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# Differential learning by native versus invasive predators to avoid distasteful cleaning mutualists

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## Abstract

1. Cleaning symbioses on coral reefs are mutually beneficial interactions between two individuals, in which a ‘cleaner’ removes and eats parasites from the surface of a ‘client’ fish. A suite of behavioural and morphological traits of cleaners signal cooperation with co-evolved species, thus protecting the cleaner from being eaten by otherwise predatory clients. However, it is unclear whether cooperation between cleaners and predatory clients is innate or learned, and therefore whether an introduced predator might consume, cooperate with or alter the behaviour of cleaners.
2. We explored the role of learning in cleaning symbioses by comparing the interactions of native cleaner fishes with both naïve and experienced, non-native and native fish predators. In so doing, we tested the vulnerability of the predominant cleaners on Atlantic coral reefs, cleaning gobies (*Elacatinus* spp.), to the recent introduction of a generalist predator, the Indo-Pacific red lionfish (*Pterois volitans*).
3. Naïve juveniles of both invasive (*P. volitans*) and native predators (*Cephalopholis* spp. groupers) initially attacked cleaning gobies and hyperventilated from a putative toxin on the gobies' skin during laboratory experiments. After one to five such encounters, invasive lionfish often approached the cleaner closely, then turned away without striking. Consistent with learned avoidance, invasive lionfish rarely interacted with cleaning gobies in the wild, either antagonistically or cooperatively, and did not affect gobies' abundance. Native predators showed little evidence of learning during early encounters; they repeatedly attacked the cleaner during laboratory experiments and hyperventilated less violently than did lionfish. However, consistent with learned cooperation, native predators rarely antagonised and were frequently cleaned by gobies in the wild.
4. We demonstrated that rapid, learned avoidance protects a distasteful cleaning mutualist from an invasive predator. The behavioural plasticity of this invader likely contributes to its success across its invaded range. Additionally, our results suggest that the cleaner's chemical defence most likely evolved as a way to deter predation and reinforce cooperation with naïve individuals of native species.

KEYWORDS
chemical defence, cleaning mutualism, invasive species, learning, novel interaction, phenotypic plasticity, predation

# 1 | INTRODUCTION

Human activities have greatly accelerated the rate of introductions of exotic species, thus generating novel interactions among plants and animals that share no history of coexistence or co-evolution (Pearse & Altermatt, 2013; Saul & Jeschke, 2015). A wealth of studies have shown the effects of invasive species through predation (Cox & Lima, 2006; Polo-Cavia & Gomez-Mestre, 2014; Sih et al., 2010), herbivory (Forister & Wilson, 2013; Pearse & Altermatt, 2013), disease (Dunn & Perkins, 2012) or competition (Alexander et al., 2015; Urban et al., 2012). Mutualisms are less well-studied within the context of species introductions, even though these reciprocally beneficial interactions are both important in shaping biological communities (Hay et al., 2004; Stachowicz, 2001) and susceptible to human-caused environmental change (Kiers et al., 2010). Also, many mutualisms are context dependent in that different conditions can shift interaction outcomes from facilitative to antagonistic (Brown et al., 2012; Chamberlain et al., 2014). The ecological importance and ambiguity of certain mutualisms may make them informative systems in which to study the insertion of a non-native species.

Cleaning symbioses on coral reefs are among the most well-studied of animal mutualisms, whereby small 'cleaner' fish and shrimp eat parasites, mucus and dead skin from the external surface of cooperative fish 'clients' (Côté, 2000). These relationships are not trivial; on the Great Barrier Reef, a single cleaner fish cleans an average 2,300 clients (Grutter, 1996) from 132 species (Grutter & Poulin, 1998) in 1 day, and individual clients can visit cleaners 144 times a day (Grutter, 1995). Cleaners are the drivers of local fish diversity, as evidenced by a decline in fish abundance and richness following the removal of a cleaner species from Pacific reefs (Grutter et al., 2003). However, cleaning symbioses can have ambiguous outcomes, that is, some interactions can result in parasitism (when the cleaner bites the client's flesh) or predation (when the client eats the cleaner), instead of mutualism (Côté & Mills, 2020; Poulin & Vickery, 1995). Coral-reef predators are very common clients of cleaners, and it remains unclear how cooperation persists in a system in which a co-evolved predator could so easily cheat. Also, it is unknown how an introduced predator might interact with a cleaner with which it could cooperate or consume, and whether this novel interaction might reveal something about the evolutionary and ecological mechanisms that give rise to cooperation between native species.

Coral reefs in the western Atlantic, Caribbean and Gulf of Mexico are host to the Pacific red lionfish (*Pterois volitans*), an invasive species and generalist predator with widespread, negative effects on invaded communities over the last decade (Albins, 2015; Benkwitt, 2015; Ingeman, 2016). The predominant, obligate cleaners on coral reefs of this region are several *Elacatinus* spp. fishes (Côté & Soares, 2011), known as cleaning gobies. These small and abundant gobies have a diverse clientele of at least 138 fish species, which they clean at specific locations on the reef known as cleaning stations (Lettieri & Streelman, 2010). The nature of novel interactions between invasive lionfish and native cleaning gobies is unclear, despite the two fishes living in close proximity on the same reefs.

In their native Pacific range, lionfish are rarely witnessed interacting with resident cleaner wrasses (A. Grutter, pers. comm.), though lionfish are relatively rare in Pacific locations where cleaning behaviour is most well-studied (Kulbicki et al., 2012). In their invaded Atlantic range, observations have not revealed lionfish being cleaned (Cure et al., 2012), and a field experiment found no effect of lionfish on the density or growth rates of the cleaner goby (*E. genie*) (Tuttle, 2017). However, one study noted lionfish approaching goby cleaning stations and consuming a juvenile bluehead wrasse (*Thalassoma bifasciatum*) at a station (Côté & Maljković, 2010). Juveniles of this wrasse resemble cleaning gobies in size, colour and pattern, and occasionally clean other fishes (Côté, 2000). Cleaning gobies have not yet been found in the guts of invasive lionfish (Albins & Hixon, 2008; Côté et al., 2013; Morris & Akins, 2009), but bluehead wrasse are common prey and cleaning shrimps have been found in lionfish guts (Eddy et al., 2016; Ellis & Faletti, 2016). Therefore, a species' status as a cleaner does not impart immunity to predation by this invader.

Interactions between cleaning gobies and native Atlantic fishes are presumed to be mutualistic, largely because predation on cleaning gobies has never been witnessed in natural conditions on the reef. We do not yet know whether cleaning mutualisms among native Atlantic species are innate or learned. One study reported hatchery-reared, predatory fish, never before exposed to cleaners, being cleaned by gobies within an hour of release into the wild (Roberts et al., 1995), suggesting that cleaning behaviour may be instinctive. However, captive predators have eaten cleaning gobies in the laboratory, and it is estimated that approximately one third of cleaning gobies' clients are potential predators (Darcy et al., 1974; Lettieri & Streelman, 2010). In fact, cleaning gobies may perceive a risk of being eaten as evidenced by their increased responsiveness and cortisol levels in the presence of predatory versus non-predatory fishes (Soares et al., 2012). There is also evidence suggesting that cleaning gobies are distasteful (Colin, 1975; Lettieri & Streelman, 2010), which may discourage predators from consuming them and incentivise cooperative behaviour. However, we know little about the mechanism(s) by which Atlantic cleaners and predatory clients cooperate.

To explore the mechanisms by which cleaning mutualisms are maintained, we compared the interactions of native Atlantic cleaning gobies with both naïve and experienced invasive and native fish predators. Using experiments both in the field and laboratory, we asked: (a) How do invasive and native predators interact with cleaners?; (b) Does the interaction of invasive and native predators with cleaners change over time with repeated exposures?; and (c) Does the invasive predator affect wild populations of cleaners?

# 2 | MATERIALS AND METHODS

## 2.1 | How do invasive and native predators interact with cleaners?

### 2.1.1 | Field

We explored how predators interact with two common cleaners on Atlantic coral reefs, the cleaner goby (*Elacatinus genie*, dominant cleaner on shallower reefs <12 m depth, but exists 0–30 m: Colin, 1975) and the sharknose goby (*E. evelynae*, dominant cleaner on deeper reefs >12 m depth, but exists 0–50 m: Colin, 1975). In the Bahamas, we observed cleaning stations at natural patch reefs where both invasive lionfish and native predators were present. A species was considered a potential predator of cleaning gobies if most of their diet is fish (Randall, 1967). From a distance of at least 3 m, in 10-min intervals, and during daylight hours (07:00 to 20:00 hours), SCUBA divers documented the nature (cleaning, predation, etc.) and duration of all interactions between cleaning gobies and other fishes that occurred at a focal cleaning station. Cleaning behaviour was presumed when a cleaning goby made contact with another fish's body for at least 1 s. We conducted our observations on four coral patch reefs (depth: 6–20 m; surface area: 200–1,300 m$^2$) in the northern Exuma Sound in the Bahamas (24°47′10″N, 76°19′33″W) during the summer of 2013. Observation time totalled 24 hr, 10 min, 13 s, with observation effort spread both across reefs (no less than 4.5 hr at a reef, with an average of 6.0 hr per reef) and within reefs (no fewer than 10 cleaning stations per reef). This resulted in no fewer than 37 observations of cleaning at a reef (76.0 ± 17.4, mean ± *SEM*) at an average frequency of one cleaning interaction every 4.75 min.

### 2.1.2 | Laboratory

We conducted a laboratory experiment to determine how invasive lionfish and two native groupers—graysby (*Cephalopholis cruentata*) and coney (*Cephalopholis fulva*)—interact with the cleaner goby (*E. genie*), during the summer of 2011 at Lee Stocking Island, The Bahamas (23°46′00″N, 76°06′00″W) and Little Cayman, Cayman Islands (19°41′56″N, 80°3′38″W). Graysby and coney are common native mesopredators, and are similar to lionfish in size and diet (Morris & Akins, 2009; Randall, 1967). Divers used SCUBA and hand nets to capture juvenile (<15 cm total length TL) lionfish, graysby and coney (hereafter ‘predators’) on nearby reefs at 3–15 m depth. Predators were held in indoor aquaria at least 72 hr prior to their experimental trial and fed live mosquitofish (*Gambusia* sp.) once daily except for the 24 hr preceding each trial.

Divers used SCUBA and hand nets to capture two goby species: the cleaner goby and the bridled goby (*Coryphopterus glaucofraenum*). The bridled goby is a known common prey of both lionfish (Albins & Hixon, 2008) and native groupers (Randall, 1967) and was therefore an indicator of predator hunger during lab trials.

To account for order of exposure to prey, we randomly assigned predators to one of two groups—(a) bridled goby then cleaner goby or (b) cleaner goby then bridled goby—such that each predator was offered exactly two gobies less than one third their total length (lionfish consume prey up to one half their own length in the wild: Morris & Akins, 2009). On the day of the trial, individual predators were placed in a transparent, 208-L indoor aquarium (122 × 33 × 51 cm) and allowed to acclimate for at least 10 min. A trial began when we released the first goby into the aquarium with the predator. We observed all subsequent behaviour of the predator for 20 min, deemed during preliminary trials as sufficient time for a predator to detect a goby of either species. We made observations from a distance of 2 m for lionfish, and from behind a viewing blind for grouper (observer could see the grouper, but the grouper could not see the observer) because grouper seldom hunted when people were visible. If the first goby was eaten, then we waited 10 min for digestion before placing the second goby into the aquarium with the same predator. If the first goby was uneaten at the end of 20 min, then we removed it from the aquarium, and replaced it with the second goby. The trial then followed the same protocol as described above.

There were no instances of cleaning, so all analyses focused on predation behaviour, for which we excluded all trials in which the predator did not strike at either goby ($n$ = 11 of 42 lionfish, 9 of 32 graysby and 18 of 30 coney). We then calculated the proportion of trials in which a predator ate a cleaner goby and compared this proportion among predator species using Fisher's exact tests. We also used binary logistic regression to determine whether predation on the cleaner goby was affected by eating the bridled goby, the order of exposure to the prey species, the total lengths of the predator and the cleaner goby, and the region (Bahamas or Cayman Islands) where the trial was conducted. A unique regression model was created for each predator species in *R* v3.2.1 (R Core Team, 2020).

## 2.2 | Does the interaction of invasive and native predators with cleaners change over time with repeated exposures?

To determine whether novel and native interactions change over short time-scales, we repeatedly exposed individual lionfish and graysby to a cleaner goby (a different goby each trial) over a 2-week period and monitored the predators' behaviours for any changes in response to cleaner versus bridled gobies. This work was done at laboratory facilities in southern Eleuthera in The Bahamas (24°49′53″N, 76°19′43″W) during the summers of 2013, 2014 and 2015, and followed approximately the same protocol as described above (differences described below). To minimise the likelihood of individual predators having prior experience with the cleaner goby and therefore maximise our ability to detect changes in the predators' behaviours, we captured predators from small, isolated patch reefs (≥100 m from nearest reef) without cleaner gobies.

To determine if the cleaner goby is distasteful to predators, as has been previously suggested (Colin, 1975; Lettieri & Streelman, 2010), we quantified predators' gill ventilation rates before and after goby consumption. On the day of each trial, we allowed each predator to acclimate to the observation aquarium for at least 10 min, then determined their baseline gill ventilation rate by counting the number of times their gill opercula beat during a 10-s interval. We then introduced the first goby to the tank and proceeded with the trial as described for the previous laboratory experiment. Immediately after a predator ate a goby, we again determined the predator's gill ventilation rate, and continued doing so every minute thereafter for no less than 3 min, and until the predator's ventilation rate returned to its baseline level. After completing a trial, we returned each predator to its holding aquarium where it was fasted until its next trial, approximately 48 hr later. To allow for learning, we exposed individual predators to a cleaner goby no fewer than four times, collecting predators' gill ventilation rates before and after goby consumption for each trial.

We conducted a separate experiment at Lee Stocking Island in 2007, during which we repeatedly exposed juvenile lionfish ($n = 9$) to a cleaner. We used a similar method as previously described except that lionfish were offered a cleaner goby a total of eight times at an interval of once every 2, 4 or 6 days, with trials lasting 1 min.

To assess predator learning, we first excluded from analyses all trials in which the predator did not strike at either goby species, and all individuals that ate during no more than one trial ($n = 0$ of 29 lionfish and 3 of 15 graysby). We then divided the predators into two mutually exclusive groups: those that struck at a cleaner goby at least once, and those that did not (i.e. the predator struck at the bridled goby but never at a cleaner goby). Of those predators that struck at a cleaner at least once, we calculated the proportion of individuals that developed an aversion to the cleaner (i.e. learned not to eat the goby). A predator was considered to have developed an aversion if after striking at or eating a cleaner goby in an initial trial (a) it did not strike at a cleaner for three trials in a row, or (b) it approached a cleaner in hunting posture then turned away without striking, even if the lionfish was hungry (as demonstrated by eating a bridled goby during the same trial). To test for learning, we used Cochran's Q test (and when significant, $p \leq 0.05$, pairwise McNemar's tests with Bonferroni adjustments for multiple comparisons) to compare the proportions of each predator species that struck at a cleaner over time, beginning with the first trial in which a predator struck at a cleaner.

Gill ventilation rates were quantified and compared in two ways: the number of gill opercular beats per minute upon consuming a goby, and the number of minutes after consuming a goby that it took for the predator's gill ventilation rate to return to ‘normal’, defined as within six gill opercular beats per minute of the baseline level. We used rank-sum tests to compare gill ventilation rates among predator species.

### 2.3 | Does the invasive predator affect wild populations of cleaners?

To determine whether invasive lionfish affect densities of *Elacatinus* spp. cleaning gobies in the wild, we conducted a manipulative experiment on eight coral patch reefs in the northern Exuma Sound in The Bahamas (24°47′10″N, 76°19′33″W) during the summer of 2013. Reefs were surrounded by sand and seagrass and the nearest hard substrate was at least 80 m away. We paired reefs by similarity in size (surface areas 200–1,300 m$^2$), depth (6–20 m), vertical relief and benthic community (coral percent cover) to create four experimental reef pairs. We randomly assigned one reef in each pair to have periodic lionfish removals (‘low-lionfish’; approaching 0 lionfish/m$^2$), and the other reef to have periodic lionfish additions (‘high-lionfish’; about 0.04 lionfish/m$^2$, similar to unmanipulated densities in The Bahamas, mean ± *SD*: 0.039 ± 0.014 lionfish/m$^2$: Green & Côté, 2009).

Before manipulating lionfish densities, we conducted full-reef censuses of the two cleaning gobies present at these reefs, namely the cleaner goby (*E. genie*) and the sharknose goby (*E. evelynae*). After baseline goby censuses were complete, we manipulated lionfish densities (low versus high) for the next 10 weeks. We conducted full-reef censuses of cleaning gobies, lionfish, graysby and coney five to six times over 10 weeks, at approximately 2-week intervals. At the end of the experiment, lionfish were removed from all experimental reefs.

We used a linear mixed-effects model (LME) to assess the effect of lionfish on changes in cleaning goby density, with *lionfish treatment* (low- versus high-lionfish densities) as a categorical fixed effect, *time* in days (day 0 was the time of baseline census) as a continuous fixed effect, the interaction between treatment and time as a fixed effect (*treatment*time*) and *reef* (eight reefs) as a random effect (with weighted terms to allow for variance among reefs) (Bolker et al., 2009). We first fitted models with and without (a) random effects (*reef* and *reef pair*, four reef pairs), (b) weighted terms to allow variance to differ among reefs and (c) AR1 structures to allow for temporal autocorrelation within reefs, using restricted maximum likelihood estimation (REML). We chose the best-performing model per Akaike's information criterion (AIC) and *p*-values from likelihood ratio tests (LRTs) (see Table S1). Residuals from the final model (full fixed effects + random effect of *reef* + *weighted* variance among reefs) indicated that all assumptions were met. We conducted our analyses using the statistical software *R* v3.2.1 (R Core Team, 2020) with the associated packages *nlme* v3.1-118 (Pinheiro et al., 2016) and *MASS* v7.3-35 (Venables & Ripley, 2002).

### 2.4 | Ethics and permits

Oregon State University's Institutional Animal Care and Use Committee (IACUC; ACUP 3886), the Department of Marine Resources of The Bahamas, and the Marine Conservation Board of the Cayman Islands Department of the Environment all approved our work.

## 3 | RESULTS

### 3.1 | How do invasive and native predators interact with cleaners?

#### 3.1.1 | Field

*Elacatinus* spp. cleaning gobies never cleaned invasive lionfish, despite inhabiting the same reefs (Table 1). After a total of 24 hr and 10 min of direct diver observations at cleaning stations, we

**TABLE 1** Interactions between *Elacatinus* spp. cleaning gobies and native non-predators, native predators and an invasive predator (*Pterois volitans*), as observed directly by divers during 10-min intervals at cleaning stations on natural patch reefs in the Bahamas

| Total observation time at cleaning stations (HH:MM:SS): 24:10:13 | NATIVE NON-PREDATORS | NATIVE PREDATORS[a] | INVASIVE PREDATOR |
|---|---|---|---|
| Number of antagonistic interactions | 0 | 1 | 1 |
| Number of cleaning interactions | 239 | 65 | 0 |
| Total observation time with cleaning behaviour (H:MM:SS) | 0:59:24 | 0:48:23 | 0:00:00 |
| Duration of cleaning interactions (sec; mean ± *SEM*) | 15.0 ± 2.7 | 44.7 ± 14.2 | n/a |
| Client species richness | 19 | 5 | n/a |
| Client total length (cm; mean ± *SEM*) | 11.9 ± 0.5 | 24.1 ± 1.1 | n/a |
| Top client species by number of cleaning interactions | 1. Creole wrasse *Clepticus parrae*, $n = 98$ | 1. Graysby *Cephalopholis cruentata*, $n = 25$ | |
| | 2. Blue chromis *Chromis cyanea*, $n = 47$ | 2. Schoolmaster snapper *Lutjanus apodus*, $n = 18$ | |
| | 3. French grunt *Haemulon flavolineatum*, $n = 42$ | 3. Nassau grouper *Epinephelus striatus*, $n = 16$ | |

[a] A native fish was considered a potential predator of cleaning gobies if its documented diet is considered mostly piscivorous (>50% fish by abundance, Randall, 1967).

witnessed only one set of interactions between an invasive lionfish and a cleaner goby (*E. genie*): three times in a 1-min period a cleaner goby nipped the pectoral fin of a resting lionfish, each time after which the lionfish turned and chased the cleaner goby before continuing to rest on the reef.

We also never observed the predation of cleaners by native species despite gobies spending 44.8% of their cleaning time with potential predators (Table 1). Cleaning gobies had 24 client species, five (20.8%) of which were potential predators (Table 1). Per interaction, gobies cleaned native predators three times longer than native non-predators (Table 1). The most frequently cleaned predator was the native graysby grouper (*Cephalopholis cruentata*), representing over one third of the observed cleaning interactions with predators ($n$ = 25 of 65). There was only one potentially antagonistic interaction between cleaners and a native predator, a small (visually estimated 7 cm total length) graysby swam in a hunting posture around the edge of a cleaning station with three cleaning gobies, all of which swam away from the graysby and then darted into nearby holes in the reef.

### 3.1.2 | Laboratory

Nearly half of juvenile lionfish ($n$ = 14 of 31, 45.2%) and graysby (11 of 23, 47.8%) and a third of coney (4 of 12, 33.3%) ate the cleaner goby in captivity. Of these predators, all lionfish (31 of 31, 100%) and nearly all graysby (22 of 23, 95.7%) and coney (11 of 12, 91.7%) ate the bridled goby. There was no difference among predator species in the proportion that ate a cleaner goby (Fisher's exact test: $p = 0.760$). There was no effect of the order of exposure to gobies or the region where the trial was conducted on the likelihood of eating a cleaner by any of the predators (binary logistic regression: all $p > 0.1$, Table S2). All predators, invasive and native, hyperventilated after consuming a cleaner goby, though gill ventilation rates were not quantified until the learning experiment (described in subsequent section 3.2).

## 3.2 | Does the interaction of invasive and native predators with cleaners change over time with repeated exposures?

During their initial encounters with the cleaner goby, most juvenile lionfish ($n$ = 27 of 29) and graysby (9 of 12) either successfully ate the cleaner goby, or grasped it and spit it out immediately, hyperventilating in either case. The proportion of trials in which a lionfish struck at a cleaner goby declined significantly after the first encounter (Cochran's Q = 24.2, $df = 3$, $p < 0.001$), and continued to decline during subsequent encounters (Figure 1). Our 2007 data also show a significant decline in the proportion of trials in which a lionfish struck at a cleaner goby after one trial (Cochran's Q = 32.4, $df = 7$, $p < 0.001$), with a continuing decline through seven subsequent trials regardless of time elapsed between encounters (2, 4 or 6 days; Figure 1). Of the 27 lionfish that struck at a cleaner goby, 20 developed an aversion (defined in Methods section 2.2) within one to five trials (2–10 days) after their initial strike at a cleaner goby (typical behaviour shown in video Figure S1). The remaining seven lionfish continued to strike at the cleaner during their subsequent trials. There was no difference between those lionfish that did and did not develop an aversion in either their gill ventilation rates after eating a cleaner goby (rank-sum test: $p = 0.63$), or the time it took for their gill ventilation rates to return to normal (rank-sum test: $p = 0.85$). After eating a cleaner goby, lionfish experienced elevated gill ventilation rates over two times as vigorous (mean ± *SEM* = 140.7 ± 7.2 versus 64.2 ± 1.1 opercular

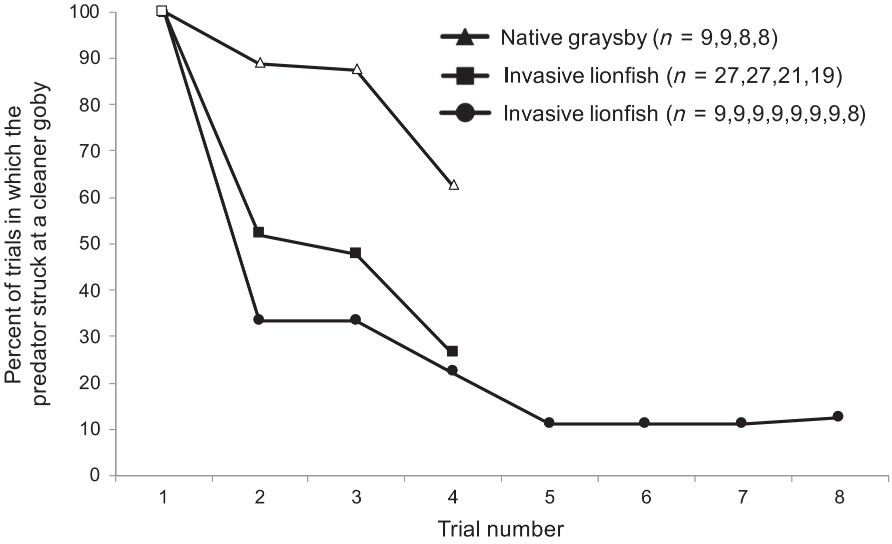

**FIGURE 1** The percent of trials in which the predator struck at a cleaner goby (*E. genie*). Trial 1 is defined as the first trial for a predator individual in which it struck at a cleaner goby. We removed all trials in which the predator was not hungry (i.e. it did not strike at the cleaner goby or the non-cleaner goby). Trials for native graysby (triangles) and invasive lionfish (squares) were conducted in 2013–2015. Trials for invasive lionfish (circles) were conducted in 2007. Sample sizes are listed respective to the trial number. Empty symbols represent non-significant differences (pairwise McNemar's test with Bonferroni-adjusted $p > 0.05$) between the first trial and corresponding subsequent trial. Filled symbols represent significant differences ($p < 0.05$)

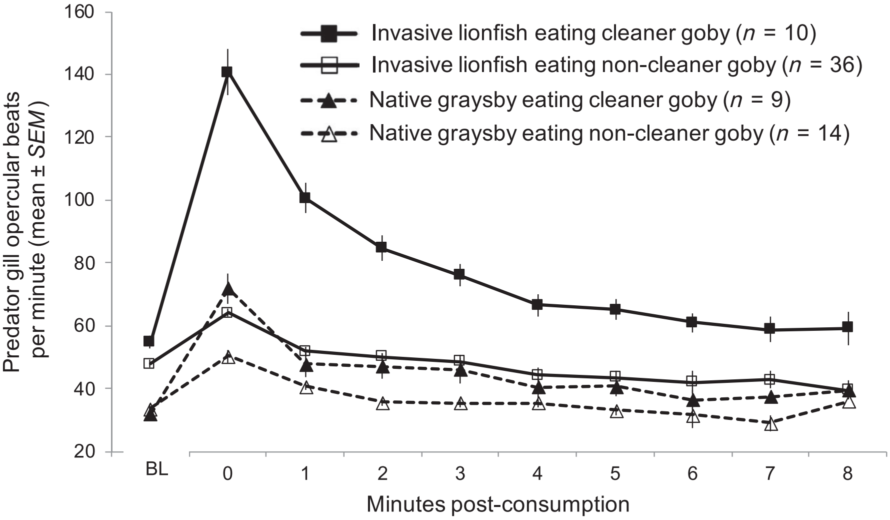

**FIGURE 2** Predator gill opercular beats per minute (mean ± *SEM*) for invasive lionfish (*P. volitans*) and native graysby grouper (*C. cruentata*), after consuming a cleaner goby (*E. genie*) or a non-cleaner goby (bridled goby, *C. glaucofraenum*). If an individual predator consumed a cleaner or a non-cleaner more than once during our trials, their individual response was averaged across predation events before being included in the calculation of means for a given predator–prey combination. BL = baseline gill opercular beats per minute; 0 = immediately post-consumption

beats/minute; rank-sum test: $W = 0$, $p < 0.001$; Figure 2) and for five times as long (mean ± *SEM* = 6.4 ± 1.1 versus 1.2 ± 0.1 min; rank-sum test: $W = 0$, $p < 0.001$; Figure 3) as those experienced after eating a bridled goby.

The proportion of trials in which a graysby struck at a cleaner goby declined over time (Figure 1), but less precipitously than with lionfish, and the decline was non-significant (Cochran's Q = 6.3, $df = 3$, $p = 0.096$). Eight of the nine graysby that initially ate a cleaner goby continued to strike at the goby during subsequent trials (typical behaviour shown in video Figure S1). Only one graysby developed an aversion to the cleaner goby. Like lionfish that avoided the cleaner goby, this graysby approached the cleaner in its second trial, and then turned away without striking. In subsequent trials, the graysby ignored the cleaner goby despite passing within 10 cm of it, and despite demonstrating hunger by eating the bridled goby within the same trials. After consuming a cleaner goby, graysby gill ventilation rates were mildly elevated as compared to those after eating a bridled goby (mean ± *SEM* = 71.9 ± 4.9 versus 50.5 ± 2.1 opercular beats/minute; rank-sum test: $W = 11.5$, $p = 0.001$; Figure 2), and for three times as long (mean ± *SEM* = 3.7 ± 0.7 versus 1.2 ± 0.2 min; rank-sum test: $W = 18$, $p = 0.004$; Figure 3).

When compared to graysby, lionfish had significantly higher gill ventilation rates after eating a cleaner goby (rank-sum test: $W = 0$, $p < 0.001$; Figure 2). It also took significantly longer for lionfish gill ventilation rates to return to normal after eating a cleaner than it did for graysby (rank-sum test: $W = 0$, $p < 0.001$; Figure 3). However,

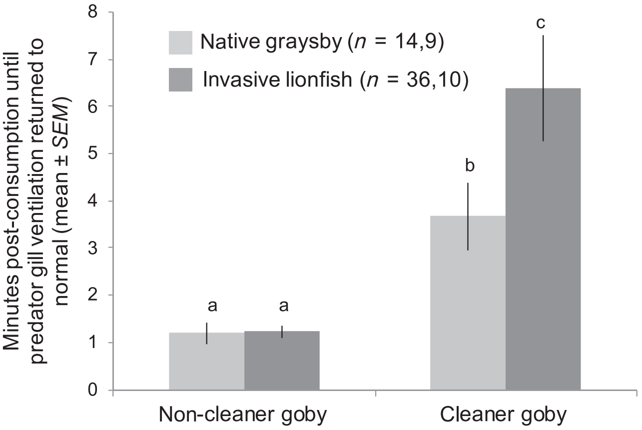

**FIGURE 3** Time in minutes (mean ± *SEM*) until predator gill ventilation rates returned to normal (within six gill opercular beats per minute), after consuming a non-cleaner goby (bridled goby, *C. glaucofraenum*) or a cleaner goby (*E. genie*). Sample sizes are listed respective to the types of goby (non-cleaner, cleaner). Different letters above the bars indicate significant differences (rank-sum tests $p < 0.05$)

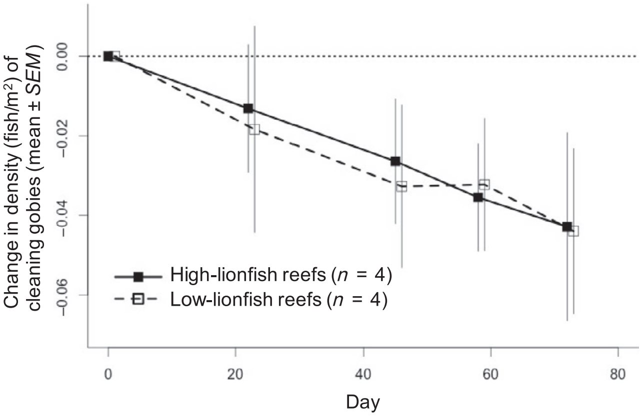

**FIGURE 4** Change in density (fish per $m^2$) of *Elacatinus* cleaning gobies (*E. genie* and *E. evelynae*) over time and by lionfish density treatment (low-lionfish reefs $n = 4$ and high-lionfish reefs $n = 4$). Results from a linear mixed-effects model revealed that time and the interaction between treatment and time significantly affected the response, but that treatment did not

there was no difference between graysby and lionfish in the time it took for their gill ventilation rates to return to normal after eating a bridled goby (rank-sum test: $W = 258.5$, $p = 0.887$; Figure 3).

### 3.3 | Does the invasive predator affect wild populations of cleaners?

The density of cleaning gobies on experimental reefs declined significantly over time (LME: $t = -12.636$, $df = 22$, $p < 0.001$; Figure 4) at a rate of −0.00075 fish per $m^2$ per day (95% CI −0.00062, −0.00087), but lionfish had no effect on the change in density (LME: $p = 0.696$; Figure 4). For an average reef with a surface area of 386 $m^2$, this decline equated to a loss of one goby every 3.45 days. Baseline (week 0) densities of cleaning gobies on the reefs ranged from 0.013 to 0.168 fish/$m^2$, and week 10 densities of cleaning gobies ranged from 0.013 to 0.078 fish/$m^2$. Over 10 weeks, we maintained lionfish densities on high-lionfish reefs at 4.9 times those on low-lionfish reefs (high-lionfish: mean ± *SEM* 0.014 ± 0.003 lionfish/m2, low-lionfish: 0.003 ± 0.001 lionfish/$m^2$). There were no differences between the average densities of *Cephalopholis* spp. (graysby and coney groupers) on low-lionfish (mean ± *SEM*: 0.017 ± 0.005 fish/$m^2$) and high-lionfish reefs (mean ± *SEM*: 0.016 ± 0.001 fish/$m^2$), regardless of whether their densities were separated into juveniles (≤15 cm TL; rank-sum test: $W = 5$, $p = 0.387$) and adults (>15 cm TL; rank-sum test: $W = 10$, $p = 0.564$) or not (rank-sum test: $W = 8$, $p > 0.1$).

## 4 | DISCUSSION

Novel biological interactions are becoming increasingly commonplace due to species introductions and climate-related range shifts. Studying novel interactions can reveal how communities and ecosystems will respond to such rapid, human-caused change. We investigated how invasive and native predators interact with *Elacatinus* spp. cleaning gobies, which are simultaneously potential prey and cleaning mutualists on Atlantic coral reefs. Our field studies indicated that invasive lionfish and native cleaning gobies rarely interact, and that lionfish do not alter the densities of cleaning gobies, consistent with previous experimental (Albins, 2015; Benkwitt, 2015; Tuttle, 2017) and observational (Green et al., 2012) studies. Our laboratory experiments indicate that learning is a plausible behavioural mechanism by which these species weakly interact; lionfish show distress and hyperventilate after ingesting a cleaner goby (*E. genie*), and thereafter do not eat the cleaner. Interactions between lionfish and cleaning gobies will likely remain neutral so long as invasive lionfish have few external parasites (Loerch et al., 2015; Ramos-Ascherl et al., 2015; Sikkel et al., 2014; Tuttle et al., 2017). Thus, the cleaner goby may be among the remarkably few small fishes on Atlantic coral reefs to escape predation by invasive lionfish, which otherwise have strong negative effects on native reef fishes (Albins, 2015; Albins & Hixon, 2008; Benkwitt, 2015; Green et al., 2019).

Other novel interactions between generalist predators and toxic prey have resulted in rapid learned avoidance (Crossland, 2001; Nelson et al., 2011; Webb et al., 2008). Being small, scaleless and lacking visible defensive features such as spines, the source of the cleaner goby's unpalatability is a putative chemical on or in its external surface, as has been found in the skin secretions of other coral-dwelling gobies (Hashimoto et al., 1974; Schubert et al., 2003). It has been suggested previously that *Elacatinus* gobies may be chemically protected (Colin, 1975; Lettieri & Streelman, 2010), and that their boldly coloured lateral stripe may have evolved as both an aposematic cue of toxicity to potential predators, as well as a cue for cooperative cleaning service to potential clients (Lettieri & Streelman, 2010). The specific chemical nature of *Elacatinus* gobies' defence is currently unknown, as is the prevalence of the defence among the 27 species in the genus, of which seven are cleaners (Taylor & Hellberg, 2005).

The same chemical defence that deters invasive lionfish from consuming the cleaner goby also affects a native predator, the graysby grouper, which hyperventilated mildly after eating a cleaner goby. In contrast to invasive lionfish, however, most native graysby did not readily learn to avoid eating the cleaner goby. Juvenile graysby may be less susceptible than lionfish to the cleaner's defence due to a co-evolved counter-defence, or graysby may simply be less quick than lionfish to learn to avoid unpalatable prey. Regardless, we found that graysby were the most frequently cleaned predator species by gobies on natural reefs. Therefore, given adequate time, environmental context (at cleaning stations on reefs) and behavioural context (e.g. swimming movements that cue cleaning), it is likely that the dominant response of graysby to cleaners switches from predation to cooperation.

The relative latency in this native predator's learned response to a cleaner suggests that the decline in goby abundance we witnessed during our 10-week field experiment may have been the result of predation by juvenile groupers and other native predators. Cleaning gobies experience similar attrition on other reefs in The Bahamas and Caribbean region (Tuttle, 2017; White et al., 2006; Wilson & Osenberg, 2002), which has been attributed to possible predation by native piscivores. Relatively high mortality rates coupled with short life spans (White et al., 2006) suggest that cleaning goby populations experience high turnover, which may prevent local extirpations in the face of predation.

Cooperation between obligate cleaners and potential clients has been thought to be instinctive due to an evolved set of visually conspicuous traits common among Pacific cleaner wrasses (Cheney et al., 2009; Stummer et al., 2004) and Atlantic cleaning gobies (Lettieri & Streelman, 2010)—a small cylindrical body and a long, lateral body stripe that is blue or yellow. Cleaners, however, are not immune to predation. There are isolated accounts of cleaner wrasses being eaten while not actively cleaning (Francini-Filho et al., 2000; Lobel, 1976; Messias & Soares, 2015), and of facultative cleaners (species that occasionally clean) being found in the gut contents of potential clients (Côté, 2000). Other laboratory studies have documented captive predators eating cleaning gobies (Darcy et al., 1974; Lettieri & Streelman, 2010) and other cleaner fish (Grutter, 2004). Laboratory experiments may overestimate the rate of predatory interactions between clients and cleaners in the wild. Even so, our results indicate that cooperation is not inherently instinctive for native and non-native predators, which both initially attacked the cleaner goby. In any case, cleaning can be risky behaviour for small reef animals that approach predatory clients.

In addition to potential clients ‘cheating’ by consuming cleaners, cleaners may cheat by consuming a client's skin and mucus instead of its parasites (Gorlick, 1980). How does cooperation thus persist? On Pacific coral reefs, there is no evidence that the dominant cleaner wrasses are distasteful, but cleaners are kept honest by clients punishing cheaters (by chasing the cleaner or switching partners) (Bshary & Grutter, 2005). On Atlantic coral reefs, however, these partner control mechanisms do not exist (Soares et al., 2008). Therefore, our results suggest that cleaning gobies may be kept honest by the threat of being eaten by juvenile predators, which have not yet learned to cooperate. In turn, novel predators may be kept honest by cleaning gobies' distastefulness. While cleaning gobies' physiological defence most likely evolved as insurance against predation by native species, it also protects gobies from an invasive predator with remarkable behavioural plasticity.

We posit that learned aversion of predatory clients to unpalatable cleaners is a prerequisite for cooperative behaviour in Atlantic cleaning goby symbioses. This learned behaviour may be the result of a cost–benefit trade-off that shifts across the ontogeny of predatory client species. A juvenile predator might benefit less from a cleaning interaction than might an adult predator (i.e., fewer parasites removed from the surface area of a smaller body) and would stand to benefit more from eating a small, cleaner-sized prey than would an adult that consumes a larger and more diverse set of prey. If this hypothesis is true and if invasive lionfish eventually accumulate external parasites, then we might expect lionfish to begin interacting cooperatively with cleaners. This could be a likely future scenario, given the invader's rapid learning capabilities and extreme negative reaction to consuming the cleaner. Additional studies should investigate the relative roles of body size, parasite load, and reaction to cleaners' defensive mucus in shaping a predatory species' likelihood to antagonise or cooperate with cleaners.

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## AUTHORS' CONTRIBUTIONS

L.J.T. and R.W.L. conceived the ideas and designed the methodology; L.J.T., R.W.L. and A.L.S. collected the data; L.J.T. analysed the data; L.J.T. led the writing of the manuscript.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.xsj3tx9f3 (Tuttle et al., 2021).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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