

Invasive Pacific lionfish *Pterois volitans* reduce abundance and species richness of native Bahamian coral-reef fishes

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ABSTRACT: Pacific red lionfish *Pterois volitans*, introduced to Atlantic waters in the 1980s, represent a particularly successful invasive marine predator with strong effects on native prey. Previous experiments examining the effects of lionfish on native fish communities have been conducted on small patch reefs. However, the effects of lionfish on native reef-fish communities over larger spatial scales — scales at which conservation and management efforts are typically applied — have not been examined experimentally. Beginning in June 2009, I conducted a large-scale field experiment near Lee Stocking Island, Bahamas. Ten large (1400 to 4000 m²) coral reefs were paired based on location and similarity of habitat, and baseline surveys of their fish communities were conducted. Quarterly removals of lionfish were then conducted on 1 reef in each pair, while the densities of lionfish on the other reefs were standardized at typical post-invasion levels. Through August 2010, quarterly surveys showed that lionfish caused significant changes in native reef-fish communities, including reductions in the total density (up to 46.3 ± 13.7 %, mean ± SEM), biomass (31.9 ± 10.7 %), and species richness (21.4 ± 9.1 %) of prey-sized fishes (all individuals <10 cm total length). Lionfish also caused substantial reductions in the biomass of prey-sized herbivorous fishes (33.9 ± 16.0 %) and piscivores (98.6 ± 39.5 %). However, these negative effects on small fish did not translate to observable declines in a larger size-class (all individuals 10 to 20 cm total length) over the 14 mo study period.

KEY WORDS: Coral reefs · Invasive species · Marine fishes · Predator effects · Prey species richness

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INTRODUCTION

Invasive species can greatly alter native communities, especially when the invader represents a novel predator archetype in the invaded community (Cox & Lima 2006, Salo et al. 2007). Such introductions have resulted in the formation of novel ecosystems and changes in ecosystem functioning, not only through the introduction of new species, but also through resultant shifts in community structure via extirpations

and reductions in the abundance of native species (Hobbs et al. 2006, Graham et al. 2014). For example, the introduction of Nile perch to Lake Victoria, a system which previously lacked a large pike-like predator, resulted in the extinction of more than 100 species of native fish (Ogutu-Ohwayo 1990). In a terrestrial example, the brown tree snake has extirpated or drastically reduced populations of 17 of 18 native birds on Guam, an island which was entirely free from snakes previous to its introduction (Wiles et al. 2003).

The Pacific red lionfish *Pterois volitans*, the first non-native marine fish to become an established invasive in the western Atlantic, is a voracious mesopredator of native coral-reef fishes (Morris & Whitfield 2009, Albins & Hixon 2013, Côté et al. 2013), and represents a novel predator type in the invaded system. Invasive lionfish have undergone a broad and rapid range expansion over the past decade, spreading along the southeastern seaboard of the USA (from Miami to Cape Hatteras), across the Bahamas, Caribbean, and Gulf of Mexico, and as far south as the coast of Venezuela (Schofield 2009, 2010). Lionfish in some invaded areas have reached densities exceeding those of all but the most common native mesopredators (Whitfield et al. 2007), and far exceeding those reported from their native Pacific (Schiel et al. 1986, Fishelson 1997, Green & Côté 2009, Kulbicki et al. 2012). Additionally, the maximum lionfish size (total length, TL) reported from the invaded Atlantic far exceeds the Pacific record (Atlantic: 52 cm, L. Akins pers. comm.; Pacific: 38 cm, Randall et al. 1996).

Invasive lionfish are generalist predators, feeding on at least 25 different families of native fishes, including the juveniles of economically and ecologically important species such as groupers and parrotfishes (Albins & Hixon 2008, Morris & Akins 2009, Muñoz et al. 2011, Layman & Allgeier 2012). Lionfish demonstrate a suite of predatory characteristics and behaviors that native prey have not encountered in their evolutionary history (Albins & Hixon 2013). Unlike many native mesopredators—which are themselves susceptible to predation by larger apex predators and so must maintain a high level of vigilance while hunting—lionfish are well defended by venomous spines and are thus free to hover above the reef, stalking their prey with apparent impunity. Their appearance—with a combination of spiny and fleshy projections on the head and face, a zebra-like barred coloration pattern, and elongated feather-like fin rays—may allow lionfish to blend with the reef background (crypsis) or appear to be a harmless invertebrate or plant (mimicry) (Albins & Hixon 2013). When hunting, lionfish flare their large fan-like pectoral fins, typically approaching prey slowly, and herding the prey into a corner of the reef or against the seafloor. During this process, lionfish often blow a jet of water directed towards the prey, which may serve to confuse the prey or to increase the probability of head-first capture (Albins & Lyons 2012). While some native mesopredators possess one or another of these traits or behaviors, none possess all the attributes of invasive lionfish. Thus, lionfish represent a

novel predator archetype, with a unique suite of morphological characteristics and behaviors in the invaded community. Evolutionary naiveté of native prey to lionfish may reduce prey vigilance and the effectiveness of prey avoidance behaviors (Anton-Gamazo 2013), conferring a high degree of predatory efficiency and contributing to the remarkable success of the lionfish invasion.

Experimental studies conducted on small (4 to 150 m²) patch reefs have demonstrated that invasive lionfish have strong effects on native prey fish populations and communities (Albins & Hixon 2008, Albins 2013, Green et al. 2014). Two short-term studies (2 mo or less) on small patch reefs (4 m²) have shown that lionfish cause reductions in the abundance (by up to 94%) and species richness of native fishes, and that lionfish have stronger effects on native fish communities than a similarly sized native predator, the coney grouper *Cephalopholis fulva* (Albins & Hixon 2008, Albins 2013). A more recent experiment carried out over a longer time period (18 mo) and on larger patch reefs (100 to 150 m²) in the Bahamas demonstrated that lionfish removals can result in increases in native prey fish biomass of 50 to 70% (Green et al. 2014).

Given that ecological processes are often scale-dependent (Levin 1992), the question remains whether and how the effects of lionfish on patch-reefs scale up to larger, more contiguous reef areas. The only study, thus far, to examine the effects of lionfish over larger, more contiguous reef areas (Green et al. 2012) followed changes in reef fish communities over time, rather than comparing treatments and controls. Therefore, while Green et al. (2012) documented a 65% decline in the biomass of prey fishes over a 2 yr period coincident with the lionfish invasion, their study did not provide unequivocal evidence of a direct causal relationship between these 2 variables. If the strong effects of lionfish demonstrated by previous small-scale experiments, and suggested by previous large-scale observations, manifest over large areas of reef and are persistent through time, then there could be important implications for the structure of native reef-fish communities and the resilience of coral-reef ecosystems as a whole, including the capacity for reefs to provide ecosystem goods and services to humans (Albins 2013, Albins & Hixon 2013).

If lionfish reduce the abundance of ecologically important species such as herbivores, which prevent fleshy macroalgae from outcompeting or otherwise inhibiting reef-building corals (Mumby 2006), then the invader could have indirect negative effects on

corals themselves (Albins & Hixon 2013). Lionfish could also have negative effects on fisheries species such as snappers and groupers in 2 ways: (1) Reductions in juvenile abundance of these species via direct predation by lionfish could translate directly into reduced adult abundances. (2) Reductions in the abundance of small fish in general via direct predation by lionfish would reduce the prey base available to native predators. These effects could have serious negative consequences for the ability of stocks to continue to support fishing rates which, in many cases, are already too high to be sustainable (Newton et al. 2007).

The current study was designed to determine how typical post-invasion densities of lionfish on large reefs affect native reef-fish communities over time scales >1 yr, encompassing the typical juvenile phase of native fishes. The specific goals of the study included determining (1) how lionfish affect the abundance, biomass, and diversity of small native fishes over large areas of reef, over an extended time period, (2) whether reductions in the abundance of small native fish translates into reduced abundances of adult native fishes (via reduced abundance of the juveniles of larger species growing into adults, or via reductions in prey availability for native mesopredators), and (3) whether lionfish have negative effects on ecologically and economically important groups of native fishes, including herbivores and predators.

MATERIALS AND METHODS

Experimental design

Ten isolated, large (ca. 1400 to 4000 m²), natural reefs, located on the Great Bahama Bank near Lee Stocking Island, Bahamas, were selected as experimental replicates. These reefs, ranging in depth from ca. 2 to 11 m, represented a diversity of habitat types and experienced a range of exposures to predominant weather patterns and tidal currents. Baseline surveys of the reef-fish communities on all 10 reefs were conducted in July 2009 (see 'Reef surveys' below). Reefs were paired based on similar charac-

teristics including depth, area, proximity to other reefs, proximity to major tidal channels, proximity to the Exuma Sound (an adjacent basin of oceanic depths), and the predominant substrate type (Fig. 1, also see Fig. S1 and Table S1 in the Supplement at www.int-res.com/articles/suppl/m522p231_supp.pdf). Reefs in each pair were then haphazardly assigned to either a low lionfish density (LLD) or a high lionfish density (HLD) treatment group. Reef-fish communities did not differ between treatment assignments at the beginning of the experiment (see 'Statistical analysis' and 'Results' sections for details). A team of SCUBA divers thoroughly searched each LLD reef and removed all lionfish using hand nets. Because lionfish densities varied considerably among (but not within) reef pairs at the baseline survey, lionfish removed from LLD reefs were tagged, measured, and transplanted onto certain HLD reefs in order to standardize lionfish densities among the high-density treatment reefs. Artificially augmented lionfish

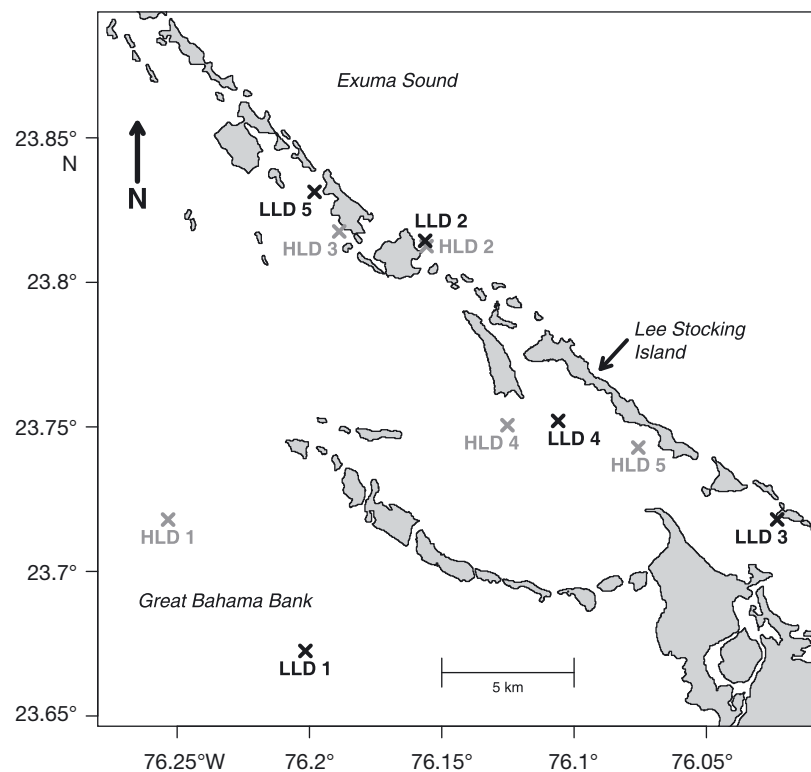


Fig. 1. Study site in the Bahamas, with pairs of low lionfish density (LLD; black labels) and high lionfish density (HLD; grey labels) reefs indicated by shared numbers. See the Supplement at www.int-res.com/articles/suppl/m522p231_supp.pdf for reef characteristics (Table S1) and satellite photos of the reefs (Fig. S1). Reef pair 2 is in relatively deep water on the fore-reef of Exuma Sound, a semi-enclosed oceanic basin, and reef pair 1 is in the relatively shallow waters of the extensive Great Bahama Bank. The remaining reefs are typical of moderate-depth habitats in the Bahamas

densities on HLD reefs were never increased beyond the highest 'natural' densities observed on similar invaded reefs in the area (see below for details). Reefs were resurveyed and treatments were maintained at 3 to 4 mo intervals for 14 mo.

Treatment effectiveness

Due to recruitment and/or immigration of lionfish onto LLD reefs between surveys (ca. every 3 mo), removals were not 100% effective (i.e. there were no reefs without lionfish). However, removals maintained lionfish density on LLD reefs at a consistent mean (\pm SEM) of 0.003 ± 0.001 fish m^{-2} over the course of the experiment (as measured by surveys conducted before removals). In comparison, lionfish density on HLD reefs was consistently an order of magnitude higher, at 0.030 ± 0.004 fish m^{-2} and was maintained by translocation as necessary. This value is well within the range, but considerably below the maximum, of densities reported on unmanipulated invaded reefs in the Bahamas (mean \pm SD: 0.039 ± 0.014 fish m^{-2} , Green & Côté 2009).

Reef surveys

Survey areas comprised a total of 400 m^2 at each experimental reef, and consisted of 6 sub-sample areas including 2 permanent square plots (10×10 m) and 4 permanent strip transects (2×25 m) placed to provide representative coverage of each reef. The 2 sub-sample types were used to assure that sub-samples did not exclude important high-relief habitat features. Thus, the 2 square plots were centered on portions of the reef with the highest apparent relief, while the strip transects were placed randomly across the remaining hard substrate. These sampling units were permanent to prevent random confounding of environmental variables among survey periods. Corners of plots and ends of strips were marked using metal stakes driven into the seafloor pavement. During each survey, pairs of SCUBA divers conducted complete censuses of each plot and strip transect, identifying and estimating the total length (TL) of all fish within each area, extending from small holes in the reef upward into the water column. Total length was estimated to the nearest cm for fish ≤ 5 cm TL, and to the nearest 5 cm for fish > 5 cm TL. Prior to conducting censuses, all observers participated in training dives to calibrate visual fish length estimates. During training dives, observers estimated

the total length of individual fish of several species, collected those individuals using hand-nets, and compared estimated length to measured length. Trained observers accurately estimated total length of a variety of fishes, with typical errors much smaller than the size bins used.

Censuses employed temporally stratified observations (Samoilys & Carlos 2000), designed to accurately sample large mobile species as well as small cryptic fishes. During each plot census, the primary observer first swam around the outside of the plot counting and sizing all large, mobile fish while a secondary observer laid a guideline between the corner stakes delineating the borders of the plot. Both observers then slowly swam in a concentric path from the outer edge of the plot to the center of the plot, maintaining positions on opposite sides of the plot, and counting and sizing all small, cryptic and site-attached fish. The primary observer was always one cycle ahead of the secondary observer, and therefore was always counting fish in undisturbed areas within the plot. During each transect census, the primary observer first swam the length of the transect, using a compass and known landmarks for navigation, maintaining a vertical position 1 to 2 m above the seafloor, and counting and sizing all large, mobile fish. The secondary observer followed behind the primary observer, deploying a guideline. Both divers then slowly swam back along the guideline, just above the seafloor, counting and sizing all small, cryptic, site-attached fish. Since the secondary observer was often less experienced than the primary observer, was occupied with deploying the guidelines for part of the survey period, and was covering areas that had potentially been disturbed by the primary observer, the secondary observer data was used primarily as a check of the primary observer data. Data were compared between observers immediately following each survey and any species or individuals missed by the primary observer, but seen by the secondary observer, were added to the master data sheet. Paired reefs (one from each treatment group) were always surveyed by the same set of divers within 24 h of one another, and the primary observer (the author) was the same for all surveys. Therefore any observer bias was distributed evenly between treatments. Surveys were conducted very slowly and carefully in order to obtain accurate counts of small, cryptic fishes such as gobies and blennies. Plot surveys took an average of 75 min each to complete (1.33 m^2 min^{-1}), while strip surveys took an average of 33 min each to complete (1.52 m^2 min^{-1}).

Response variables

The primary response variables included change in density and change in biomass of small (<10 cm TL) and medium (10 to 20 cm TL) sized fishes in 3 categories: all species combined, all herbivores combined, and all piscivores combined. Based on previous gut-content studies on lionfish in the Atlantic, I assumed that fish in the small size class included the vast majority of prey items of lionfish, while fish in the medium size class were rarely, if ever, consumed. This assumption was based on the findings of previous gut content studies. Morris & Akins (2009) found that although lionfish in the Bahamas were capable of consuming prey up to half their own length, the average ratio between prey TL and lionfish TL was approximately 0.14, and the average size of prey consumed varied between 1.5 and 3.0 cm TL, depending on lionfish size. A more recent gut-content study conducted off the southeast coast of the USA, including lionfish ranging in size from 14.5 to 45 cm TL, reported a mean fish prey size of ca. 4.4 cm TL and a maximum of 10 cm TL (Muñoz et al. 2011). Lionfish on experimental HLD reefs ranged in size from new recruits (ca. 2 cm TL) to large adults (ca. 35 cm TL), with the majority measuring from 15 to 25 cm TL. Therefore, I chose 10 cm TL, the maximum prey size observed in the Muñoz et al. (2011) study, as the cut-off between potential lionfish prey and fish that were likely too large to be consumed by lionfish in substantial numbers.

Biomass was calculated using published species-specific length-weight conversions when available, and by using parameters for closely related or similarly shaped species when not available (Table S2 in the Supplement at www.int-res.com/articles/suppl/m522p231_supp.pdf). When lionfish affected density or biomass of all species combined, I calculated the contribution of individual species to these overall effects. I also examined the effect of lionfish on the change in species richness (number of species per sample unit), species evenness measured as Pielou's J (Pielou 1966), and species diversity measured as Shannon-Wiener's H' (Pielou 1966). Richness, evenness, and diversity were calculated separately for the 2 size categories.

Changes in density and biomass within each size class were calculated separately for each species by subtracting the baseline value for each sub-sample from the value measured during subsequent surveys of that sub-sample. Similarly, changes in species richness, evenness and diversity within each size class were calculated as the difference in each metric

between the baseline survey and each subsequent survey for each sub-sample. I subtracted the baseline values from those measured during the post-treatment surveys in order to calculate a measure of how much each response variable had changed since the treatments were applied.

Statistical analyses

To assess the effects of lionfish on the primary response variables, I used linear mixed effects models (LMMs) with Sub-sample (6 sub-samples per reef) nested within Reef (10 reefs) as random effects, and with Time (5 levels: Aug 2009, Nov 2009, Jan 2010, Jun 2010, and Aug 2010) and lionfish Treatment (2 levels: HLD and LLD) as categorical fixed effects (Pinheiro & Bates 2000, Bolker et al. 2009, Zuur et al. 2009). I used Time as a categorical rather than continuous predictor because there was no *a priori* reason to assume linear relationships between response variables and time. I fitted models with and without random effects using restricted maximum likelihood estimation (REML), and compared them using Akaike's Information Criterion (AIC) and likelihood ratio tests (LRTs) with an adjustment for testing-on-the-boundary (Verbeke & Molenberghs 2000, Zuur et al. 2009). Models including random effects always resulted in better fits than models without them (Table S3 in the Supplement).

Visual examination of the residuals from these models indicated departures from the assumptions of homogenous variance among reefs and independence with respect to time. Therefore, I allowed variance to differ among reefs by including weighted terms in the models, and allowed for temporal autocorrelation within sub-samples using AR1 structures (Zuur et al. 2009). For all response variables, allowing variance to differ among reefs improved the model fits based on both AIC and LRTs (Table S3). Inclusion of autocorrelation was found to improve several, but not all, model fits. However, I included an AR1 structure in all models to allow for a more direct comparison of results (e.g. between density and biomass models). Re-examination of the residuals from the final models indicated that all assumptions, including homogeneity, independence, and normality, were met.

Once the best-fitting models in terms of random structure, variance structure, and temporal correlation were selected, I refit each using maximum likelihood estimation (ML) and used LRTs to assess the significance of the fixed effects (Zuur et al. 2009). Once the best-fitting models in terms of fixed effects

were selected via LRTs, models were refit using REML in order to estimate the fixed-effects parameters and associated effect sizes. When LRT results suggested that the Treatment \times Time interaction was significant, I used the methods described by Hothorn et al. (2008) to make simultaneous inferences about the marginal effects of the lionfish treatment at each survey period by adjusting the p-values associated with these specific linear combinations to maintain an approx. 5% family-wise error rate. When the Treatment \times Time interaction was not found to be significant based on LRT results, it was dropped from the model and each of the main effects were tested using LRTs. Whether or not the Treatment \times Time interaction was found to be significant, I used models with the full set of fixed effects (interaction term included) to estimate expected values and uncertainties (SEMs) for each of the response variables in each treatment at each survey period. To determine whether response variables differed between the groups of reefs assigned to each lionfish treatment at the baseline survey, I also fit models similar to those described above, but with density, biomass, richness, evenness, and diversity (rather than the change in each of these) as response variables.

All statistical analyses were conducted in the R language and software environment, v. 2.14.2 (R Development Core Team 2012) using add-on packages nlme v. 3.1-103 (Pinheiro et al. 2012) and multcomp v. 1.2-9 (Hothorn et al. 2008).

RESULTS

Baseline surveys

None of the response variables—including density, biomass, richness, evenness, and diversity in each size class, as well as density and biomass of herbivores and piscivores in each size class—differed between reefs assigned to the 2 treatments at the baseline survey before the lionfish manipulations (Table S4, Fig. S2 in the Supplement at www.int-res.com/articles/suppl/m522p231_supp.pdf).

Density and biomass of native reef fishes

There were substantial fluctuations in the mean density of small (<10 cm TL) native reef fishes (all species combined) through time, with large increases during the Aug 2009, Nov 2009, and Aug 2010 (recruitment season) surveys and a decrease during

the Jan 2010 winter survey (Fig. 2a). The effect of lionfish on the density of small fish changed over the course of the experiment (Treatment \times Time interaction: LRT $p = 0.015$), so this effect was evaluated separately for each survey period. Lionfish caused reductions of $38.3 \pm 16.5\%$ (2.20 ± 0.95 fish m^{-2}) during the Aug 2009 survey ($p_{\text{cor}} = 0.049$) and $46.3 \pm 13.7\%$ (3.22 ± 0.95 fish m^{-2}) during the Aug 2010 survey ($p_{\text{cor}} = 0.010$). The mean change in density was always lower on HLD reefs than on LLD reefs, but not significantly so for other survey periods (Table S5 in the Supplement).

Similarly, the biomass of small native fishes fluctuated over the course of the experiment, with peaks during the Nov 2009 and Aug 2010 surveys (Fig. 2c). Lionfish reduced the biomass of small fishes by $31.9 \pm 10.7\%$ (3.26 ± 1.10 g m^{-2} ; LRT $p = 0.007$), and this effect did not vary detectably over time (Table S5). With the exception of an initial increase on all reefs, both the density and biomass of medium sized (10 to 20 cm TL) native fishes remained fairly constant across time (Fig. 2b,d), with no evidence of an effect of lionfish treatment (Table S5).

Lionfish had no detectable effect on the density of small herbivores (Table S5), but did reduce small herbivore biomass by $33.9 \pm 16.0\%$ (0.36 ± 0.18 g m^{-2} ; LRT $p = 0.044$). Lionfish also had no discernible effect on the density or biomass of medium sized herbivores (Table S5). Herbivorous species observed on experimental reefs included 3 surgeonfishes (Acanthuridae), one chub (Kyphosidae), and 10 parrotfishes (Scaridae), most of which appeared in both size classes (Table S6 in the Supplement).

While there was no evidence that lionfish had an effect on the density of small piscivores, lionfish did cause a reduction in small piscivore biomass (Table S5). For each of these response variables, the effect of lionfish varied through time (Treatment \times Time interaction: density LRT $p < 0.001$, biomass LRT $p < 0.001$). In the case of density, none of the marginal effects of treatment were significant (Table S5), but by the final survey, lionfish had reduced the biomass of small piscivores by $98.6 \pm 39.5\%$ (0.10 ± 0.04 g m^{-2} ; $p_{\text{cor}} = 0.047$). As with herbivores, lionfish had no detectable effect on density or biomass of piscivores in the medium size class (Table S5). Piscivorous species observed on experimental reefs included a hawkfish (Cirrhitidae), a trumpetfish (Aulostomidae), a flounder (Bothidae), several groupers (Serranidae), a snapper (Lutjanidae), 2 scorpionfishes (Scorpaenidae), and lizardfishes (Synodontidae), with most of these species appearing in both size categories (Table S6).

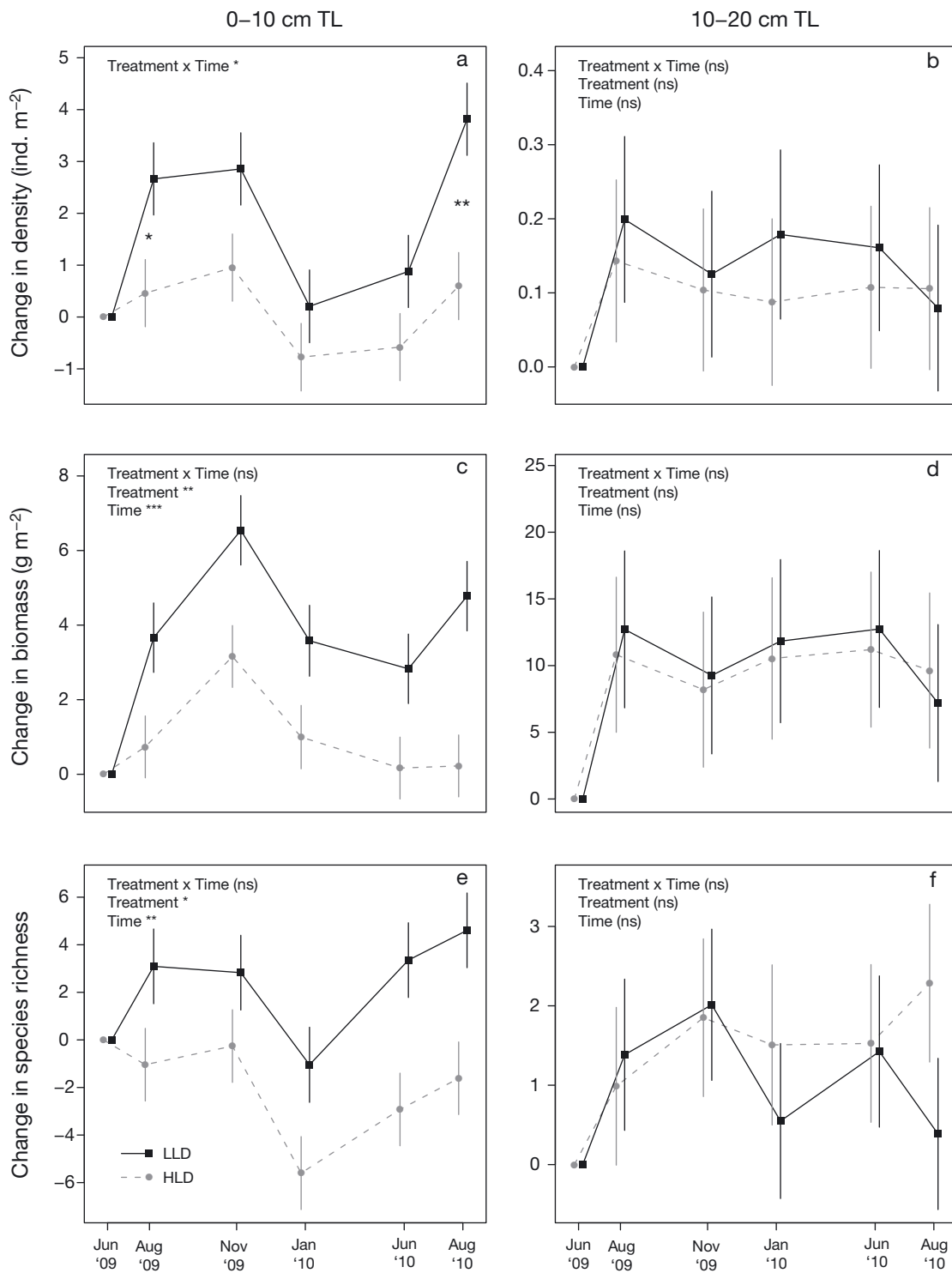


Fig. 2. Total change in (a,b) density, (c,d) biomass, and (e,f) species richness in the 2 size classes (<10 cm total length [TL] and 10 to 20 cm TL) of all reef-fish species combined. Data estimated from the full random- and fixed-effects models; dots represent means, and error bars are SEM. LLD: low lionfish density treatment reefs ($n = 5$); HLD: high lionfish density reefs ($n = 5$). Results of likelihood-ratio tests for the fixed effects are shown at the top left of each panel. When Treatment \times Time interactions were significant, the marginal effects of Treatment were examined individually for each survey date. In this case, significant p -values were adjusted to obtain an approximate family-wise error rate of 5% and are displayed for each survey date. Note different y -axis scales for the 2 size classes. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Of the 144 species of fish in the small size class (<10 cm TL) observed on experimental reefs, lionfish reduced the density of 84 species (averaged over the 2 late-summer survey periods) and reduced the biomass of 83 species (averaged over all post-baseline time steps). Of these, 2 species combined—the bridled goby *Coryphopterus glaucofraenum*, and the bluehead wrasse *Thalassoma bifasciatum*—contributed 52.9% of the total lionfish effect on density and 54.8% of the total lionfish effect on biomass, with the remaining top 20 species contributing an additional 37.4% of the total density effect and 37.8% of the total biomass effect (Fig. 3). The top twenty contributors to the effects of lionfish on overall native fish density and biomass included 3 herbivores, but did not include any piscivores (Fig. 3).

The top 2 contributors to overall native fish reductions, the bridled goby and the bluehead wrasse, were the most abundant species on the experimental reefs. While these species comprised the bulk of the overall lionfish effects on density and biomass of small reef fishes, their shares of these effects were not in proportion to their density. In fact, species with the highest effect-to-density and effect-to-biomass ratios (ratio of the difference between the mean response on LLD reefs and the mean response on HLD reefs to the mean response on LLD reefs) were very different from those described above, and were dominated by rare species rather than common ones (Table S7 in the Supplement). The species with the top 20 highest effect-to-density ratios included 2 herbivores (*Acanthurus bahianus* and *Scarus vetula*), 2 piscivores (*Scorpaenodes caribbaeus* and *Cephalopholis cruentatus*), several cardinalfishes (*Apogon maculatus*, *A. binotatus*, and *A. townsendi*), and several rare cryptic species, among others (Table S7). The species with the top 20 highest effect-to-biomass ratios did not include any herbivores, but did include 2 piscivores (*Scorpaenodes caribbaeus* and *Mycteroperca tigris*), several grunts (*Haemulon album*, *H. melanurum*, and *H. parra*), and several other relatively rare species (Table S7).

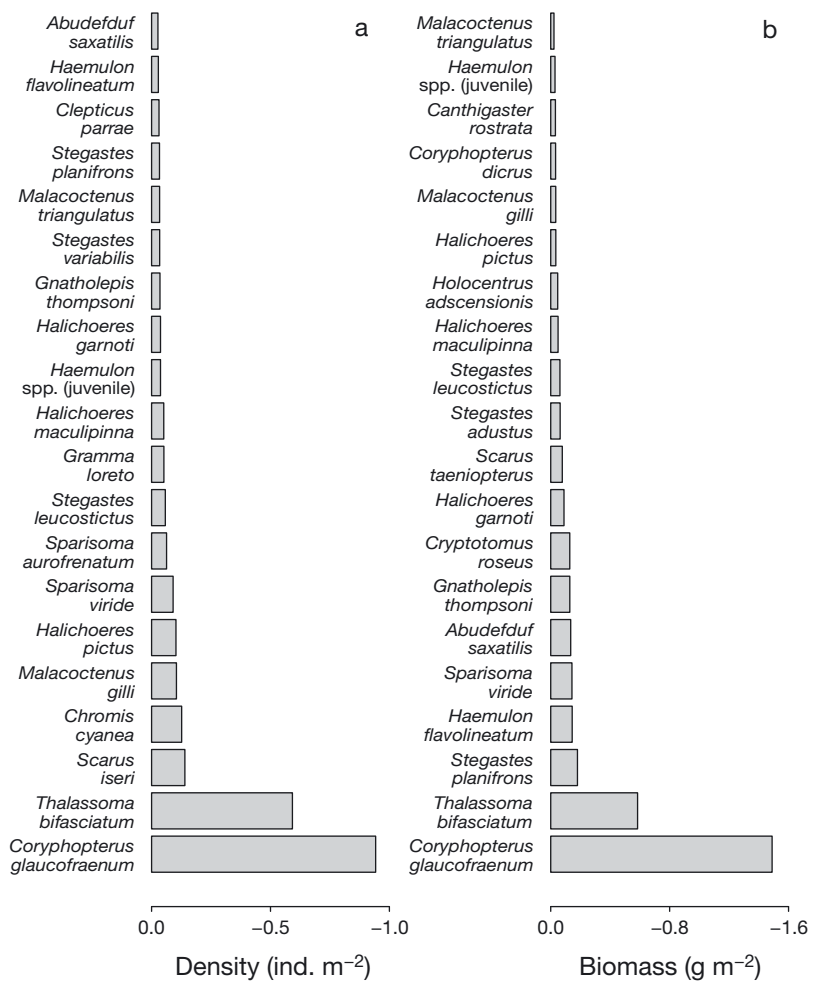


Fig. 3. Individual contributions of the top 20 species to the overall effect of lionfish on (a) density and (b) biomass of small (<10 cm total length) native reef fishes. The total contributions of all species sum to the overall lionfish effect on that response variable. Negative values indicate negative effect of lionfish treatment on response variables

Local diversity of native reef fishes

Species richness of small (<10 cm TL) fish fluctuated somewhat across time (Fig. 2e) with slight increases during the summer and fall recruitment periods (Aug 2009, Nov 2009, Jun 2010, and Aug 2010) and a decline during the winter period (Jan 2010). Lionfish reduced species richness by an average (\pm SEM) of 4.92 ± 2.09 species (LRT $p = 0.022$), and this effect was consistent through time (Table S5). This effect was due in part to a loss of richness on HLD reefs, yet primarily due to a gain in richness on LLD reefs (Fig. 2e).

The pattern seen in the change in richness at the sub-sample scale (the scale of individual plots and transects) was also reflected in richness measured at

the whole-reef scale (when data from plots and transects were aggregated). At this whole-reef scale, during the baseline survey, 72 species were observed on HLD reefs, and 60 were observed on LLD reefs. By the final survey the average number of species lost on HLD reefs was 9.8 ± 2.9 (mean \pm SEM), and the number of species gained was 6.6 ± 1.5 , while the number of species lost on LLD reefs was only 4.0 ± 1.0 , and the number of species gained was 10.6 ± 0.9 species (see Tables S8 & S9 in the Supplement for a list of species losses and gains).

There was no evidence that the species richness of medium-sized fish differed between treatments (Table S4). There was also no effect of lionfish treatment on the evenness (J) or diversity (H') of either small or medium sized fish (Table S5, Fig. S3).

DISCUSSION

Effects of lionfish on density and biomass of native reef fishes

I had assumed that differences in density or biomass of small fish (<10 cm TL) between the HLD and LLD reefs would primarily be the direct result of lionfish predation, and that any differences in density or biomass of large fish would be indirectly caused by one or both of 2 potential mechanisms: reductions in the numbers of juveniles surviving to grow into larger size classes, or other carryover effects that occurred between juvenile and adult stages (competition, etc.). There was clear evidence that lionfish reduced the density and biomass of the small size class, but no evidence of an effect on the larger size class was detected over the period of the study.

Many, if not most, coral-reef fish populations are regulated, at least in part, by early post-settlement mortality due to predation (reviews by Hixon & Webster 2002, Osenberg et al. 2002, Hixon & Jones 2005, Hixon et al. 2012). Based on the results of this study, and those of earlier small-scale experiments (Albins & Hixon 2008, Albins 2013), it is clear that lionfish cause a substantial increase in mortality of small fish, in some cases causing local extirpations. The density of small fish on low lionfish density (LLD) reefs varied seasonally as expected, with high levels of summer recruitment resulting in increased density during the summer months, and mortality exceeding recruitment during the winter months. The peaks in these seasonal fluctuations were greatly attenuated on the high lionfish density (HLD) reefs compared to the LLD reefs due to the effects of lionfish (most likely via

predation) on small native fish. The difference in density between HLD and LLD treatments largely disappeared during the winter months, although biomass remained consistently higher on LLD reefs, indicating a larger average size of native fish (within the <10 cm size class) on reefs with low densities of lionfish. This difference in biomass, despite a lack of evidence for a difference in density during the winter surveys, could have been caused by some combination of the following: a non-lethal effect of lionfish resulting in higher growth rates in the absence of the constant threat of predation by lionfish (cf. Lima 1998), increased survival of fish recruiting during the previous summer pulse, particularly if ongoing winter recruitment was density-dependent, and/or a relative increase in lionfish predation on larger fish (within the <10 cm size class) during that time period. Each of these mechanisms could have resulted in a higher ratio of large to small fish on LLD reefs during the winter months.

The small size category included both juveniles and adults of small-bodied fishes, as well as juveniles of larger species. The effect of lionfish on the density and biomass of small-bodied species (those with maximum sizes <10 cm TL) could have substantial ecological implications for the coral-reef community. Many of these small-bodied species have important roles in the ecosystem, including participation in cleaning mutualisms (reviews by Losey et al. 1999, Côté 2000). For example, the bluehead wrasse—the second most important species in terms of its share of the overall effects of lionfish on density and biomass—is a known facultative cleaner (Limbaugh et al. 1961), and has been observed being consumed by lionfish at cleaning stations (Côté & Maljkovi 2010).

Many other small-bodied species negatively affected by lionfish also represent important forage fish for native piscivores. Reductions in this prey base are likely to lower prey availability to important commercial and subsistence fisheries species, such as snapper and grouper. While lionfish had no observable effect on medium-sized piscivores in this study, competitive or other deleterious effects could emerge over longer time scales and broader spatial scales than those examined here. Competition with native mesopredators would likely result in reduced vital rates, such as growth and reproduction, rather than immediate reductions in density. In fact, if by reducing the available prey base, lionfish cause reductions in the fecundity of native piscivores, then such an effect would likely be detectable only at the metapopulation scale, as offspring are typically not retained locally in most coral reef fishes (Caley et al. 1996).

The negative effect of lionfish on juveniles of large-bodied species could result in reductions in the number of juveniles surviving to grow into larger size classes. However, lionfish had no effect on the larger size class of fishes, at least over the time horizon of the current study. There are at least 3 possible explanations for this result. First, increased predation on juveniles of large-bodied species caused by lionfish may not be additive with other sources of mortality. In other words, lionfish predation may affect only the 'doomed surplus' (sensu Errington 1956)—those individuals that would otherwise have died due to some other mechanism—and will therefore not have an effect on the realized adult population sizes of large-bodied fishes. Second, increased mortality due to the lionfish invasion may be additive with other sources of mortality, and may translate into reduced adult population densities, but this effect might not be detectable at the local habitat-patch scale due to compensatory ontogenetic movement of adult fishes among patches. While many coral-reef fishes are relatively site-attached, others demonstrate movement patterns beyond the spatial scale examined here and well beyond the scale at which manipulative experiments are logistically feasible. For example, 3 species of parrotfish whose juveniles were negatively affected by lionfish, *Scarus iserti*, *Sparisoma aurofrenatum*, and *S. viride*, have been reported to move over substantial distances as adults (Munro 1998). Third, mortality due to lionfish predation may be additive with other sources of mortality, and may translate into reduced adult population densities—but this experiment did not run long enough to detect this effect. Species in this system have a wide range of generation times and ontogenetic growth patterns, making it difficult to estimate the time required for such effects to appear. However, based on a selection of available von Bertalanffy growth parameters for some of the large-bodied herbivorous species common in the system (Choat & Robertson 2002), estimates of the time expected for individuals to attain standard lengths of 10 cm (putting their total lengths well into the 10 to 20 cm TL size class of this study) ranges between 5 and 16 mo. Given that this experiment ran for 14 mo, and the initial recruitment pulse occurred 3 mo into the study, it is possible that a longer time series, ideally one including several seasonal recruitment pulses (i.e. multiple years), would eventually have detected effects on large-bodied species. Unfortunately, the isolated marine lab where this experiment was conducted has closed, precluding such a prolonged study.

Effects of lionfish on local diversity of native reef fishes

In addition to reductions in density and biomass, lionfish caused substantial reductions in native species richness in the small size class. This was the case whether richness was measured at the sub-sample level or at the whole-reef level. Native reef-fish species with the highest lionfish effect-to-density and effect-to-biomass ratios were among the rarest species, 18 of which were extirpated from all of the HLD reefs on which they were originally found. It should be noted that high effect-to-density or effect-to-biomass ratios for individual species are driven largely by the rarity of these species and may be influenced largely by low detection probabilities. Therefore, it is more important to look at the overall number of species losses and gains for each of the treatments rather than losses and gains of specific species. If all losses and gains were attributable to low detection, one would expect magnitudes to have been similar between the treatments. While individual species were lost and gained on reefs in both treatments, more species were lost, and fewer gained on HLD reefs than on LLD reefs. In addition to a mean loss of species on HLD reefs, the difference between the treatments was largely due to gains in species on the LLD reefs, a common feature of reef-fish communities in the seasonal environment of the Bahamas, where settlement occurs largely during the summer (M. A. Hixon pers. comm.).

Predatory fishes can have a range of effects on the species richness and evenness of prey communities (reviewed by Hixon 1986). If predators disproportionately consume the most abundant prey species, as might be expected based on predator foraging theory (Schoener 1971) and the preponderance of density-dependent prey mortality in reef fishes (Hixon & Jones 2005), then the effect of predation would tend to increase the evenness of a community, and would not typically result in extirpations of rare species. Alternatively, predation can reduce the abundance of all potential prey species in proportion to their densities, thereby having no effect on the equitability among species in the prey community, and causing extirpations only occasionally, when the last of a rare species happens to be consumed. A third possibility, in which rare species are targeted at rates disproportionately large compared to their densities, has been documented in coral-reef fishes in 2 geographically distinct systems (Almany & Webster 2004), and results in strong negative effects of predation on species richness. The strong negative effect of

lionfish on the richness of small native reef-fish, along with the disproportionately large effect-to-response ratios for both density and biomass of rare species, suggest that lionfish may fall into this third category. In addition to causing substantial reductions in the abundance of common species, the lionfish invasion may represent a serious threat to native reef-fish biodiversity and the continued existence of globally rare species on Atlantic and Caribbean coral reefs.

Comparison to previous studies

The results of this experiment corroborate and greatly extend those of earlier experiments by showing that the effects of lionfish on small native coral-reef fishes scale up spatially to levels typical of management and conservation efforts, and that lionfish have negative effects on ecologically important groups of reef fishes such as herbivores, predators, and facultative cleaners. However, the magnitudes of the effects of lionfish on the density and biomass of prey-sized native fishes demonstrated by the current experiment were smaller (by a factor of approx. 0.5) than those reported from previous small-scale experimental studies (Albins & Hixon 2008, Albins 2013, Green et al. 2014), suggesting that the effects of lionfish on native prey may be scale-dependent with stronger effects occurring on small isolated habitat patches than on larger, more contiguous reefs. The effects demonstrated in the current study also appear to be smaller (by a factor of approx. 0.5) than those from an earlier observational study conducted on contiguous reefs (Green et al. 2012). This suggests that there may be an explanation other than scale-dependence for the discrepancy in effect-size between the current study and previous ones.

CONCLUSIONS

This large-scale, controlled field experiment demonstrated that lionfish at typical invasion densities had negative effects on the density, biomass, and local species richness of small (<10 cm TL) native coral reef fishes on large patches of reef habitat. However, after 14 mo, these patterns were still not detectable on larger (10 to 20 cm TL) fish. While it is possible that the latter result is due to the fact that the experiment had not run long enough, it is also possible that lionfish were merely consuming the 'doomed surplus' of juveniles of large-bodied species, or that

the effects of lionfish on larger-bodied species manifest at spatial scales beyond those amenable to manipulative experiments.

The results of this study suggest that the negative effects of the lionfish invasion on large contiguous reefs may be somewhat lower than would be expected based on previous small-scale studies. Nevertheless, lionfish appear to represent a substantial threat to coral-reef ecosystems via a variety of mechanisms, including large reductions in the abundance of small-bodied, but ecologically important species, such as herbivores and facultative cleaners, substantial loss of native reef-fish biodiversity, and declines in the prey base available to native piscivores, including important fisheries species. This study supports the general conclusion that invasive predators representing novel archetypes in the invaded community can have large effects on native populations and communities.

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