Invasive lionfish (*Pterois volitans*) learn to avoid a "spicy" prey fish

El pez león invasor (*Pterois volitans*) aprende evitar un pez presa "picante"

La Rascasse volante invasive (*Pterois volitans*) apprend eviter une proie "épicée"

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EXTENDED ABSTRACT

Biological invasions afford a unique opportunity to witness novel encounters between species without the complications of coevolution. Studying these interactions is also relevant to the conservation of native fauna, which can be threatened via predation (Fritts and Rodda 1998), competition (Byers 2000), and/or indirect effects (White et al. 2006) on food webs and behavior caused or initiated by exotic species. Of particular conservation concern in the greater Caribbean region is the invasion of western Atlantic coral reefs by the Indo-Pacific red lionfish (*Pterois volitans*) (Green et al. 2012, Albins 2015). As voracious, generalist predators of fishes, lionfish may eat and/or alter the behavior of cleaner gobies (*Elacatinus* spp.): ubiquitous, conspicuous, and ecologically important species that clean parasites off of other reef fishes. If lionfish do affect cleaner gobies, then cleaning mutualisms among native species could be weakened, leading to increased transmission of parasites on invaded reefs. We conducted two laboratory experiments to test whether or not juvenile lionfish and native groupers (1) eat the cleaner goby, *E. genie*, and (2) learn not to eat *E. genie*, which have a putative skin toxin.

Each lionfish was acclimated to an aquarium before we introduced a cleaner goby and recorded subsequent behavior for up to 20 minutes. If the goby was still alive after that time, we removed it and placed a similarly sized and shaped bridled goby (*Coryphopterus glaucofraenum*) into the aquarium, also up to 20 minutes. This was done in random sequence so that either the cleaner or the bridled goby was the first offered to the lionfish. The bridled goby is a known common prey of lionfish (and native groupers), and was therefore an indicator of predator hunger during our trials. We replicated this experiment for juveniles of two native groupers – the graysby (*Cephalopholis cruentata*) and the coney (*Cephalopholis fulva*) – and at two locations in the invaded range: The Bahamas and the Cayman Islands. As Atlantic coral-reef mesopredators, graysby and coney are the most ecologically similar native species to lionfish. Nearly half of tested lionfish (n=14 of 31) and graysby (11 of 23), and a third of coney (4 of 12) ate the cleaner goby. Every predator, invasive and native, had greatly increased gill ventilation rates after consuming a cleaner goby, although no cleaner was ever regurgitated. A predator never hyperventilated after consuming a bridled goby.

Given the apparent distastefulness of the cleaner goby, we tested whether lionfish and native grouper learn not to eat them. We repeatedly exposed individual lionfish and graysby to a cleaner goby over a two-week period in The Bahamas (following the same protocol as described above), and monitored their behavior for any changes in response to cleaner versus bridled gobies. Most lionfish (33 of 37) and graysby (9 of 14) either successfully ate the cleaner goby, or ate it and spit it out immediately, hyperventilating in either case. After eating a cleaner goby,

lionfish experienced elevated ventilation rates over twice as vigorous (mean±SEM = 140.7±7.2 vs. 64.2±1.1 opercular beats/minute, Fig. 1) and for 5 times as long (mean±SEM = 6.4±1.1 vs. 1.2±0.1 minutes) as those experienced after eating a bridled goby. After consuming a cleaner, graysby ventilation rates were only mildly elevated (mean±SEM = 71.9±4.9 vs. 50.5±2.1 opercular beats/minute, Fig. 1) and 3 times as long (mean±SEM = 3.7±0.7 vs. 1.2±0.2 minutes) as those experienced after eating a bridled goby. Over the course of two weeks, the proportion of trials in which a lionfish struck at a cleaner goby declined precipitously (Fig. 2). An experienced lionfish, i.e., one that had eaten or regurgitated a cleaner goby in a previous trial, would approach a cleaner goby closely, but turn away without striking, even if the lionfish was hungry (as demonstrated by eating a bridled goby in the same trial). The proportion of trials in which a graysby struck at a cleaner goby also declined over time (Fig. 2), but less precipitously than with lionfish. In fact, only one graysby (of the 9 that ate a cleaner) showed signs of learning, i.e., consistently turning away from cleaners after having eaten one in a previous trial.

Our results are consistent with those of multiple experiments that found no effect of lionfish on *Elacatinus* spp. abundance (Green et al. 2012, Albins 2015, *unpublished data*). Therefore, learned avoidance is the likely behavioral mechanism by which invasive lionfish and native cleaner gobies weakly interact. It has been assumed that cleaning mutualisms are instinctive for fishes, but our results with a native predator indicate that cooperation might be learned, especially between native piscivores and cleaner fish. The native predator showed little evidence of short-term learning in our experiment, unlike the lionfish, but they also experienced less intense reactions upon consuming the cleaner goby than did the lionfish. Possible explanations for the observed difference in hyperventilation rates between the invasive and native predators are that the native grouper has coevolved some degree of immunity to the compound on the cleaner's skin, and/or that there is a mechanical difference in how the two predators ingest their prey, which affects contact with the compound. In either case, the cleaner goby is distasteful, which may be an evolved defense given their vulnerability to predation while cleaning inside the mouths and gills of piscivores. Ours is the first study to quantify the effect of a putative *Elacatinus* skin toxin on predator behavior, although the compound has yet to be isolated or identified. Nevertheless, due to their distastefulness, *E. genie* may be one of the few small fishes on Atlantic coral reefs that escapes the jaws of invasive lionfish.

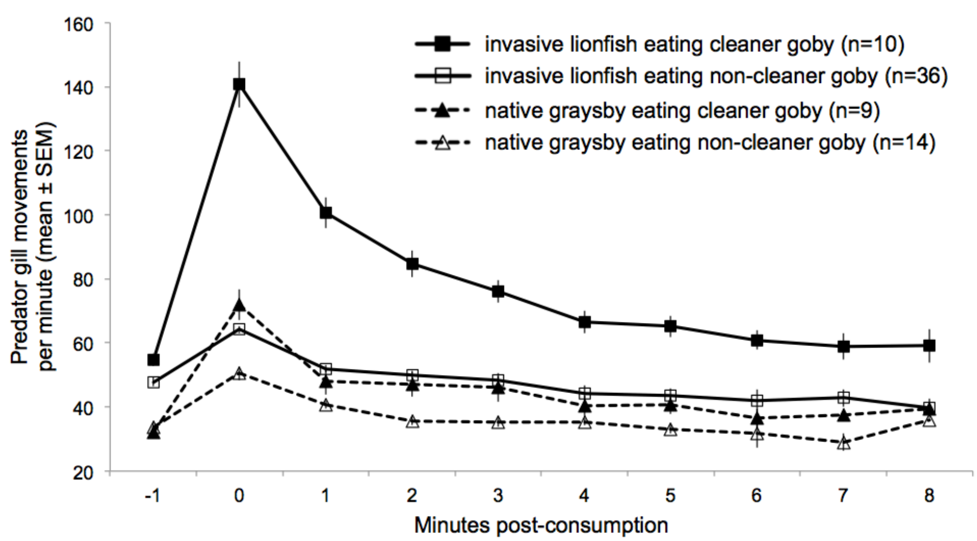

Figure 1. Mean predator gill movements per minute (± SEM) for invasive lionfish (*Pterois volitans*) and native graysby grouper (*Cephalopholis cruentata*), after consuming a cleaner goby (*Elacatinus genie*) or a non-cleaner goby (bridled goby, *Coryphopterus glaucofraenum*). If an individual predator 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.

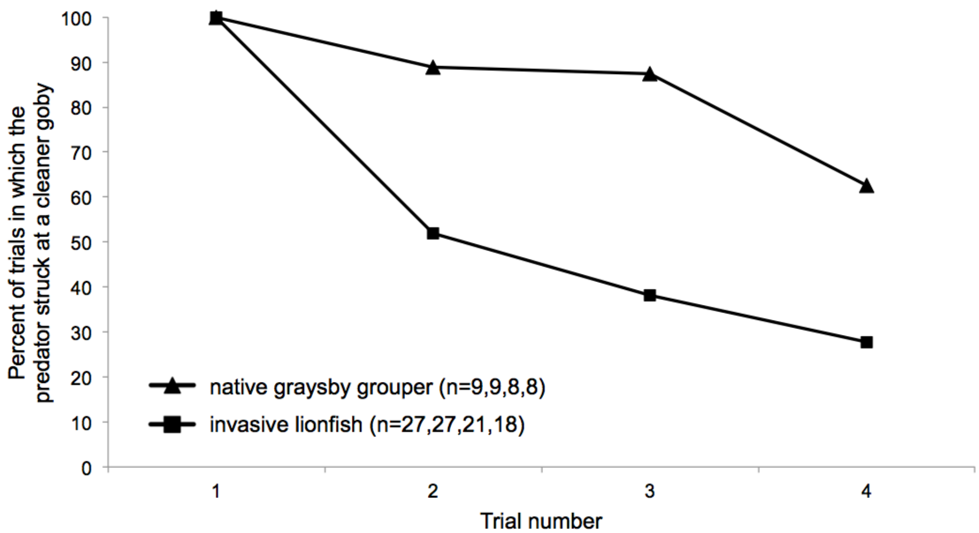

Figure 2. The percent of trials in which the predator (native graysby grouper *Cephalopholis cruentata* or invasive lionfish *Pterois volitans*) struck at a cleaner goby (*Elacatinus genie*). Trial 1 is defined as the first trial for a predator individual in which it struck at a cleaner goby. We removed all trials in which the predator was not hungry (i.e., it did not strike at the cleaner goby or the non-cleaner goby). Sample sizes are listed respective to the trial number.

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