DNA extraction (Qiagen DNeasy Blood and Tissue Kit) and genetic analysis. The timing of the before and after sampling (i.e. months apart) along with the lethal sampling of juveniles ensured that we did not sample the same individual twice and minimized the possibility of resampling from the same recruitment cohort in the before and after sampling, thereby ensuring the independence of the samples for statistical purposes.

We chose 12 microsatellite loci from Williams et al. (2003) and Thiessen & Heath (2007) to estimate genetic variation in this species (see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m558p223_supp.pdf). We used polymerase chain reaction (PCR) to amplify microsatellite loci using dye-labeled forward primers in 10 µl reactions comprising ca. 100 ng template DNA, 10 µM of dye-label forward primer, 10 µM of unlabeled reverse primer, 200 µM of each dNTP, 0.1 U Flexi GoTaq (Promega) polymerase, 1× PCR buffer, and locus-specific concentrations of $MgCl_2$. PCR conditions were 94°C for 2 min, followed by 29 to 40 cycles of 94°C for 15 s, locus-specific annealing temperatures (see Williams et al. 2003, Thiessen & Heath 2007 for details) for 15 s, 72°C for 30 s, and a final extension of 72°C for 90 s. The sizes of the PCR products were estimated using an ABI 3730xl genetic analyzer. We used GeneMapper v. 4.0 software for genotyping microsatellite fragments (Applied Biosystems). We calculated standard indices of genetic diversity, including observed and expected heterozygosity (H_O and H_E) and allelic richness (A).

To test the hypothesis that lionfish predation was strong enough to cause declines in genetic diversity of bicolor damselfish recruit cohort populations, we first tested for changes within sites in allelic frequencies between pre- and post-removal samples with an exact *G*-test using GENEPOP on the web (version 1.2, dememorization: 1000, number of batches: 100, iterations per batch: 1000; Raymond & Rousset 1995); p-values were adjusted for multiple comparisons using the sequential Bonferroni method. A significant change in allele frequencies may indicate an effect of removal. Secondly, we calculated the change (difference) in genetic diversity indices (H_O, H_E, and A) between before and after lionfish removals at all 3 sites. If lionfish are having an impact on genetic diversity of these recruit cohorts, then we expect to see a positive change in diversity indices at the treatment sites where lionfish predation has been alleviated, but no significant change at the control site. To test if changes in diversity indices were significantly different from 0, we used 1-sample *t*-tests in R (R Core Development Team); p-values were corrected for multiple comparisons using the sequential Bonferroni method. Lastly, to test whether the invasion of the lionfish has led to declines in genetic diversity in Panamanian populations of bicolor damselfish, we compared our measured values of H_O, H_E, and A to the same diversity indices published previously by Salas et al. (2010). They sampled bicolor damselfish before the lionfish invasion in 2009 from fringing and patch reef sites in the same bay system in Bocas del Toro, Panama, named 'Coral Key' and 'Bocas' and spaced ~20 and 45 km, respectively, from our sites at Tiger Rock. They used 9 of the same microsatellite markers that we used in our study, and we compared the same diversity indices with a 1-sample *t*-test in R. We pooled our control and treatment site samples together for this comparison. If lionfish predation has influenced bicolor damselfish genetic diversity since the invasion, our samples should be lower in diversity than those of Salas et al. (2010). We used 1-sample *t*-tests implemented in R to test the hypothesis that genetic diversity has declined in Panamanian populations since the invasion of the lionfish in 2009; p-values were corrected for multiple comparisons using the sequential Bonferroni method.

RESULTS

Lionfish density

The average densities of lionfish prior to removal were 0.02, 0.03, and 0.04 fish m^{-2} at T1, T2, and C, respectively. These densities are comparable to natural reefs in the Bahamas (0.04 m^{-2}; Green & Cote 2009) but lower than artificial reefs in the Gulf of Mexico (0.14 m^{-2}; Dahl & Patterson 2014). After the removal, lionfish density at both treatment sites was reduced by an order of magnitude (Fig. 2; see Table S3 in the Supplement for all fish densities). Control site density decreased by 27%. Post-removal, lionfish density at this site was an order of magnitude greater than at T1 and T2 (Fig. 2). After initial lionfish removal efforts, no lionfish were seen at T1 for 6 mo or at T2 for remainder of the experiment (3 mo; Fig. 2).

Effects of lionfish on the density of native reef fishes

After the removal of lionfish at T1, the average recruit density of bicolor damselfish more than doubled (130% increase, Fig. 3A; Table S3). The control site, by comparison, experienced a 14% increase in density over the same period (Fig. 3A). The post-removal density of recruits at T1 was more than double (122% more) the density at C (Fig. 3A). The model that exhibited the best combination of fit and parsimony was Model (5), which indicates a linear increase in the difference in densities between the control and treatment site after removal (Fig. 3A; Table S4). A 95% credible interval for the slope of the increase is (0.06, 0.30) recruits m^{-2} mo^{-1}.

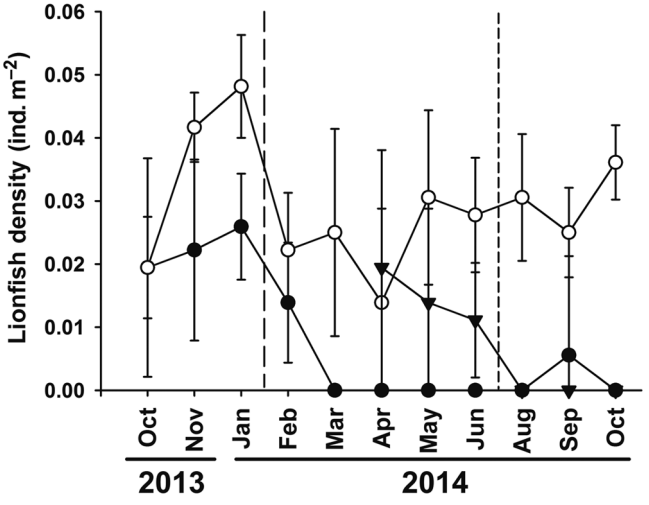

Fig. 2. Density of lionfish *Pterois volitans* from monthly surveys at 3 study sites (Control: open circles, Treatment 1: filled circles, Treatment 2: filled triangles). Long- and short-dashed vertical lines indicate the timing of lionfish removal in Treatments 1 and 2, respectively. Error bars indicate SD. Sites could not be surveyed in December 2013 and July 2014 due to weather; these dates are omitted for simplicity

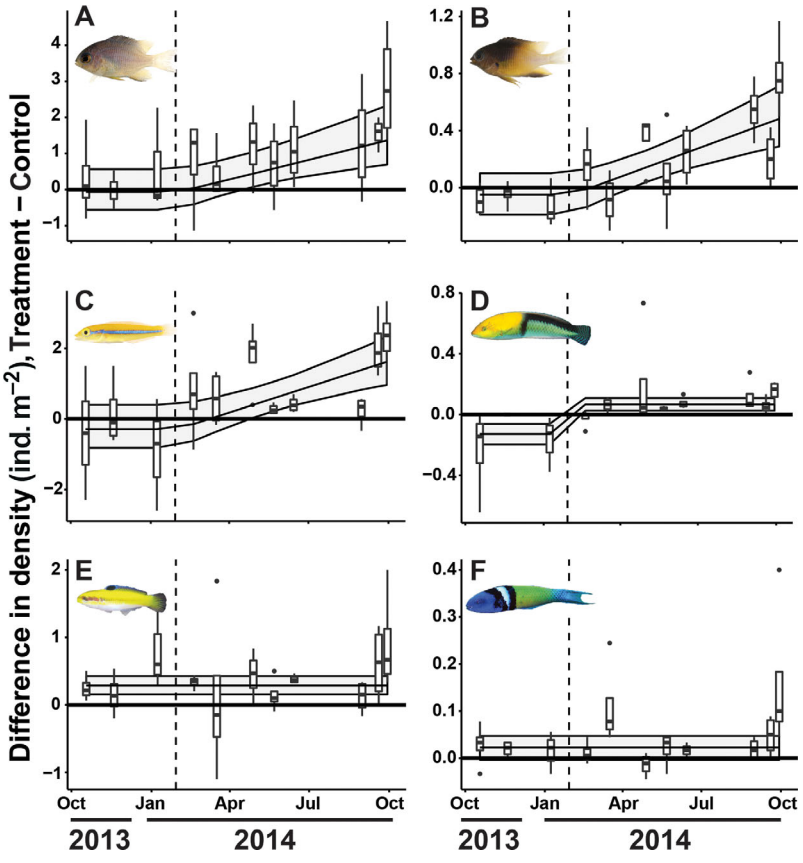

Fig. 3. Difference in density of recruits and adults of 3 reef fish species between 2 sites (Treatment 1 and Control) from monthly surveys. (A,B) *Stegastes partitus*, (C,D) *Halichoeres garnoti*, (E,F) *Thalassoma bifasciatum*; recruits and adults, respectively. Vertical dashed lines indicate the timing of lionfish removal from the treatment site. Note the differences in the scales on the *y*-axes. Box-plots were constructed using the standard quantile-based definition (e.g. Quinn & Keough 2002, p. 60). Shaded regions around trend lines are 95% credible intervals for the mean

Bicolor damselfish adult density increased by 520% at T1 after lionfish removal (Fig. 3B). In comparison, density of adults increased by 65% at C (Fig. 3B). The post-removal density of bicolor adults at T1 was 97% greater than the density at C over the same period (Fig. 3B). The model chosen for bicolor damselfish adults was again Model (5), a linear increase in the difference in densities between the control and treatment site after removal. The difference in density between T1 and C increased rapidly after lionfish removal (Fig. 3B). A 95% credible interval for the slope of the increase is (0.03, 0.10) adults m^{-2} mo^{-1}.

In contrast, mean recruit density of yellowhead wrasse remained stable (~2% increase) between pre- and post-removal of lionfish at the treatment site (Fig. 3C). However, the density of recruits dropped markedly (74%) at Site C after the removal period (Fig. 3C). As with bicolor damselfish, the post-removal density of yellowhead wrasse recruits at T1 was nearly double their density at C (188% greater; Fig. 3C). The model that exhibited the best combination of fit and parsimony was Model (5), which indicates a linear increase in the difference in densities between the control and treatment site after removal (Fig. 3C, Table S4). A 95% credible interval for the slope of the increase is (0.12, 0.36) recruits m^{-2} mo^{-1}.

Yellowhead wrasse adult density increased by 150% at T1 following lionfish removal. In contrast, density decreased by nearly an order of magnitude (850%) at C (Fig. 3D). The density of adults was 400% greater at T1 compared to C after lionfish removal (Fig. 3D). The model chosen for yellowhead wrasse adults was Model (3), a single constant increase in the difference in densities between the control and treatment site after removal (Fig. 3D, Table S4). A 95% credible interval for the increase is (0.12, 0.28) adults m^{-2} mo^{-1}.

After the removal of lionfish at T1, the average recruit density of bluehead wrasse increased slightly by 34% (Fig. 3E). However, the density of recruits also increased 88% at the control site after the removal period (Fig. 3E). As a result, the difference in recruit density between the treatment and control sites was nearly constant before and after removal of lionfish, and the preferred model was the null model (Model 1) (Fig. 3E). Bluehead wrasse adult density was quite low across all sites and times but did increase by 75% after lionfish removal at T1; densities did not change at C (Fig. 3F). Again, the preferred model was the null model (Model 1).

Effects of lionfish on the genetic diversity of *Stegastes partitus*

We found little evidence of systematic changes in allele frequencies in response to lionfish removals. We found small, significant changes in allele frequencies in some loci in samples from C and T2, but not T1 after the removal of lionfish based on exact

G-tests (Table S2). Only 6 of 36 site-by-locus comparisons indicated a significant change in allele frequency after lionfish removal; this was reduced to 3 significant comparisons after correction for multiple comparisons.

Additionally, there was no clear increase in diversity at the treatment sites compared to the control site after lionfish removals (Fig. 4, and see Tables S1, S5, S6 & Fig. S1 in the Supplement). The multi-locus average H_O showed small changes from pre- to post-removal, with all sites showing a mean increase in diversity ranging from 1 to 5% change in the frequency of observed heterozygotes (Fig. 4A). However, none of the changes was significant, and the responses of individual loci varied (i.e. both negative and positive changes were seen; see Fig. S1). Mean H_E increased very slightly (<1%) at C and T2 after removals, but declined (<1%) at T1 after removals; the effect was only significant for the control site at $\alpha = 0.05$; however, after correction for multiple comparisons, this was no longer significant (Fig. 4; H_E: *t*-test: df = 10, $t = 2.47$, p = 0.033; see Table S5 for all tests). As with H_O, individual loci varied in their response to removals exhibiting increases and decreases in H_E (Fig. S1). Lastly, allelic richness increased after lionfish removals from C and T2, but decreased after removals from T1. The effect was only significant at $\alpha = 0.05$ for the control site, and the effect remained significant after Bonferroni correction (df = 10, $t = 3.43$, p = 0.007). As observed with H_E and H_O, individual loci varied in their response to removals, exhibiting increases or decreases in allelic richness depending on the locus (Fig. S1).

We found that all 3 multi-locus diversity indices were on average very slightly higher in this study compared to previous samples taken by Salas et al. (2010) before the lionfish invasion in 2009 (Fig. 5 and see Fig. S2); none of these comparisons was significant at $\alpha = 0.05$ (Table S6). Again, responses of individual loci varied for each diversity index, with some loci showing increases and some showing decreases in diversity compared to the samples from Salas et al. (2010).

DISCUSSION

Predators can have 2 kinds of effects on their prey species populations, affecting their genetic composition through selection and genetic 'drift' effects. If predation is severe and a large portion of the prey population is removed, changes in gene frequencies and a reduction in genetic diversity can be observed across the entire genome (drift) caused by a genetic bottleneck. In this study, we looked for evidence of a genetic bottleneck in bicolor damselfish as a result of lionfish predation; however, we did not find one despite a large suppression effect of lionfish predation on the population.

In order for a genetic bottleneck to occur, lionfish must reduce the size of the populations substantially (Peery et al. 2012). Recruit and adult densities of bicolor damselfish rose linearly and significantly after lionfish removal at our treatment sites, indicating that lionfish were having a significant effect on their population demographics. The lionfish effectively reduced recruit populations by 55% and adult populations by 84%. If lionfish have an effect on genetic diversity, we would expect to see increases in diversity indices or changes in allelic frequencies between

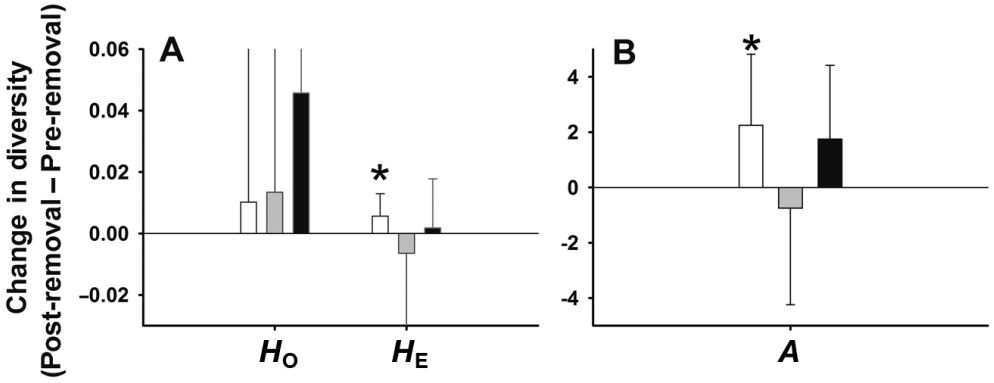

Fig. 4. (A) Changes in genetic diversity of *Stegastes partitus* pre- to post-removal of lionfish *Pterois volitans* across all sites. (A) Differences in mean observed heterozygosity (H_O) and expected heterozygosity (H_E); (B) differences in mean allelic richness (*A*) for 12 microsatellite loci between pre- and post-removal samples. Control: white bars; Treatment 1: grey bars; Treatment 2: black bars. Error bars are multi-locus SD. Asterisks (*) indicate significant changes within sites between the pre- and post-removal periods

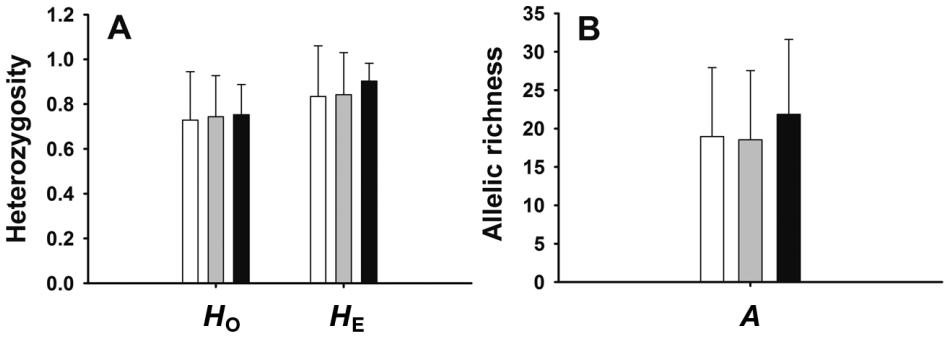

Fig. 5. Genetic diversity indices of Panamanian samples of *Stegastes partitus* pre- and post-invasion of lionfish *Pterois volitans*. (A) Mean observed heterozygosity (H_O) and expected heterozygosity (H_E); (B) mean allelic richness (A), for 12 microsatellite loci. Pre-invasion samples from Salas et al. (2010): Coral Key (white bars); Bocas Island (grey bars). Post-invasion samples from this study: mean for all Tiger Rock samples (black bars). Error bars are multi-locus SD

pre- and post- lionfish removal within treatment sites. While the allelic frequencies changed significantly in the control site, they did not change significantly in either treatment site. Similarly, genetic diversity increased slightly at the control site after lionfish removal but not at the treatment sites as expected.

Several factors may explain why bicolor damselfish populations have not experienced a significant decline in genetic diversity despite obvious predation effects of lionfish. The bicolor damselfish is a highly fecund species that reaches maturity quickly, which can lead to rapid population replenishment (Wilson & Meekan 2002). High fecundity and short generation times can result in sufficiently large genetic population sizes that a 55% reduction in density is not extreme enough to leave a detectable genetic signal. Peery et al. (2012) reported that many studies fail to detect bottlenecks in populations known to have experienced significant population declines. They stated that populations must be reduced by 2 to 3 orders of magnitude before a bottleneck can be detected through heterozygosity and allelic richness measures. The magnitude of reductions in population size observed in this study is not this extreme; therefore, we conclude that the effect of lionfish predation was not sufficient to cause a genetic bottleneck in these recruit cohorts.

It is possible that lionfish populations that have been established in this region since 2009 (Schofield 2010) have already caused a reduction in genetic diversity of bicolor damselfish in all possible recruit source populations—given their widespread nature—thereby limiting the possibility of genetic diversity recovering after lionfish removal. However, the levels of genetic diversity found in our samples were slightly higher, albeit not significantly, than estimates of genetic diversity of Panamanian populations of bicolor damselfish taken in the Bocas del Toro region prior to the lionfish invasion (Salas et al. 2010). This suggests that there has not been a widespread decline in genetic diversity of this species associated with the lionfish invasion in this region. In contrast to our findings, previous studies have demonstrated that reductions in genetic diversity of native prey species can be caused by invasive predators (Gasc et al. 2010, Iwai & Shoda-Kagaya 2012). However, these studies were conducted on terrestrial species on islands, where dispersal is more limited and population sizes are smaller than in the marine environment (Kinlan & Gaines 2003). Bicolor damselfish, like many marine fishes, have a high dispersal capability. With a pelagic larval stage duration of 27 to 31 d (Wellington & Victor 1989), the larvae of this species disperse on average 77 km, and up to 180+ km, from the natal reef (Hogan et al. 2012). Due in part to this tremendous dispersal capacity, the genetic effective population size of this species must be very large, with genetic populations spanning much of the Caribbean (Purcell et al. 2009). Connectivity among populations of this species is likely strongest within the same ecoregion (southwestern Caribbean; Schill et al. 2015) from locations 10s to 100s of km away (Hogan et al. 2010, 2012, Salas et al. 2010). Therefore, the genetic diversity in Panama (and elsewhere in the Caribbean) can be readily replenished from dispersal from sometimes distant source populations. Thus, marine populations of this size might be well buffered from reductions in genetic diversity caused by the introduction of invasive species.

Many species in the Caribbean have large effective population sizes and have widespread genetic connectivity, similar to the bicolor damselfish, which may make them particularly immune to genetic re-

ductions from lionfish predation. Yellowhead and bluehead wrasse, studied here, both show high levels of genetic connectivity and diversity across large areas (Rocha 2004, Purcell et al. 2006). However, many species show population subdivision within the Caribbean (Shulman & Bermingham 1995, Purcell et al. 2006), and some have small populations and may be endemic to a particular region or reef system (Taylor & Hellberg 2003). Life-history traits that affect dispersal and recruitment may also have an effect on recovery from predation and can affect genetic diversity loss. Bicolor damselfish and yellowhead wrasse have shorter pelagic larval durations (28 and 26 d, respectively; Victor 1986, Wellington & Victor 1989) than bluehead wrasse (49 d; Victor 1986), perhaps contributing to their more rapid recolonization responses. Furthermore, recruitment patterns can also differ; some species experience regular bouts of recruitment, while others experience more of a boom-and-bust recruitment. For example, Victor (1982) showed that most summertime recruitment of bluehead wrasse in San Blas, Panama, occurred over a 2 wk period in late June/early July, with very low levels of recruitment outside of that window. The recruitment of bluehead wrasse in our study appeared to be episodic with low levels of recruitment year round except for large increases in recruitment in March and again in October 2014 and at both treatment and control sites. Species with episodic or low levels of recruitment, those that show lower standing diversity, have short dispersal distances, have restricted ranges, and have strong population structure within the Caribbean may be more susceptible to genetic diversity loss as a result of lionfish predation.

Additionally, species clearly differ in their response to lionfish predation, which may make them more or less susceptible to reductions in genetic diversity. Here we observed variable responses to lionfish predation among the 3 native species that we monitored. Juvenile and adult densities of bicolor damselfish and yellowhead wrasse increased when lionfish predation was alleviated; however, bluehead wrasse densities were not affected by removals. Species characteristics may play a large role in susceptibility to lionfish predation and subsequent diversity loss. Small, shallow-bodied, solitary fishes found resting on or just above reefs appear to be most susceptible to lionfish predation (Green & Cote 2014). Our 3 species here all have traits making them susceptible to lionfish: they are all small and shallow-bodied (at least in the juvenile form), and they are all closely associated with the reef. Bicolor damselfish are solitary and territorial, while bluehead wrasse tend to be solitary or aggregate in small shoals, and yellowhead wrasse tend to aggregate in small shoals of conspecifics (J. D. Hogan pers. obs.).

In conclusion, predation by the invasive lionfish was not strong enough to cause a genetic bottleneck in populations of the prey species investigated here. The size of these populations and the scale of genetic connectivity in the bicolor damselfish may have buffered against widespread losses in genetic diversity perpetrated by the lionfish. Species that demonstrate a susceptibility to invasive predators and those that have small, geographically restricted genetic populations may be at greater risks of reductions in genetic diversity from predation. However, this is not to say that this invasive predator has not left a genetic mark on these populations. Lionfish predation had a significant effect on population size, and lionfish could impose a selective effect on the genetic structure of these prey populations. Future studies should look for evidence of selection effects of invasive predators on prey species. These effects could be observed at lower rates of predation than drift effects. Next-generation sequencing technologies could be used to discover single nucleotide polymorphisms in genes under selection by predators.

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