

Direct and indirect effects of invasive lionfish on coral-reef cleaning mutualists

Lillian J. Tuttle^{1,2,*}

¹Department of Integrative Biology, Oregon State University, Corvallis, Oregon 97331, USA

²Present address: Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822, USA

ABSTRACT: Cleaning mutualisms are ubiquitous interactions on coral reefs involving cleaner fishes and shrimps that remove external parasites from cooperative fish clients. Despite their ecological importance, nothing is known regarding how cleaning mutualisms are affected by the invasion of Atlantic coral reefs by the Pacific red lionfish *Pterois volitans*. Lionfish are generalist predators that may consume both cleaners and clients, with potential cascading effects on native communities. To determine whether invasive lionfish affect cleaning mutualists, I conducted a before-after-control-impact experiment manipulating the presence of lionfish on patch reefs in the Bahamas. The addition of lionfish to reefs did not significantly affect the survival and growth rates of the predominant obligate cleaner on experimental reefs, the cleaner goby *Elacatinus genie*. However, lionfish affected juvenile bluehead wrasse *Thalassoma bifasciatum*, a facultative cleaner whose density was 33% less on reefs with lionfish versus those without. The decline of bluehead wrasse was most likely due to predation by lionfish. Also, the presence of lionfish affected large transient clients, i.e. species that move among reefs; their density was 78% less on reefs with lionfish versus those without. The decline of transient species may be an indirect effect of lionfish consuming non-goby cleaners and prey, fishes that would otherwise cue transient species to aggregate at reefs. The cleaner goby is among the few small fishes on invaded reefs to escape predation by lionfish. However, by consuming other cleaners, invasive lionfish may nonetheless alter native reef communities and ecosystems.

KEY WORDS: Indirect effects · Cleaning symbiosis · Obligate cleaner · Facultative cleaner · Aggregative response · Predator–prey interaction · Non-native

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INTRODUCTION

Cleaning symbiosis is a ubiquitous interaction on coral reefs, during which a cleaner fish or shrimp removes ectoparasites, dead skin, or other particles from the body surface of a larger client (Losey et al. 1999, Côté 2000). These interactions are often mutually beneficial to the cleaner and client (Grutter 1999, Sikkell et al. 2004), and can have community-level effects on reef fishes (Bshary 2003, Grutter et al. 2003, Waldie et al. 2011). On small Pacific reefs where a single cleaner species (bluestreak cleaner wrasse *Labroides dimidiatus*) was excluded for 18 mo, mobile reef fish abundance and species richness

decreased by 75% and 50%, respectively (Grutter et al. 2003). Over an additional 7 yr, reefs without the cleaner had smaller, slower growing, and more parasitized client fishes, and less diverse communities of resident and mobile predators and herbivores (Clague et al. 2011, Waldie et al. 2011). These experiments revealed that despite their relatively small size, cleaners may have large ecological effects on the fitness and diversity of reef fishes.

Cleaners may be ecologically important, but we do not know how they may be affected by the local and global stressors that modern coral reefs face (Carpenter et al. 2008). Among these stressors is the invasion of Atlantic coral reefs by the Indo-Pacific red

*Corresponding author: tuttlel@hawaii.edu

lionfish *Pterois volitans*, which is a growing conservation concern in a region already experiencing environmental degradation (Burke & Maidens 2004, Sutherland et al. 2010, Albins & Hixon 2013). Lionfish first appeared off Florida in the 1980s, and have since spread throughout the western tropical and subtropical Atlantic, Caribbean, and Gulf of Mexico, reaching densities of greater than 400 fish per hectare (Schofield 2009, Green & Côté 2009). The reason for concern is that invasive lionfish are voracious, generalist predators of native fishes (Albins & Hixon 2008, Morris & Akins 2009, Côté & Maljković 2010). On large reefs in The Bahamas, lionfish have caused declines in total biomass (by 32%), density (by up to 46%), and species richness (by 21%) of reef fishes (Albins 2015). However, we do not know whether or to what extent lionfish have affected native Atlantic cleaning mutualisms.

On Atlantic coral reefs where the lionfish are invasive, there are several species of obligate cleaners, i.e. species that rely largely on cleaning for food, and facultative cleaners, i.e. species that occasionally obtain food from cleaning. The predominant, obligate cleaners are *Elacatinus* spp. cleaning gobies (Côté & Soares 2011), which have a diverse clientele of at least 138 species of reef-associated fishes (Lettieri & Strelman 2010). *Periclimenes* spp. cleaning shrimps are also obligate cleaners on invaded reefs (Côté 2000). Most abundant of the facultative cleaners are the banded coral shrimp *Stenopus hispidus*, and juveniles of the bluehead wrasse *Thalassoma bifasciatum* and Spanish hogfish *Bodianus rufus* (Côté 2000).

Invasive lionfish could affect these native cleaners in several ways. First, lionfish could directly interact with cleaners by engaging in cleaning behavior. However, extensive observations in the invaded range have not revealed lionfish being cleaned, despite lionfish and cleaners inhabiting the same reefs (Cure et al. 2012). A more likely direct interaction is lionfish consuming cleaners. In particular, lionfish have strong negative effects on the density and biomass of juvenile bluehead wrasse, likely due to predation (Albins & Hixon 2008, Albins 2015). On the other hand, cleaning gobies and shrimps have not yet been identified in the gut contents of invasive lionfish (Albins & Hixon 2008, Morris & Akins 2009, Muñoz et al. 2011, Côté et al. 2013), and field studies have not revealed an effect of lionfish on the density of cleaning gobies (Albins & Hixon 2008, Green et al. 2012, Albins 2015, Benkwitt 2015). However, these cleaning gobies and shrimps are small-bodied and may be digested quickly in the guts of predators, thus making it difficult to definitively assess whether lionfish eat them or

not. In fact, banded coral shrimp have been found in lionfish stomachs (A. Sellers pers. comm.) and there are anecdotes of captive, juvenile lionfish eating cleaning gobies in aquaria. These contradictory accounts warrant further examination into the direct effects of invasive lionfish on the abundance of different cleaner species.

Lionfish may also interact with native cleaners in ways other than predation. First, there is anecdotal evidence to suggest that lionfish may disrupt cleaning behavior on invaded reefs. Côté & Maljković (2010) noted 13 instances during which hunting lionfish approached aggregations of client fishes formed around cleaning gobies; the aggregations of clients dispersed and, in one case, a lionfish ate a juvenile bluehead wrasse. Second, lionfish could indirectly affect cleaners by reducing the abundance of client fish. An increase in lionfish abundance in the Bahamas coincided with a 44% decline in the biomass of large-bodied presumed competitors (Green et al. 2012), which are the most common clients of cleaning gobies. If lionfish interrupt cleaning behavior and reduce the abundance of client fish, then cleaners might have decreased growth rates. Slower growth rates might be especially pronounced among smaller goby recruits, which compete for foraging opportunities with larger conspecifics, as has been documented with another *Elacatinus* goby, *E. prochilos* (Whiteman & Côté 2004). If cleaning gobies have fewer foraging opportunities in the presence of lionfish, they may also have higher mortality rates, even if lionfish do not eat them. Additionally, if lionfish consume some cleaners more than others, the resulting shift in relative abundance of cleaner species on invaded reefs would alter the remaining cleaners' access to clients.

The complexity of species interactions on coral reefs makes it difficult to predict how lionfish affect cleaning mutualisms, and whether there will be cascading effects on the broader ecosystem. As a first step in determining whether and how invasive lionfish might affect native cleaners and clients, I conducted a before-after-control-impact (BACI) study (review by Smith 2002) in which I added lionfish to patch reefs in the Bahamas. I asked 2 questions: (1) Do lionfish affect the survival and growth rates of the cleaner goby *E. genie*? (2) Do lionfish affect the densities and diversity of non-goby cleaners and clients? To best detect any effects of lionfish on the survival and growth of groups of the cleaner goby that I transplanted to experimental reefs, I conducted this study immediately after the annual season of peak settlement of *Elacatinus* gobies, when mortality naturally

exceeds recruitment. Therefore, I predicted a decline in cleaner goby density over time, regardless of lionfish presence. If there was a further decline in the density and diversity of clients after the addition of lionfish to patch reefs, then I predicted that the growth rates of the cleaner goby would subsequently further decline in the presence of lionfish. I also predicted that lionfish would negatively affect the survival of non-goby cleaners, especially of juvenile bluehead wrasse, thus changing the relative abundance of cleaner species on reefs with lionfish.

MATERIALS AND METHODS

Study design

To determine whether lionfish affect the cleaner goby *Elacatinus genie* and communities of other cleaners and clients on invaded reefs, I conducted a manipulative experiment at 12 coral patch reefs in Rock Sound in the Bahamas (24.843°N, 76.267°W; Fig. 1) from late June through August 2014. Rock Sound is a shallow network (3 to 5 m deep) of sand and seagrass with small coral patch reefs interspersed. I paired reefs by similarity in size (surface areas 12 to 35 m²), vertical relief, benthic community (coral per-

cent cover), and baseline communities of cleaners and client fishes to create 6 experimental reef pairs.

The experiment followed a BACI design, for which I maintained low lionfish densities (approaching 0 lionfish m⁻²) at all reefs for 4 wk before randomly assigning 1 reef in each pair to continue having lionfish periodically removed for an additional 5 wk ('control' reefs, n = 6), and the other reef to have lionfish added to levels that naturally occur in the region, also for 5 wk ('impact' reefs, n = 6; about 0.30 lionfish m⁻²; Green & Côté 2009). For the duration of the experiment, reefs were visited every 1 to 3 d to either remove or add lionfish, as necessary. Thus, lionfish were present on impact reefs after the addition of lionfish, at densities an order of magnitude higher than at (1) control reefs both before and after the addition of lionfish to impact reefs, and (2) impact reefs before the addition of lionfish (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m569p163_supp.pdf). Control and impact treatment reefs were physically distributed across an area of approximately 11 × 4 km, and were no closer to each other or to another reef than 400 m, thus limiting the potential for lionfish and other resident fishes to move among patch reefs (Fig. 1).

Lionfish size on the reefs ranged from 5 to 30 cm total length (TL) (mean TL = 17.3 cm), and most lionfish (>85%) were between 11 and 25 cm TL. For 1 mo before and 1 mo after the addition of lionfish, I repeatedly measured the densities (a proxy for survival) and TL of the cleaner goby, and conducted community surveys of non-goby cleaners, potential clients (native fish >10 cm TL), and prey-sized fishes (≤10 cm TL) on all reefs (see below for details).

Effects of lionfish on the cleaner goby

The cleaner goby resides primarily on live coral in discrete groups and recruits to reefs in cohorts, often from sources less than 23 km away (Taylor & Hellberg 2003). In this region, the cleaner goby experiences its highest recruitment during the spring (L. J. Tuttle unpubl. data). Mimicking a natural recruitment pulse, a pair of SCUBA divers transplanted groups of small cleaner goby to experimental reefs, and measured the groups' survival and growth rates over time. This was done in the early summer, the time immediately following peak recruitment, to limit the number of incoming cleaner goby recruits that could confound the study's ability to detect lionfish effects on transplanted gobies. Transplantation standardized the starting density of the cleaner goby

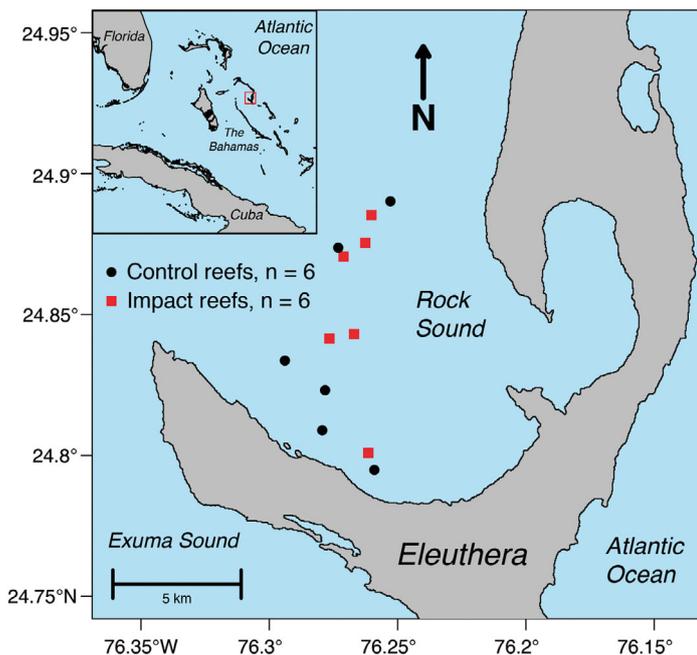


Fig. 1. Reefs in Rock Sound, off southern Eleuthera in the Bahamas, location of a before-after-control-impact experiment into the effects of invasive lionfish on cleaning mutualisms. See 'Materials and methods' for experimental procedures at 'control' and 'impact' reefs

on my experimental reefs, and should have also standardized any density-dependent predation by lionfish and other potential predators (Ingeman & Webster 2015, Ingeman 2016). To transplant gobies, divers first captured them with small aquarium nets from source reefs in the nearby (5.5 km away) northern Exuma Sound (24.820° N, 76.346° W). Because congeneric group-living gobies *E. prochilos* have dominance hierarchies that affect foraging rates of group members (Whiteman & Côté 2004), divers (1) visually located and removed all pre-existing *Elacatinus* gobies from experimental reefs to avoid previous hierarchies from affecting transplanted gobies, and (2) captured entire groups of gobies from source reefs to ensure that any within-cohort hierarchies would remain intact after transplantation to experimental reefs. The number of transplanted gobies per reef ranged between 8 and 15, with a target starting density of approximately 0.50 gobies m⁻². Transplants occurred once at the beginning of the experiment (Time-step 0), at which point every goby was measured to the nearest 0.1 cm TL. Follow-up surveys revealed that 80% of all cleaner goby were still present 2 d after transplantation.

The density and size of the cleaner goby were measured 3 times before (Time-steps 1 to 3) and 3 times after lionfish were added to half the reefs (Time-steps 4 to 6), at approximately 10-d intervals. At each time-step and reef, divers captured all gobies with aquarium nets, placed them in water-filled plastic bags, and recorded the TL of each goby to the nearest 0.1 cm before releasing it to the location on the reef where it was found. Divers were confident that they captured all cleaner goby individuals present on a reef at a given time-step because the goby is visually conspicuous in color (contrasting black and yellow), pattern (lateral stripes), and habit (mostly found either on the bodies of other fishes, or on the surface of corals). It was not possible to track the growth of individuals because many of the gobies were too small (<1.5 cm TL) and slender to reliably tag without affecting their survival or behavior. Therefore, the metric of growth was change in mean length, calculated as the mean TL of all gobies at a reef at a given time-step minus the mean TL of all gobies on that reef at Time-step 0 (day of transplantation).

Effects of lionfish on cleaner, client, and prey communities

A pair of divers on SCUBA recorded the species, abundance, and body size (TL estimated to the near-

est cm) of all fishes and cleaner shrimps present at each reef on 3 dates before the addition of lionfish (Time-steps 1 to 3), and 3 dates after the addition of lionfish (Time-steps 4 to 6), at approximately 10-d intervals. According to standard protocol (Hixon & Beets 1989, 1993), divers slowly approached and circled a reef from approximately 3 m away, counting all planktivorous and other active species that hovered above the reef. Divers then slowly circled the reef from 1 m away, counting demersal fishes and invertebrates. Lastly, divers used lights to count cryptic species living in reef holes.

'Non-goby cleaners' included the banded coral shrimp *Stenopus hispidus* (facultative), Pederson cleaner shrimp *Periclimenes pedersoni* (obligate), spotted cleaner shrimp *P. yucatanicus* (obligate), and juveniles (≤ 10 cm TL) of the bluehead wrasse *Thalassoma bifasciatum* (facultative) and Spanish hogfish *Bodianus rufus* (facultative).

Fish smaller than 10 cm are occasionally cleaned, but an order of magnitude less frequently than fish greater than 10 cm on experimental reefs (L. J. Tuttle unpubl. data). Therefore, 'clients' were defined as all native fish >10 cm TL. The 7 species of *Elacatinus* gobies that clean have at least 138 documented client species (Lettieri & Streelman 2010), and there are no accounts of any fish consuming *Elacatinus* gobies in the wild. Thus, my assumption was that all large native fishes were potential clients of the cleaner goby, not predators. Each client species was designated as either resident or transient based on the relative size of the species' home range (one patch reef vs. many patch reefs, respectively; see Table S1 in the Supplement).

'Prey' were defined as all fish ≤ 10 cm TL (excluding the cleaner goby), and were censused to determine whether lionfish affect small fish, as has been previously well documented on patch reefs in the region (Albins & Hixon 2008, Albins 2013, Benkwitt 2015).

Statistical analyses

I used linear mixed effects (LME) models to assess the effects of lionfish on native cleaners, clients, and prey-sized fishes. I created a unique model for each of the following response variables: (1) cleaner goby density, (2) cleaner goby change in mean length, and the densities of (3) all non-goby cleaners, (4) juvenile bluehead wrasse, (5) banded coral shrimp, (6) *Periclimenes* cleaner shrimps, (7) resident clients, (8) transient clients, (9) yellowtail snapper *Ocyurus chrysurus* (the most abundant

transient client), and (10) prey-sized fishes. Each model had 'treatment' (categorical: control versus impact), 'period' (categorical: before versus after the addition of lionfish), and the interaction of treatment \times period as fixed effects, and 'reef' ($n = 12$) as a random effect (Zuur et al. 2009, Bolker et al. 2009). To test for the effect of lionfish on a response variable, in 'Results' I report the LME estimate for the treatment \times period interaction, which represents how much more different the control and impact reefs were from each other after the addition of lionfish, given how different they were before the addition of lionfish.

I first fitted models with and without (1) the random effect of reef, (2) first order autoregressive (AR-1) structures to allow for temporal autocorrelation by time-step (Time-steps 1 to 6) within reef (thus avoiding pseudoreplication due to repeated measures), and (3) weighted terms to allow variance to differ among reef using restricted maximum likelihood estimation (REML) (Zuur et al. 2009). I chose the best-performing model per Akaike's Information Criterion (AIC) and p-values from likelihood ratio tests (LRTs) (Table S2 in the Supplement). Two response variables, the densities of resident clients and prey-sized fishes, were log-transformed to meet the assumptions of normality and equal variance, which were visually assessed with residual plots for all models. I conducted the analyses with the statistical software R, v3.2.1 (R Core Team 2016) and the associated package nlme v3.1-118 (Pinheiro et al. 2016).

To test for changes in community structure (i.e. species composition and relative abundance) of non-goby cleaners, resident clients, and transient clients, I used permutation-based multivariate analysis of variance (PERMANOVA; Anderson 2001). As with univariate analyses, I tested for the significance of treatment, period, and the treatment \times period interaction. I $\log(x+1)$ transformed species abundance data to reduce the influence of the most abundant species, and used Bray-Curtis distance and 1000 permutations constrained within 'reef'. Multivariate analyses used the vegan v.2.2-0 package (Oksanen et al. 2016) in R (R Core Team 2016).

Permits

Our work was approved by Oregon State University's Institutional Animal Care and Use Committee (IACUC; ACUP 3886), and with permission from the Department of Marine Resources of The Bahamas.

RESULTS

Effects of lionfish on the cleaner goby

The addition of lionfish to impact reefs did not significantly affect the density (LME, treatment \times period: $t = 0.806$, $df = 69$, $p = 0.423$), or the change in mean length (LME, treatment \times period: $t = 0.300$, $df = 57$, $p = 0.765$) of the cleaner goby (Fig. 2, Table S3 in the Supplement). On all reefs the density of the cleaner goby decreased through time (Fig. 2a; LME, period: $t = 3.586$, $df = 69$, $p = 0.001$), as expected regardless of lionfish treatment. The change in mean length of the cleaner goby increased through time (Fig. 2b; LME, period: $t = -4.052$, $df = 57$, $p < 0.001$) regardless of lionfish treatment.

Effects of lionfish on cleaner, client, and prey communities

After the addition of lionfish, there was a suggestive but nonsignificant trend that impact reefs had fewer non-goby cleaners than control reefs (Fig. 2c, Table S3; LME, treatment \times period: $t = -1.836$, $df = 58$, $p = 0.071$). Among the non-goby cleaners, the juvenile bluehead wrasse *Thalassoma bifasciatum* was significantly affected by lionfish (Fig. 2d). Before the addition of lionfish, juvenile bluehead wrasse represented approximately half the non-goby cleaners by number, with average densities (\pm SEM) of $0.17 (\pm 0.09)$ and $0.15 (\pm 0.05)$ fish m^{-2} on control and impact reefs, respectively (Table S3). After the addition of lionfish, the mean density of juvenile bluehead wrasse on impact reefs fell to $0.10 (\pm 0.04)$ fish m^{-2} (Table S3). This was 0.05 fish m^{-2} fewer than on control reefs after the addition of lionfish, after accounting for the difference between the same reefs before the addition of lionfish (95% CI: 0.00 to 0.10; LME, treatment \times period: $t = -2.151$, $df = 58$, $p = 0.036$). Lionfish did not significantly affect the densities of the facultative cleaner shrimp *Stenopus hispidus* (Table S3; LME, treatment \times period: $t = 0.779$, $df = 58$, $p = 0.439$), or of obligate cleaner shrimps *Periclimenes* spp. (Table S3; LME, treatment \times period: $t = -1.062$, $df = 58$, $p = 0.293$). There were only 2 Spanish hogfish *Bodianus rufus* (a facultative cleaner) counted during the experiment, therefore the density of this fish was not analyzed separately from other non-goby cleaners. While the community structure (i.e. species composition and relative abundance) of all non-goby cleaners changed significantly through time (Table S4 in the Supplement; PERMANOVA, period: $F = 1.252$, $df = 1$,

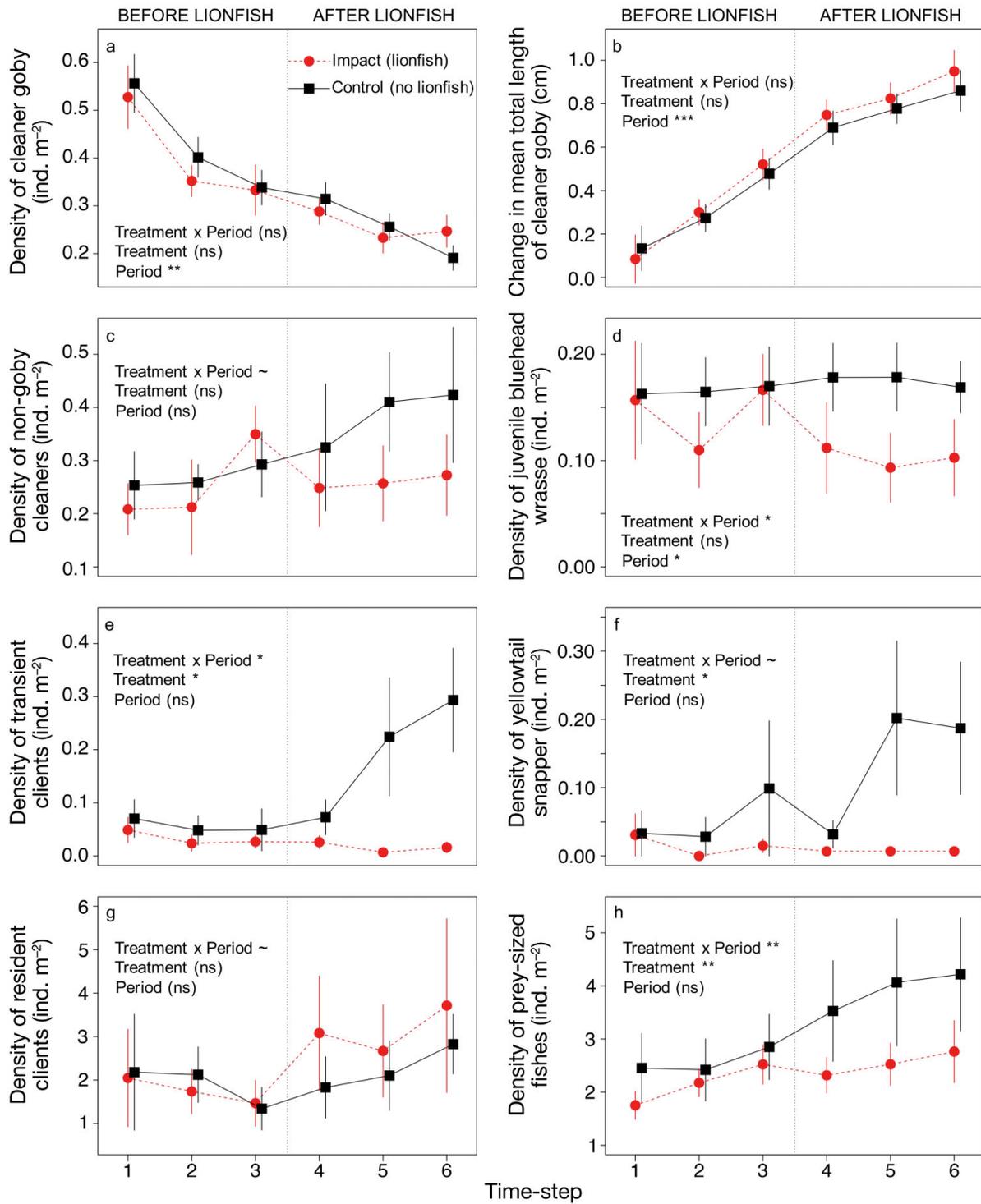


Fig. 2. Effects of invasive lionfish over time on cleaning mutualisms on patch reefs in The Bahamas on: (a) density of cleaner goby, (b) change in mean total length (TL) of cleaner goby, and densities of (c) non-goby cleaners, (d) juvenile bluehead wrasse (most abundant non-goby cleaner), (e) transient clients, (f) yellowtail snapper (most abundant transient client), (g) resident clients, and (h) prey-sized fish (≤ 10 cm TL). Dots represent means and error bars are SEM; note different y-axis scales. The vertical dashed line is the time at which lionfish were added to 'impact' reefs ($n = 6$) and excluded from 'control' reefs ($n = 6$). Results from linear mixed models (LME) for fixed effects are shown in each panel for 'treatment' (impact vs. control reefs), 'period' (before vs. after the addition of lionfish) and treatment \times period (indicating the effect of lionfish on the response): (ns) $p > 0.1$; (~) $0.1 \geq p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

$p = 0.037$), there was no evidence of a lionfish effect (PERMANOVA, treatment \times period: $F = 0.624$, $df = 1$, $p = 0.278$).

Lionfish significantly affected the density of transient clients (Fig. 2e, Tables 1 & S3). After the addition of lionfish, the mean density of transient clients on impact reefs was $0.02 (\pm 0.01 \text{ SEM}) \text{ fish m}^{-2}$, which was 0.07 fish m^{-2} fewer than on control reefs, after accounting for the difference between control and impact reefs before the addition of lionfish (95% CI: 0.01 to 0.12; LME, treatment \times period: $t = -2.073$, $df = 58$, $p = 0.043$). Among the transient client species that were observed during reef censuses, yellowtail snapper *Ocyurus chrysurus* was the most abundant and commonly counted (Table 1). After the addition of lionfish, there was a suggestive but nonsignificant decline in abundance of yellowtail snapper on impact reefs, compared to control reefs (Fig. 2f, Table S3; LME: treatment \times period $t = -0.095$, $df = 58$, $p = 0.060$). While the community structure of all transient clients changed significantly through time (Table S4; PERMANOVA: period $F = 2.264$, $df = 1$, $p = 0.022$), there was no evidence of a lionfish effect (PERMANOVA: treatment \times period $F = 0.755$, $df = 1$, $p = 0.481$).

Lionfish did not significantly affect the density of resident clients (Fig. 2g, Table S3; LME, treatment \times period: $t = 2.41$, $df = 60.00$, $p = 0.090$). The community structure of resident clients changed significantly through time (Table S4; PERMANOVA, period: $F = 0.647$, $df = 1$, $p = 0.026$); but again there was no evidence of a lionfish effect (PERMANOVA, treatment \times period: $F = -0.10$, $df = 1$, $p = 0.993$).

Table 1. Potential, transient client species (>10 cm TL) counted during an experiment into the effects of invasive lionfish on cleaning mutualisms, conducted on 12 patch reefs in Rock Sound, Eleuthera, The Bahamas. The table shows mean abundances across all reefs (during surveys when that species was present) and the number of surveys (out of 72) in which the species was seen. These fishes were deemed 'transient' because their typical daily home range is estimated to be at least 1000 m², and therefore encompasses many patch reefs

Transient client species	Mean abundance \pm SEM	No. of surveys seen (%)
Yellowtail snapper <i>Ocyurus chrysurus</i>	4.8 \pm 1.3	16 (22.2)
Bar jack <i>Caranx ruber</i>	2.0 \pm 0.7	7 (9.7)
Mutton snapper <i>Lutjanus analis</i>	1.1 \pm 0.1	9 (12.5)
Great barracuda <i>Sphyrna barracuda</i>	1.0 \pm 0.0	9 (12.5)
Ocean triggerfish <i>Carantheris sufflamen</i>	1.0 \pm 0.0	5 (6.9)
Nurse shark <i>Ginglymostoma cirratum</i>	1.0 \pm 0.0	3 (4.2)
Cero mackerel <i>Scomberomorus regalis</i>	1.0 \pm 0.0	2 (2.8)
Atlantic spadefish <i>Chaetodipterus faber</i>	1.0 \pm 0.0	1 (1.4)
Southern stingray <i>Dasyatis americana</i>	1.0 \pm 0.0	1 (1.4)
Sharksucker <i>Echeneis naucrates</i>	1.0 \pm 0.0	1 (1.4)

Schools of hundreds of juvenile grunts *Haemulon* spp. inhabited some of the reefs and outnumbered all other prey-sized fishes by up to an order of magnitude. When these grunts were excluded from analyses because of their influence on prey counts (cf. Shulman 1985), lionfish negatively affected the density of prey-sized fishes (Fig. 2h, Table S3). After the addition of lionfish, the mean density of prey-sized fishes on impact reefs was $2.02 (\pm 1.13) \text{ fish m}^{-2}$, which was 0.53 fish m^{-2} fewer than on control reefs, after accounting for the difference between control and impact reefs before the addition of lionfish (95% CI: 0.33 to 0.85; LME, treatment \times period: $t = -2.693$, $df = 58$, $p = 0.009$).

DISCUSSION

Invasive lionfish are a major conservation concern because they are generalist predators of ecologically and economically important native species on Atlantic coral reefs (Sutherland et al. 2010, Albins & Hixon 2013). However, we do not yet know how lionfish affect species that engage in cleaning mutualisms, a common interaction with the potential to affect the structure and function of coral-reef communities. I found that lionfish had no detectable effects on the survival and growth rates of transplanted groups of the cleaner goby *Elacatinus genie*. However, reefs with lionfish had fewer juvenile bluehead wrasse *Thalassoma bifasciatum*, the most common facultative cleaner on patch reefs, and fewer transient client fishes. The addition of lionfish to patch reefs did not affect the density of all other non-goby cleaners or of resident clients, nor did lionfish alter ordinated community structures of non-goby cleaners and clients.

In previous field studies, lionfish also had no effect on the density of *Elacatinus* spp. cleaning gobies on small patch reefs (Albins & Hixon 2008, Benkwitt 2015), large patch reefs (Albins 2015), and continuous reefs (Green et al. 2012). The mechanism by which cleaning gobies seem to escape the direct effects of lionfish is unknown, especially because these gobies are small (prey-sized) and conspicuous fishes that commonly co-occur with lionfish throughout its invaded range. One possible explanation is that lionfish recognize gob-

ies as cleaning partners, and therefore avoid eating gobies. However, behavioral observations of lionfish have never revealed lionfish being cleaned by gobies or any other cleaner on Atlantic coral reefs (Cure et al. 2012). In addition, lionfish have remarkably few external parasites in their invaded range (Sikkel et al. 2014, Ramos-Ascherl et al. 2015, Tuttle et al. 2017), so there is little need for them to be cleaned. A more likely explanation is that *Elacatinus* spp. gobies might be distasteful (Colin 1975, Randall & Lobel 2009), and therefore lionfish avoid eating them. Future studies should examine the physiological and/or behavioral mechanisms by which cleaning gobies presumably escape predation by lionfish (and other reef predators).

While lionfish did not affect the cleaner goby, lionfish did decrease the density of juvenile bluehead wrasse, a facultative cleaner. Declines were most likely caused by predation, as the bluehead wrasse is a very common prey item of lionfish in the region (Albins & Hixon 2008, Morris & Akins 2009, Côté et al. 2013). Few studies have compared the behaviors of obligate cleaners and facultative cleaners on Atlantic coral reefs (Darcy et al. 1974, Johnson & Ruben 1988). Therefore, it is difficult to predict what will be the repercussions of reduced abundance of facultative cleaners on invaded reefs. If lionfish do not affect obligate cleaners, which tend to clean more clients more often (Johnson & Ruben 1988), but do affect facultative cleaners, there may be little change to the ectoparasite loads and distribution of reef clients. However, if lionfish have strong, negative effects on particular facultative cleaners that strongly interact with particular clients, then changes to the clients' ectoparasite loads and visitation rates to cleaning stations could occur. Future studies should investigate the ecological importance of cleaning by juvenile bluehead wrasse to better predict how invasive lionfish might indirectly affect cleaning mutualisms via predation of bluehead wrasse.

While there was no clear effect of lionfish on resident clients (those species that spend most of their time at one patch reef), lionfish did affect the density of transient clients (those species that move among patch reefs over the course of a day). There was a large increase in the density of transient clients on control reefs without lionfish, but no increase on impact reefs with lionfish. Transient clients most likely came from neighboring patch reefs interspersed among the experimental patch reefs, all of which were spread across an area of approximately 11×4 km. Because of the relatively even distribution

of control and impact reefs across the study area, there was no indication that their relative locations should have influenced the presence versus absence of transient clients. During visits of transient species to patch reefs used in this study, these fishes are often cleaned by obligate and facultative cleaners. Over the course of the experiment, there were increasing trends in the densities of non-goby cleaners and prey-sized fishes on all reefs due to the accumulation of new settlers during the summer recruitment season, since most species in this region, except cleaning gobies, experience recruitment peaks in July to August (Webster 2003, Hixon et al. 2012, M. Hixon pers. comm.). These increases were greater on control versus impact reefs (Fig. 2c,h). As a result, the increase in transient species, especially yellowtail snapper *Ocyurus chrysurus* (Fig. 2f), on control reefs only is likely attributable to an aggregative response of transient species to cleaners and/or prey, as has been described on coral patch reefs in the region (Hixon & Carr 1997, Hixon 1998). Lionfish may have suppressed the number of cleaners and prey below a threshold that would otherwise induce aggregative responses by transient species to patch reefs. This is the first documentation of lionfish affecting the density of transient reef species, indicating that the effects of lionfish may extend beyond those already documented for resident reef species.

While I did not observe any effects of invasive lionfish on the native cleaner goby over the experimental period (the goby was in the presence of lionfish for 1 mo), this outcome does not preclude longer-term effects on the goby via relatively prolonged, reduced densities of potential clients on patch reefs. Future studies should investigate the effects of lionfish on cleaning rates, especially on patch reefs where the aggregative response of transient species might be most affected. At present, however, there is little evidence to suggest that lionfish affect the cleaner goby. Lionfish may have similarly weak interactions with other *Elacatinus* spp. cleaning gobies throughout the invaded western Atlantic and Caribbean region, where there are 27 species in the *Elacatinus* genus, 7 of which are obligate cleaners (Taylor & Hellberg 2005). If this is true, then cleaning gobies are among the remarkably few fishes on invaded reefs to escape the effects of lionfish. The continued presence of cleaning gobies on invaded reefs should limit cascading effects that are mediated by these cleaners. However, by consuming other cleaners, invasive lionfish may alter the structure and function of native reef communities.

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