

Predator-prey interactions between native snappers (Family: *Lutjanidae*) and juvenile red lionfish (*Pterois volitans*) in the Western Pacific

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Abstract

Over three decades ago, two species of lionfish (*Pterois volitans* and *P. miles*) native to the Indo-West Pacific were introduced to the Western Atlantic and have since become widespread and invasive. Their impact on local reef fish populations has been devastating, leading to a substantial decline in ecosystem health. In contrast, lionfish are relatively rare in the Indo-West Pacific for which the underlying mechanisms are poorly understood. Biological resistance possibly plays an important role in stabilizing lionfish populations in their native range, yet natural predators remain largely unknown. In an effort to contribute towards filling this knowledge gap, I conducted laboratory predation trials in the Western Pacific using juvenile lionfish and three native snapper species, which are common meso-predators and have been suggested as a potential biological control for lionfish. Snappers showed signs of indifference and avoidance behaviour when presented with juvenile lionfish (in a transparent tube) in a single prey-choice experiment. After adding a natural prey species (*Gambusia sp.*) in a double prey-choice setup, snappers actively attempted strikes towards the *Gambusia*, yet never towards lionfish. In addition, snappers never shared shelter with free-swimming lionfish and mainly kept a distance, suggesting a combination of visual and chemical cues causing lionfish avoidance. In turn, lionfish showed no response when snappers were nearby. Instead, lionfish remained mostly stationary which suggests that lionfish either do not recognize snappers as a threat or rely heavily on their venomous defensive mechanism. These results indicate that snappers do not readily prey upon juvenile lionfish in their native range and congeneric species in the Western Atlantic are unlikely to be an effective biological control there in the near future.

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1. Introduction

1.1 – Brief history of the invasion and driving mechanisms

Biological invasions caused by the introduction of non-indigenous species are a significant component of man-made global change and have been increasingly harmful to flora and fauna around the world (Vitousek, 1997). While marine invasions constitute a major part of global biological invasions, they have traditionally received less attention than their terrestrial counterparts. A possible explanation for this knowledge gap includes the difficulty of identifying introduced marine species due to poor knowledge of their taxonomy and native ranges (Ruiz et al., 2000). Many early marine invasions may simply remain undetected and cryptogenic for long periods of time, only to be recognized as a potential threat after high dispersal rates have irreversibly led to the establishment of the invasive species. In addition, invasions within terrestrial and freshwater environments can often be detected on a much earlier stage due to their relatively confined nature as opposed to the openness and degree of connectivity within the marine realm which hampers early detection and eradication of invaders (Olenin et al., 2011).

While marine introductions can take place through various pathways such as shipping and discharging of ballast water, aquaculture, aquarium trade and artificial canals (Knapp et al., 2017), not all recipient environments facilitate survival and establishment of non-indigenous species. A combination of biotic (e.g. competition, predation and parasites) and abiotic factors (e.g. temperature and habitat availability) can provide sufficient resistance to invaders within the recipient environment (Ruiz et al., 2000). However, global changes and disturbances of marine environments (e.g. habitat alteration, fisheries exploitation, chemical pollution and global warming) are predicted to diminish the capability of these ecosystems to resist invaders with increasing magnitude over time (Stachowicz et al., 2002; Freestone et al., 2013; Kimbro et al., 2013). As such, marine invasions are posing a growing conservation and socio-economic threat worldwide and studying their underlying mechanisms is crucial for the mitigation of related negative effects and the preservation of marine ecosystems.

One of the most dramatic marine invasions to date is currently taking place in the Caribbean and Western Atlantic (hereafter: WA). Two species of lionfish, the red lionfish (*Pterois volitans*) and the devil firefish (*Pterois miles*), both belonging to the family Scorpaenidae, have successfully made their way from their native ranges into the WA.

Initially, it was unclear whether the introduction of these exotic marine species to the WA was triggered by Hurricane Andrew or aquarium releases, a debate which was later settled in favour of aquarium releases (Semmens, 2004; Morell, 2010).

Prior to the

introduction, the native range of *Pterois miles* was exclusively the Indian Ocean from South-Africa to the Red Sea and east to Sumatra, whereas *P. volitans* was found throughout the western Pacific from southern Japan to Western Australia and east to the Pitcairn Group (Fig. 1; Kulbicki et al., 2012). However, after the first sighting by a local fisherman in 1985 off Dania, on the east coast of Florida (Morris & Akins, 2009), populations of *P. miles* and *P. volitans* (hereafter: lionfish) began to increase rapidly from the year 2000 (Fig. 2a, b; Whitfield, 2002; Whitfield et al., 2007; Schofield, 2009).

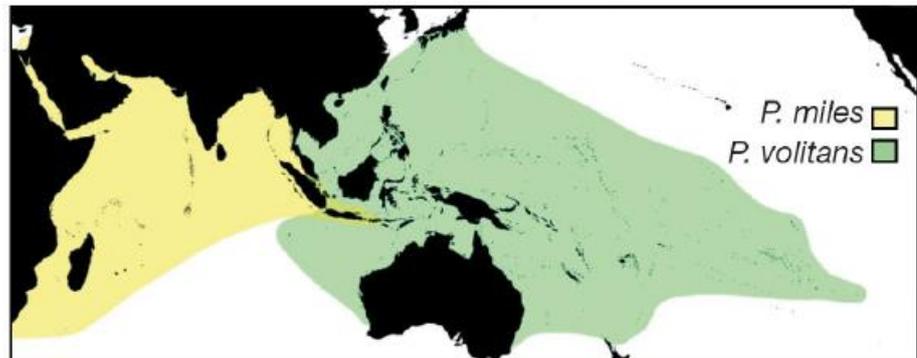


Fig. 1 – Native ranges of *P. miles* and *P. volitans*. From Kulbicki et al. (2012).

Despite the presence of the northward flowing Gulf Stream, which has been shown to form an effective barrier for many species between Florida and Bahamian waters (Briggs, 1995), lionfish have succeeded in establishing geographically widespread populations between the regions.

Strong reproductive traits have since been reported for lionfish, i.e. a batch fecundity of 1.800-41.945 eggs and the potential in adults to spawn every 2-3 days year round while reaching adulthood within their first year of life (Gardner et al., 2015). These high reproduction rates, in combination with successful egg and larval dispersal in the region (Freshwater et al., 2009), have likely been the most important factors in contributing to the rapid spread during the early stages of the lionfish invasion.

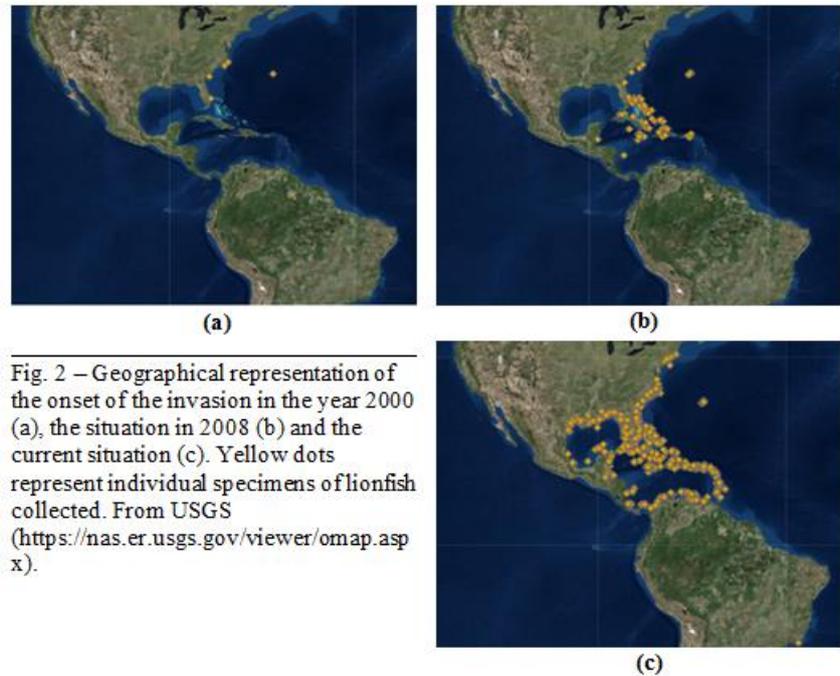
Today, the invasive range of lionfish is continuing to expand with reported summer recruits as far north as Rhode Island, establishment in the Gulf of Mexico (Fig. 2c; Schofield, 2010) and Brazil (Fig. 2c; Ferreira et al., 2015).

In parallel, a second lionfish invasion event appears to be taking off in the Mediterranean Sea (Kletou et al., 2016), although it remains unclear whether the invasion will be as dramatic there as oceanographic conditions were found to be less favourable for widespread larvae dispersal in that region (Johnston & Perkins, 2014). Nevertheless, the high success rate of lionfish in the WA is evident and their presence is already having major deleterious effects on native marine species and their habitats.

1.2 – Invasion dynamics

Invasive species are often successful in their introduced habitat if the invaded community lacks a species similar to the invader (Sih et al., 2010). Although lionfish rely on the technique of ram-suction feeding which is often observed in teleost fish (van Leeuwen & Muller, 1984), lionfish utilize an array of additional tactics to put other WA predators at a disadvantage. The ram-suction technique involves a wide expansion of the buccal cavity combined with fast forward motion, which allows for rapid ambushes and even enables lionfish to consume prey of up to two-thirds of their own length (Albins & Hixon, 2008). However, owing to their cryptic nature, lionfish employ considerably less ram movement and can approach their prey more closely before initiating a strike than native WA piscivores e.g. groupers (Epinephelidae), sea basses (Serranidae) and trumpetfish (Aulostomidae) (Ingeman, 2016; Muller & Osse, 1984). In addition, lionfish have been observed hunting in groups by using conspicuous flares of their feather-like pectoral fins to either communicate with conspecifics or herd their prey while blowing jets of water to confuse them (Lönnstedt et al., 2014; Albins & Lyons, 2012). These techniques clearly illustrate the novelty advantage of lionfish in the WA and have facilitated their success through a lack of effective defensive strategies from native prey species.

The negative impact of the invasion success has been widely documented over the past decade in terms of direct effects, e.g. a 79% reduction of native fish recruitment in just five weeks during a controlled field experiment (Albins & Hixon, 2008), a 65% decline in total biomass of 42 Atlantic prey species combined on nine large coral reefs in the Bahamas (Green et al., 2012) and significant reductions of total density, biomass and species richness of prey species across a similar study area



(Albins, 2015). A generally accepted hypothesis to explain the driving mechanism behind these catastrophic effects is the theory of prey naïveté. A lack of evolutionary co-existence of native prey species with the invader often causes weak or non-existent anti-predator responses (Sih et al., 2010). While naïveté can go both ways and predators may sometimes adapt poorly to novel prey as well, this certainly is not the case for the lionfish invasion.

To illustrate the level of naïveté of a native reef inhabitant, Kindinger (2015) showed that the behavioural response of 40 three-spot damselfishes (*Stegastes planifrons*) towards lionfish (the latter contained in a clear bottle) was not significantly different from the response towards the empty control bottle, whereas they exhibited strong species-specific behaviour towards native predators in the bottle. Similarly, white grunts (*Haemulon plumieri*) failed to adapt their approaching distance towards the exotic invaders and members of two families (Labridae & Pomacentridae) kept greater distance from lionfish in the Pacific compared to the Atlantic region (Anton et al., 2016). While native prey species are sometimes able to gradually develop appropriate anti-predator responses through behavioural adaptation, in the meantime they might be experiencing a significant reduction in genetic variation.

In general, the obvious negative impact of any predator on the biomass and density of prey populations through direct consumption, i.e. consumptive effects (CEs), often obscures important non-lethal effects of this predator on the ecosystem (Lima, 1998). In order for scientists to fully comprehend the impacts of the lionfish invasion, changes in prey behaviour (e.g. feeding strategies and courtship) due to the mere presence of this exotic invader, i.e. non-consumptive effects (NCEs), cannot be left out of the equation. Animals always face a traditional trade-off between predation risk and energy intake, which is typically the case for foragers when the most energetically profitable space is also the most dangerous one (Lima, 1998). This is illustrated in a recent study, where Bahamian reefs with high lionfish-densities were linked to a significant decrease in grazing intensity by both small and large herbivorous fishes (Kindinger & Albins, 2017) leading to increased algal abundance. In this study, parrotfishes (Labridae: Scarinae) were driving the overall response of herbivorous fishes by decreasing their visitation rate (i.e. numbers of fish/minute) to high lionfish-density reefs and causing a subsequent reduction of 66-80% in the removal of algae, thus increasing the risk of a phase shift towards algal-dominated reefs in the WA (see also Lesser & Slattery, 2011). Results from such studies highlight the importance of considering NCEs when trying to implement management actions to minimize potential effects of the lionfish invasion.

1.3 – Biotic resistance

A key factor in trying to understand biological invasions is to determine why particular species are so successful in their invaded range, i.e. their invasion success. One possible explanation is linked to the biodiversity of an ecosystem, with numerous studies providing evidence of a positive relation between the diversity of systems and their resistance towards exotic species (e.g. Stachowicz et al., 1999; Kennedy et al., 2002; Levine et al., 2003). The underlying theory here is that species-rich communities make the most efficient use of both resources and space, thus limiting the exotic invader in its ability to successfully settle and become invasive (Stachowicz et al., 1999). As a result, the relatively weak system of the WA in which biodiversity has already been severely affected by overfishing, pollution and global warming (Schofield, 2010; Mumby et al., 2011) might have proven to be the ‘ideal’ habitat for a widespread marine invasion.

The enemy release hypothesis (ERH) is another proposed theory (e.g. Keane & Crawley, 2002; Sih et al., 2010) in which the invasive species escapes its native enemies and faces little predation risk or competition in the invaded range. Especially when the invader does not resemble native species morphologically, it may escape predation by naïve top-predators or gain a significant advantage over competitors through prey naïveté (Sih et al., 2010). Although ambush predators were already present in the WA prior to the lionfish invasion (e.g. Epinephelidae, Serranidae, Antennariidae and Scorpaenidae), none of these native species possess both the unique refined hunting techniques and venomous defensive mechanism of the lionfish combined (Hare & Whitfield, 2003), which makes the ERH a plausible explanation for the invasion success of lionfish.

Escape from parasites is also included in the ERH and may be further limiting resistance to lionfish in the WA. Although there is very little information on parasites of invasive marine fishes in general,

there seems to be an increasing focus on this aspect of biotic resistance for the lionfish invasion. Parasite species richness and abundance of lionfish in the WA was recently compared to that of two native hosts (*Cephalopholis cruentata* and *Synodus intermedius*) and found to be significantly lower in lionfish with no direct negative effects in the condition of the invasive host (Sellers et al., 2015). Furthermore, an extensive comparison between parasite susceptibility of lionfish in the WA and Western Pacific revealed that lionfish were less likely to host a parasite in their invaded range, mainly driven by relatively high infection rates in their native range (Tuttle et al., 2016). As such, the release from parasites might imply a certain degree of energy conservation by lionfish in their invaded range which instead can be invested into growth and reproduction to further contribute to their invasion success.

In order to further investigate the validity of the ERH for the lionfish invasion it is important to consider 'native enemy effects'. The existence of native enemies to be released from in the first place is a crucial condition of the ERH, which is overlooked in many studies on invasive species (Prior et al., 2015). Alternatively, species can be controlled by other ecological factors in their native range (e.g. limited food resources or habitat space) and become invasive elsewhere as a result of release from these limiting factors rather than from predation (e.g. Prior & Hellmann, 2013). To date, evidence of predation on lionfish is limited to a few anecdotal reports (Bernadsky & Goulet, 1991; Maljković & Van Leeuwen, 2008) and it remains unclear whether lionfish have any native enemy effects. Therefore, it has been suggested that future studies should focus on interactions between meso-predators (e.g. snappers) capable of consuming venomous scorpaenids (Muñoz et al., 2011) and lionfish in their native range, particularly post-larvae and small juveniles which may be more susceptible to predation (Donaldson et al., 2010).

1.4 – Goal of this study

In an attempt to fill this knowledge gap, I used a combination of field observations and predation experiments at the University of Guam (UOG) Marine Laboratory (the Marianas, USA) to examine potential predator-prey interactions between three species of native Pacific snapper (family: Lutjanidae) and juvenile red lionfish. Specifically, I aimed at answering three research questions:

1) In a single prey-choice setup (lionfish only), do snappers exhibit any form of predatory behaviour towards juvenile lionfish following a 24-h starvation period?

Hypothesis: Snappers show predatory behaviour towards juvenile lionfish after the starvation period.

2) In a double prey-choice setup (lionfish and natural prey species), do snappers show complete avoidance of juvenile lionfish or rather a more opportunistic predatory style?

Hypothesis: Snappers display opportunistic predatory behaviour instead of completely avoiding juvenile lionfish.

3) Do juvenile lionfish exhibit anti-predator behaviour towards snappers when free-swimming and is there a correlation with lionfish total length (TL)?

Hypothesis 1: Juvenile lionfish show anti-predator responses towards snappers (e.g. hiding in provided shelter or flaring of pectoral fin).

Hypothesis 2: The degree of anti-predator behaviour is negatively correlated with lionfish TL (i.e. smaller juveniles spending more time in hiding or flaring).

2. Materials & Methods

2.1 – Field observations

Observing and collecting juvenile lionfish on Guam proved challenging, both because of their cryptic nature (Morris et al., 2009) and relatively low densities of *Pterois* species in the Central Pacific (Donaldson et al., 2010; Kulbicki et al., 2012). After some weeks of intensive searches, during which a pair of scuba divers would scan as many holes and crevasses of different reefs around the island as possible, three juveniles were found in the shallow Paseo Channel near the Hagåtña River mouth (Fig. 3; 13° 28' 45.9" N, 144° 45' 19.0" E). Conditions at which *P. volitans* juveniles were encountered typically were high in turbidity, with runoff from a nearby river and high sedimentation load causing relatively low levels of visibility. This significantly limited the ability to observe interactions between juvenile lionfish and potential predators in their natural habitat, which made it unrealistic to obtain a sufficient amount of data from *in-situ* observations and led to the decision to focus on lab experiments instead.

2.2 – Sampling methods

Lionfish were collected using small hand nets and stored in a bucket attached to a floating device. The juveniles measured 4.51 cm, 6.23 cm and 8.72 cm TL respectively. After being transported back to the UOG Marine Laboratory in a large cooler containing an aeration device, lionfish were kept in an outdoor holding tank (188 x 69 x 76.2 cm) which was covered by a shade cloth to block incoming sunlight; large bricks and pieces of coral rubble were placed on the floor of the tank. Lionfish were fed one small live mosquitofish (*Gambusia sp.*, Poeciliidae; Hereafter: *Gambusia*, TL ~ 1.5 cm) each per day, which were caught by hand net from the nearby Masso Reservoir (Fig. 3; 13° 27' 24.3" N, 144° 41' 42.5" E) and kept in a freshwater tank which was replenished in stock on a monthly basis.

Three species of snapper (family: Lutjanidae) were caught by rod and reel, either from land or boat, from various sites on Guam (see Fig. 3). Specifically, mangrove snappers (*Lutjanus argentimaculatus*) were caught from Talofoyo River (13° 20' 20.2" N, 144° 45' 42.6" E) and Ylig River (13° 23' 38.4" N, 144° 46' 11.5" E), while blacktail snappers (*Lutjanus fulvus*) were caught in the Cabras Marina mangrove area (13° 27' 36.4" N, 144° 41' 00.6" E) and a one-spot snapper (*Lutjanus monostigma*) was



Fig. 3 – Map of Guam showing various sites where specimens were collected. The location of two prey species is shown in red; *P. volitans* (circle) and *Gambusia sp.* (triangle) whereas predators were collected from yellow areas; *L. argentimaculatus* (squares), *L. fulvus* (circle) and *L. monostigma* (triangle). From Google Maps Satellite View.

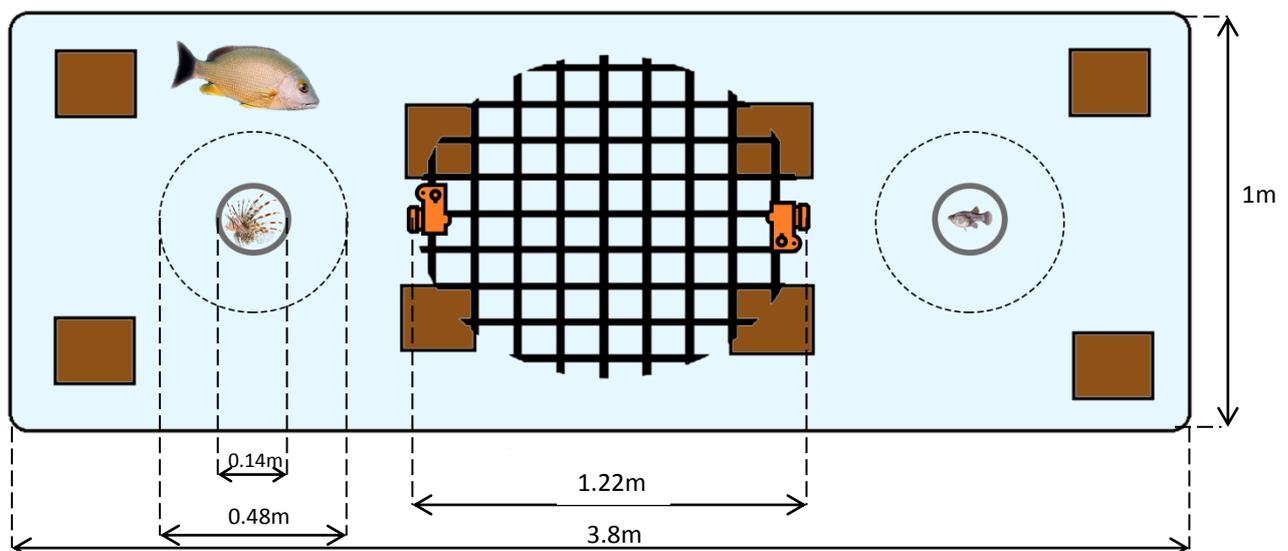
caught in Apra Harbour (13° 26' 38.5" N, 144° 39' 22.1" E, see Table A1 for complete overview of the species used). Transportation was carried out in a similar manner as with lionfish and the snappers were kept in a large holding tank (380 x 100 x 96 cm) containing lots of structure (e.g. tubes, coral, rubble and bricks), water pumps for flow and aeration. Snappers were fed twice a day with chunks of frozen lake smelt or shrimp.

2.3 – Experimental setup

Laboratory predation trials were conducted in a large rectangular experimental tank (380 x 100 x 96 cm) which is part of the outdoor research facilities at the UOG Marine Laboratory. A large circular grid (122 cm in diameter) was placed in the centre of the tank on top of four small hollow bricks to serve as a large covered area for shelter. Also, one hollow brick was placed in each corner to provide for additional shelter away from the centre of the tank (Fig. 4). For each trial, one snapper was used as a predator species and placed in the tank two days prior to the trial to acclimate. Predators were collected from their holding tank by near-full drainage after which they could easily be scooped up with a hand net and the amount of stress involved was minimized. Once acclimated to the setup, snappers were then starved for a 24 hour period to standardize hunger levels for all trials.

The experimental tank was covered by a large tarp and smaller shade cloths to block direct sunlight and prevent the water from heating up rapidly. Seawater flow was temporarily turned off during trials to allow for better quality of overhead videos and prevent predators from favouring the inlet side of the tank over the other one or vice versa. Experiments were carried out either early morning or late afternoon which is when snappers were feeding and most active. This way, the high midday temperatures were also avoided and background noise from people visiting the surrounding area was minimized. For all predation trials, two hollow transparent acrylic tubes were used to create a physical barrier between predator and prey while allowing for visual cues (measurements and setup provided in Fig. 4a, b). A camera (GoPro Hero 2.0) was mounted above the tank to capture overhead footage of all trials, while two additional cameras (GoPro Hero 2.0 in underwater casing and an Olympus Stylus Tough TG-3) were attached to weights and mounted on top of the grid facing one of the tubes each.

a)



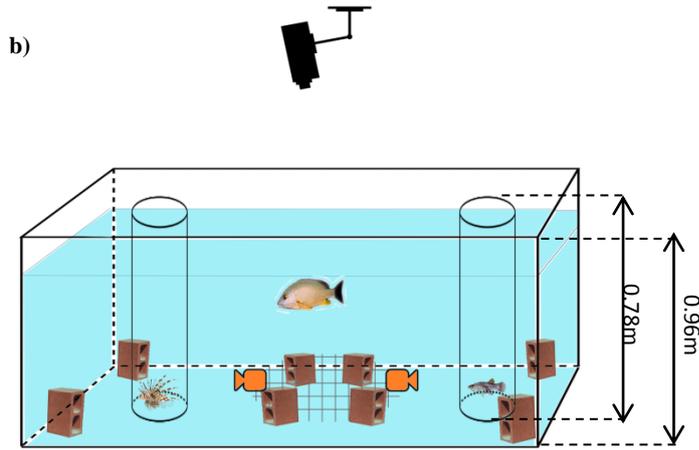


Fig. 4 – Schematic view of the experimental setup used for studying predator-prey interactions, with a) the overhead view and b) the 3D side view. One transparent acrylic tube is placed at each side of the tank (grey circles in a) to prevent direct physical contact between predator and prey but allow for visual cues. This specific overview represents the setup for a double prey-choice experiment, during which a snapper is presented with both a lionfish and *Gambusia*. The setup for the remaining two experiments is described in section 2.2. The dashed circles around the tubes in a) represent the prey zones (PZs) defined in section 2.3. The brown hollow bricks together with the large grid in the centre provide shelter for the predator to acclimate and for juvenile lionfish to hide during the free-swimming experiment. Two underwater cameras (orange) were positioned on top of the grid facing one of the tubes each, while a third camera was mounted above the tank for overhead footage.

In order to assess the degree of predatory behaviour, a virtual ‘prey zone’ (hereafter: PZ) was created in ImageJ video analysis software (dashed circles in Fig. 4). This zone encompassed each tube with an area of 72.5 cm, resulting in 19.1% of area coverage per tube which closely resembles the area of 17% used in similar predation trials (Morris, 2009).

First, control experiments were carried out to test for the experimental setup and the efficacy of the hollow acrylic tubes. To test whether predators did not show a preference for either the left or right PZ, the tubes were left empty and the mean time the predator spent in both zones was compared. Similarly, to test whether predators would respond to the visible cues provided by the transparent tubes and spent more time near the tube containing natural prey species, one *Gambusia* was added to either the left or right tube (assigned randomly) while the other one was left empty, after which both means were once again compared. Both these control experiments were done for all three snapper species, with $n = 7 - 10$ for both controls and all species.

Three experiments were then performed consecutively on the same day, after a brief acclimation period of ten minutes in between each experiment. For the first experiment only one of the tubes contained a juvenile lionfish while the other tube was left empty as a control (single prey-choice setup; position of the lionfish was determined at random prior to each trial). After ten minutes, one *Gambusia* was placed in the remaining empty tube to serve as a natural prey species and start the second experiment (double prey-choice setup shown in Fig. 4). For this experiment, only the largest *Gambusia* were used ($n = 16$, TL: 4.2 ± 0.3 cm) in order to closely resemble the length of the juvenile lionfish and prevent size related bias in selection by predators.

Finally, after another ten minutes, the *Gambusia* were caught with a small hand net and returned to the holding tank after which both tubes would be removed from the experimental tank for the third experiment.

2.4 – Variables measured

First, the amount of time a snapper spent in either PZ was measured for which the duration was determined to the nearest half second since one ‘visit’ would often be very short in duration. A snapper was marked as being inside the PZ upon entrance of the anterior part of the body (position of the eyes used as reference) into the designated area and lasted until the same reference point left the area again. In addition, an assessment of the predation intensity was obtained by tallying the amount

of strikes made directly towards the tubes, as well as the frequency of the predator entering the PZ. A successful strike was defined as a short acceleration of the predator aimed at the tube followed either by visible contact (i.e. bumping into the tube) or reaching a complete standstill in the final instant, which possibly occurred due to conditioning from previous bumps and was thus accounted for by still including these observations in the analysis.

Finally, at the onset of the third experiment, the *Gambusia* was removed and barriers were lifted in order to allow direct predation upon the free-swimming lionfish. The behaviour of lionfish was closely analysed by making use of the overhead camera and the underwater cameras combined. After an initial ten minutes for acclimation, the amount of time a juvenile lionfish spent hiding in one of the shelters was measured. Juveniles were divided into three separate classes, being small-, medium- and large-sized juvenile in order to investigate the relationship between lionfish TL and the degree of anti-predator behaviour. Any form of predatory behaviour of the snapper towards the freely swimming juvenile during this stage was recorded and the juvenile was kept in the tank for an additional 12 hours (overnight period) to make a final assessment on prey survival.

2.5 – *Data analysis*

To measure and analyse these variables, ten minute overhead videos of both experiments were recorded for all trials and converted from MP4 to AVI format to enable video analysis in ImageJ software. To import the relatively large files, virtual stacking was allowed resulting in a frequency of 30 frames per second of the original video and enabling precise determination of the position of the predator by frame-to-frame analysis.

Since different snappers were used between trials with unequal sample sizes (see Table A1 for an overview of the individuals used), an unpaired t-test assuming unequal variance (Welch's t-test) was used to compare means. Analyses were performed in Microsoft Excel 2010 using the Analysis Toolpak Add-in with a confidence interval of 95%. For the freely-swimming lionfish experiment, a single-factor ANOVA was used to determine whether a significant difference was found in mean time spent hiding between the three juvenile classes. A Welch's t-test was then used again to clarify which classes were significantly different, since sample sizes were unequal between the juveniles.

3. Results

3.1 – Control experiments

For both control experiments, the total time a snapper spent next to each tube was measured and is shown in Figure 5. During the control for the experimental tank setup with both tubes left empty, no significant differences were found between the time spent near the left and right tube for all snapper species (Fig. 5A, see Table A2 in appendix for means and deviations). As for the second control experiment to test the efficacy of the transparent tubes, all snapper species clearly show a strong preference for the tube containing a natural prey species as opposed to the empty tube (Fig. 5B, Table A2). Strikes towards the *Gambusia* tube were observed continuously during the trials of the second control experiment. Overall swimming activity was found to be relatively low when both tubes were left empty, but increased drastically after randomly adding a *Gambusia* to one of the tubes.

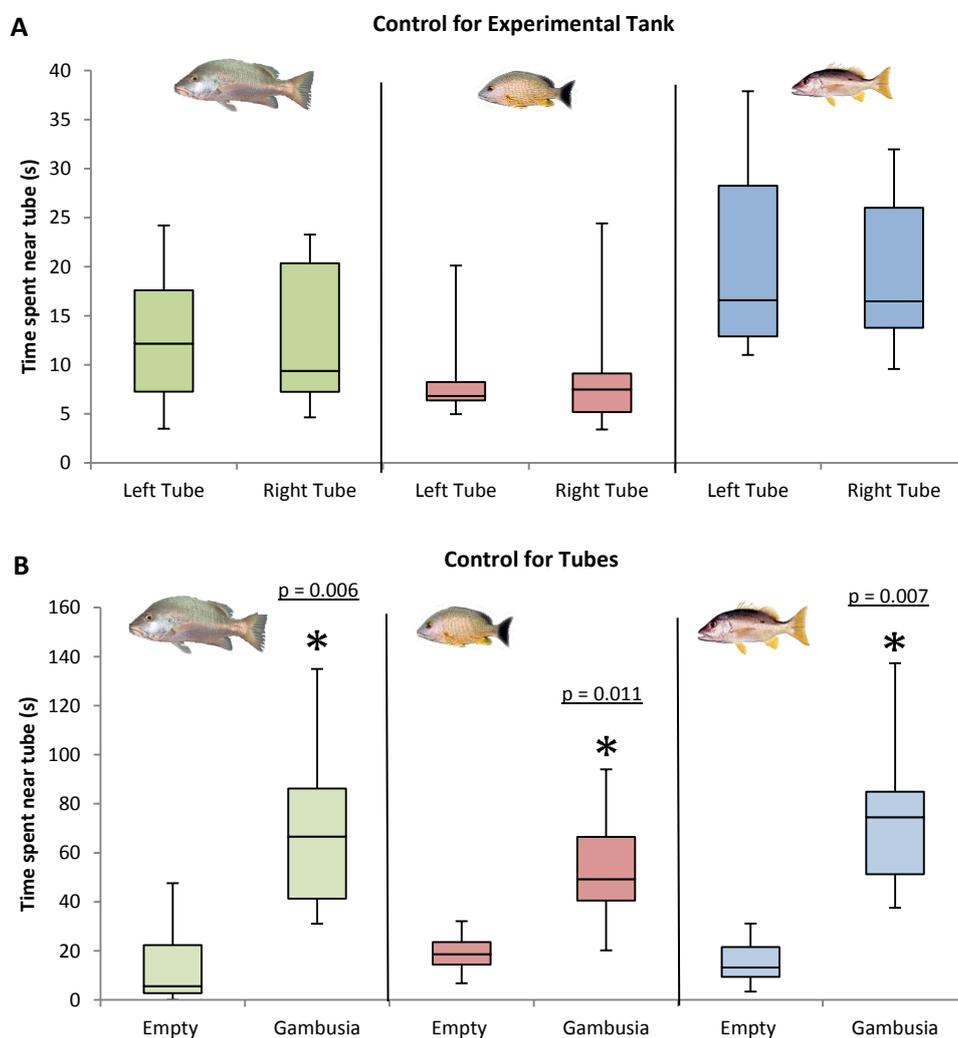


Fig. 5 – Control experiments. Box plots of two control experiments to test (A) the setup of the experimental tank and check for any unexpected side bias within predator species by leaving both tubes empty and (B) the design of the two acrylic tubes to see whether predators can perceive visual cues through the transparent boundaries and show predatory behaviour towards the tube containing a natural prey species (*Gambusia sp.*). Three snapper species were studied: *Lutjanus argentimaculatus* (green bars), *Lutjanus fulvus* (red bars) and *Lutjanus monostigma* (blue bars). *Significant difference between the amounts of time spent in both zones.

3.2 – Experiment 1: Single prey choice

When presented with a single choice of prey (juv. lionfish only), no significant differences were found between the time spent near the lionfish tube or the empty tube for any of the snapper species. Both *L. argentimaculatus* and *L. fulvus* spent an equal amount of time near both tubes on average (Table A2), while *L. monostigma* showed a strong preference for the empty tube which came very close to being significant ($p = 0.056$, Fig. 6A). No significant differences were found in the frequency of visits to the PZs either (Fig. 6B, Table A2), however a trend of *L. fulvus* entering the empty PZ more frequently on average was clearly visible. Swimming activity was found to be relatively low in general during Experiment 1, with multiple trials resulting in 5 or less visits to the PZs for all three snapper species. Finally, as opposed to the tube control experiment with *Gambusia* earlier, not a single strike was ever observed towards the lionfish tube during this experiment.

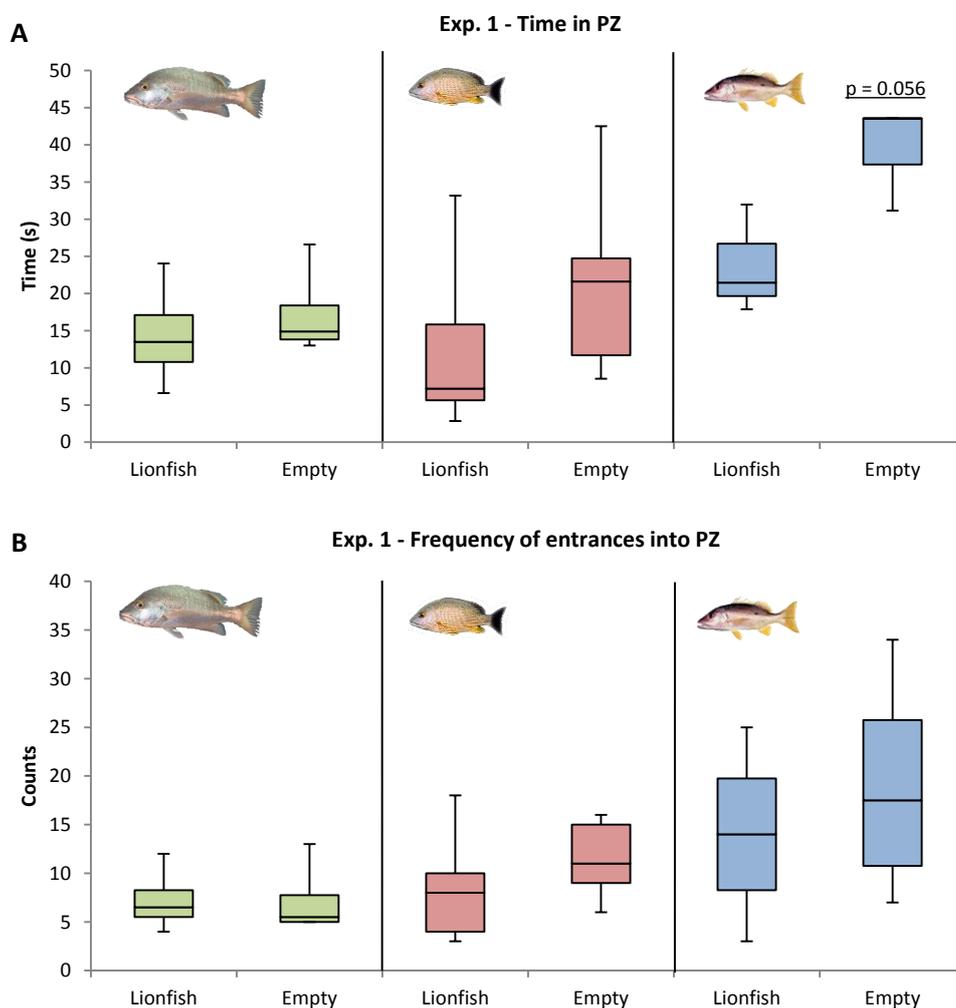


Fig. 6 – Experiment 1 (single prey choice). Box plots of snapper predatory behaviour during Experiment 1, where the snapper has the choice between an empty tube and a tube containing one prey species (juv. lionfish). Three snapper species were studied: *Lutjanus argentimaculatus* (green bars), *Lutjanus fulvus* (red bars) and *Lutjanus monostigma* (blue bars) for which (A) elapsed time in the designated PZs (prey zones) was measured (see Fig. 4 for description of PZs) and (B) the number of visits to the PZs was counted.

3.3 – Experiment 2: Double prey choice

The addition of a *Gambusia* to the empty tube for the second experiment, increased overall swimming activity for all snappers and two out of three species showed a strong preference for the *Gambusia* PZ (Fig. 7). When looking at the time spent in each PZ (Fig. 7A), *L. argentimaculatus* spent longer in the *Gambusia* PZ ($p = 0.028$), whereas *L. fulvus* and *L. monostigma* showed a clear trend in favour of the same PZ which fell just short of being significant ($p = 0.059$ and $p = 0.079$ respectively). The number of visits between the two PZs on the other hand, was only found to be significantly different in *L. fulvus* ($p = 0.013$), while the remaining two snapper species visited both PZs equally (Table A2).

Interestingly, whereas again no strikes were ever made towards the juvenile lionfish, they were not completely avoided either with all snapper species still paying a substantial number of visits to the lionfish PZ on average (Fig. 7B, see Table A2 for the amount of strikes on *Gambusia*). On one occasion a snapper was seemingly observing the juvenile lionfish for a short period, yet never attempting a single strike.

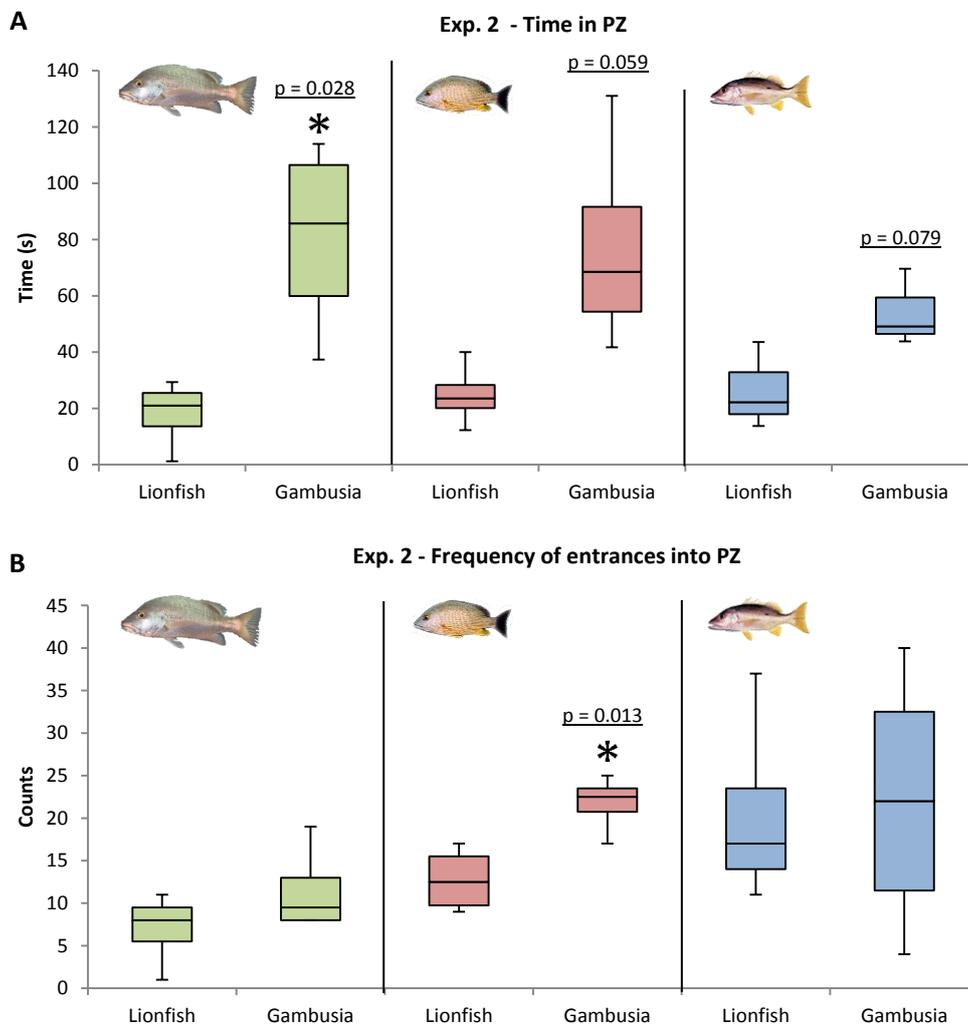


Fig. 7 – Experiment 2 (double prey choice). Box plot of snapper predatory behaviour during Experiment 2, where the snapper has the choice between two prey species (*Gambusia* sp. and juv. lionfish) presented separately in tubes at either side of the tank. See Fig. 6 for explanation of the box plots. * Significant difference in either the amount of time spent in each zone or the number of zone visits.

3.4 – Experiment 3: Lionfish flight response

The third and final experiment, during which *Gambusia* and both tubes were removed from the tank, aimed at measuring the amount of time a lionfish spent in hiding as an indication of defensive behaviour. A single factor ANOVA determined a significant difference between the three different size classes of juvenile lionfish ($p = 0.001$) in the amount of time spent hiding. Specifically, the small juvenile spent longer in hiding ($81.22 \pm 15.25\%$) than both the medium-sized ($48.46 \pm 14.02\%$; $p = 0.003$) and the large juvenile ($34.24 \pm 19.01\%$; $p = 0.006$). No significant difference was found between the large and medium-sized juvenile ($p = 0.26$, Fig. 8). After removing the tube and allowing a ten minute acclimation period, the large and medium-sized juvenile were often observed to still be stationary or swimming rather slowly in a nearby open area despite the presence of an actively swimming snapper. The small juvenile on the other hand, would swim immediately for the nearest shelter after removal of the tubes during all trials. Occasionally, the small juvenile was observed to leave its shelter and swim in the open for a brief period but always returned rather quickly or swam straight for the next brick to hide in. Snappers never shared the shelter with juvenile lionfish and not a single strike was ever observed during the ten-minute trials. Even after allowing a twelve-hour overnight period without protective barriers all lionfish juveniles survived.

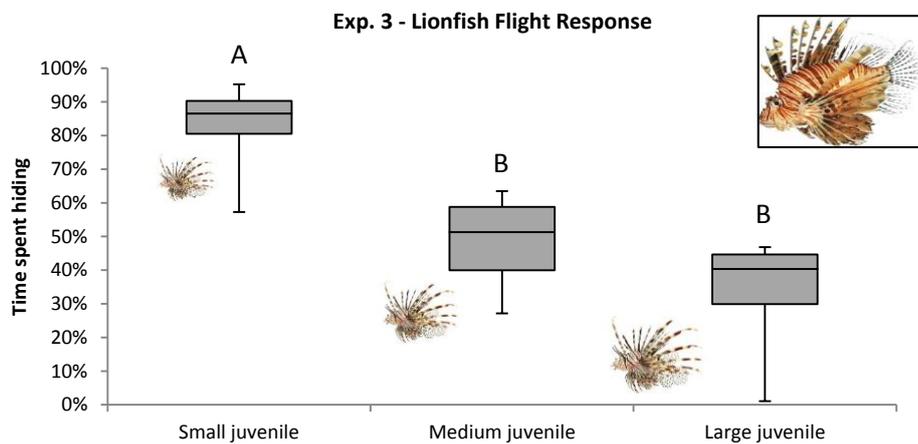


Fig. 8 – Experiment 3 (lionfish flight response). Box plot of Experiment 3, during which the tubes are removed from the experimental tank and lionfish are able to swim freely through the tank, yet no longer protected by the barrier of the tube. As an indicator for juvenile defensive behaviour, the proportion of the time spent in hiding (either under the large grid or inside the hollow bricks, see Materials & Methods section for tank setup) was measured. Three individual juveniles (TL: 4.51 cm, 6.23 cm and 8.72 cm) were divided into the classes small, medium and large juvenile respectively to test the hypothesis that there is a negative relation between body size and time spent hiding from a predator. Different letters above the plots indicate a significant difference ($p < 0.05$, see section 3.4 for exact p-values) between the two classes. An image of an average-sized adult lionfish (*Pterois volitans*, TL: 35 cm) has been added to the upper right corner of this graph to give an indication of the size differences with the three juvenile classes.

4. Discussion

This study aimed at contributing towards filling the large knowledge gap on the ecology of lionfish in their native range. To date, there have been no other quantitative studies, either field or tank experiments, on potential predators of lionfish from this region (see Table C1 in the appendix for main topics of past studies and summaries of major findings). The few studies that have examined lionfish in their native range have focused on either hunting behaviour, venomology or anti-predator behaviour of native prey species. Some possible explanations for the lack of biocontrol studies from this region include: 1) the relative rarity of lionfish in their native range, which makes it more challenging to collect enough specimens to perform predation trials with a decent sample size, 2) the difficulty of performing predation trials in general, specifically when using large predatory species in confined spaces, and finally 3) the limitations in both logistics and infrastructure often associated with tropical islands around which native lionfish can be studied in their native range. Nonetheless, a handful of facilities on locations such as Guam and the Philippines provide the rare opportunity for scientists to gain more insights into the biology and ecology of the native lionfish, which unfortunately is not often enough taken advantage of.

The experimental setup used in this study, with transparent tubes acting as a physical barrier, was designed to closely resemble the setup used for a similar study in the Caribbean (Morris, 2009) to allow an accurate comparison of results. To test whether this setup served the purpose of effectively studying predator-prey interactions, control experiments were performed for both the experimental tank and the transparent tubes. The control experiment for the tank eliminated the possibility of side bias in any of the predation trials, by showing that no preference for either the left or right side of the tank was found in any of the snapper species. Similarly, results from the tube control implied that the visual cues provided by the transparent tubes were sufficient in triggering predatory behaviour in all three snapper species and thus confirmed this setup to be effective for studying predator-prey interactions.

The difference between these two control experiments can also be shown from a slightly different perspective by considering the total time budget of the snappers for each trial. In addition to the two PZs, there naturally is a third 'neutral zone' in which the snapper spends the majority of the time when either hiding or swimming outside the two PZs. The time spent in this neutral zone is highest when both tubes are left empty and snappers are not yet in hunting mode, with all data points lying roughly near the 600s mark with little deviation (Fig. B1a in appendix). After adding a *Gambusia* to one of the tubes however, the snapper switches to hunting mode and the time spent in the neutral zone becomes less with higher deviation between trials (Fig. B1b). The neutral zone was left out in the main result section to better focus on the differences between both PZs, but nonetheless shows the complete time budget of the three snapper species during the trials and should therefore not be excluded entirely.

Results from the predation trials that followed suggest that snappers exhibit indifference and possibly avoidance behaviour towards juvenile lionfish. No predatory behaviour was observed in the single prey-choice experiment, with snappers ignoring and even avoiding the lionfish tube by spending more time near the empty tube. Therefore, the first hypothesis of snappers showing predatory behaviour towards lionfish after starvation was rejected. Under the double prey-choice setup, snappers significantly preferred the *Gambusia* tube by actively attempting strikes and entering the PZ for prolonged visits. No strikes were ever observed towards the lionfish tube and while they were not avoided entirely (snappers were still briefly visiting the prey zone, see Table A2), these findings also reject the second hypothesis of an opportunistic predatory style.

The difference in swimming behaviour between the three snapper species had important implications for their respective time budgets. While *L. argentimaculatus* was observed to be particularly inactive in absence of natural prey species and generally slower in movement, the other two species were more active swimmers regardless of the presence of potential prey species. Specifically, *L. argentimaculatus* was often observed hanging near its prey of choice for prolonged periods while attempting strikes, whereas the other species would actively swim in- and outside of the PZ while striking which implies a much higher frequency of entrances into the PZ. Once again, the difference in snapper activity between these two experiments is elucidated when looking at the complete time budget of the three snapper species (Fig. B2). The mean time spent in the neutral zone during the single prey-choice experiment is relatively high, indicating more time is spent in hiding or slow swimming. However, less time is spent in the neutral zone during the double prey-choice experiment after the *Gambusia* is added and snappers naturally become more active and spent longer inside the *Gambusia* PZ.

From his predation trials in the Caribbean, Morris (2009) concluded that four species of grouper (family: Serranidae) do not readily prey on lionfish. A strong contrast was observed in behaviour of groupers towards a natural prey species (pinfish) and juvenile lionfish, with groupers clearly preferring the pinfish. Specifically, black sea bass (*Centropristis striata*) on average spent 53% of the time near the pinfish tube compared to 3% near the juvenile lionfish (Morris, 2009). In comparison, the three snapper species in this study on average spent between 3-5% of the time near the lionfish. Since the PZs were nearly equal in proportional area (17% compared to 19.1% in this study), these studies show very similar results and both strongly suggest that the predator species studied do not regard lionfish as being palatable prey species. However, in one occasion in Morris (2009), a juvenile lionfish was consumed by the black sea bass during a 48-h predation period in which the tubes were removed from the tank. While consumption was never observed in this study and might hint on natural predation in the Caribbean study, the single observation of consumption can by no means be regarded as naturally occurring since it took place after a total starvation period of 84 hours while being in isolation after which predators will likely resort to desperate strategies in order to survive.

The few reports that have been published on lionfish predation thus far are all based on either stomach analyses or anecdotal evidence. A common lionfish (*Pterois miles*) was found in the stomach of a single bluespotted cornetfish (*Fistularia commersonii*) in the Red Sea (Bernadsky & Goulet, 1991) while both a tiger grouper and two Nassau groupers were found with a red lionfish (*P. volitans*) in their stomachs in the Caribbean (Maljković & Van Leeuwen, 2008). Anecdotal evidence often comes from spear fishermen and divers, with a spotted moray eel (*Gymnothorax moringa*) preying on a speared and injured lionfish in the Bahamas (Pimiento et al., 2013) and a dusky grouper chasing and consuming a lionfish in the Mediterranean (Turan et al., 2017). In addition, during the time spent on Guam for this project, I obtained additional anecdotal evidence of predation by the moray eel *Gymnothorax flavimarginatus* (T.J. Donaldson, pers. comm.), by the stonefish (*Synanceia verrucosa*) and blue octopus (*Octopus cyanea*) on healthy lionfish adults (J. Cummings & D. Burdick, pers. comm.). Finally, video surveillance from the Caribbean of tethered lionfish documented ‘multiple’ occasions of predation by nurse sharks and Nassau groupers (Diller et al., 2014), yet fails to mention the number of occasions.

These observations may seem indicative of natural predation on lionfish in different areas, yet they represent the only evidence of this kind in over two decades. Extensive field studies investigating the potential of biological control found no relationship between the density or biomass of lionfish and that of native predators (Hackerott et al., 2013; Valdivia et al., 2014). Both the present study and the Caribbean study by Morris (2009) provide evidence of tank experiments in agreement with results

from these large field studies. Predation on lionfish, whether in their native or invaded range, does currently not seem to play a significant role in controlling their populations.

Possible explanations for this lack of lionfish predation have widely been speculated upon and likely are the result of a combination of factors. Lionfish are equipped with venomous dorsal, anal and pelvic spines capable of extracting a toxin that highly affects neuromuscular transmission (Halstead et al., 1955; Cohen & Olek, 1989). The observation of tail first consumption by *F. commersonii* of a lionfish led to the suggestion that an attack from the rear would possibly prevent the spines from puncturing and injecting venom while being swallowed (Bernadsky & Goulet, 1991). However, an examination of the spine rotation range by Morris (2009) discarded this theory by showing that the dorsal and anal spines are positioned in such a way that discourages a tail-first consumption of the lionfish. Interestingly, the Indo-Malaysian mimic octopus was found to mimic a lionfish by hovering just above the seafloor with arms trailing from the body, likely indicating that predation is low on lionfish which would make it an appropriate mimicking model (Norman et al., 2001).

Further predator deterrents are probably related to a combination of visual cues, i.e. 'bold' appearance by flaring and extending the pectoral fins and warning potential predators with aposematic (red) coloration, together with chemical cues. Although there have been no studies to date investigating the role of chemical cues or odour in deterring predators by lionfish, snappers in this study seemed to show stronger avoidance of lionfish after removal of the tubes (which would act as an effective barrier of chemical cues). Snappers would often closely approach the lionfish tube, albeit for very short periods, which was never observed again after removal of the tubes where snappers would typically use different shelter space or swim higher in the water column to avoid the lionfish. These observations might be indicative of the presence of strong odour or other chemical cues in lionfish, to further deter an approaching predator.

Removal of the tubes also allowed for quantification of lionfish anti-predator behaviour by measuring the amount of time a juvenile spent in hiding. Results from this final experiment showed that all juveniles used the provided shelter to some degree when no longer protected by the tubes. The small juvenile was found to spend significantly longer in hiding than both the medium- and large-sized juvenile, confirming the last two hypotheses of section 1.4. Possible flares of the pectoral fins could not be included in the analysis however. While fin extensions in a position perpendicular to the transverse plane are a good indicator for defensive behaviour in adult lionfish (Côté et al., 2010), the juveniles in this study were too small to show distinctive fin extensions in the video footage.

Recently, Raymond et al. (2015) studied the use of shelter in Nassau groupers and lionfish under both isolation and interaction periods. They performed trials on small juveniles (ranging in size from 5.6 to 12.3 cm TL) and adult lionfish (up to 31.7 cm TL). The proportion of time their small juveniles used shelter when in presence and interacting with large groupers was slightly larger than results of this study, with their juveniles using shelter 58.9% of the time compared to 48.5% and 34.2% for medium- and large-sized juveniles respectively in this study. This small difference is possibly related to predator size with Nassau groupers ranging from 18.4 to 42.1 cm TL in their study, whereas snappers used in this study were relatively small and ranged from 24.7 to 32.3 cm TL in size. After removal of the protective tubes in this study, medium- and large-sized juveniles were often observed to remain stationary for a substantial period of time or show minimal movement by hovering over the bottom rather than swimming towards the nearest brick for shelter, even when the snapper was swimming nearby. Furthermore, lionfish were observed to switch readily between bricks in far corners of the tank and the large grid in the centre when hiding, often crossing the entire length of the tank (~ 4m) multiple times yet never swimming up and down in the water column but always hovering over the bottom instead. In comparison, lionfish were observed to swim up and down the

water column in their holding tanks in addition to hovering on the bottom, especially after 6pm which is also when the third experiment took place. This difference in behaviour might indicate a certain level of awareness in lionfish of a potential predator during this experiment in which the juveniles chose to remain near the bottom instead, possibly to limit the directions in which the predator can approach. Nonetheless, the relatively low proportions of time that juvenile lionfish spent in hiding, in combination with the lack of response towards a nearby snapper, clearly shows that juveniles do not recognize snappers as an immediate threat and possibly rely on their venomous defence mechanism in the off chance a predator decides to strike.

Similarly, Morris (2009) reported that juvenile lionfish showed no flight response when potential serranid predators approached in his study, yet there is no mention of any form of shelter provided for lionfish (or grouper) in the experimental tank. This raises the question as to how anti-predator behaviour could have been quantified at all and how species could have been acclimated in the first place.

The avoidance behaviour observed in snappers when exposed to free-swimming lionfish is also in agreement with results for groupers in Raymond et al. (2015), with no aggressive behaviour ever observed towards juvenile lionfish and the predator keeping a relative distance. The general trend in previous studies on competition for shelter in fishes was typically that of larger individuals dominating the smaller ones (e.g. Shulman, 1985; Buchheim & Hixon, 1992), which clearly is not the case for lionfish. Instead, Raymond et al. (2015) found that Nassau groupers would change their shelter use when interacting with lionfish, implying that lionfish could cause displacement of Nassau groupers on Caribbean reefs. This is unlikely to happen to native snappers in the West-Pacific however, due to the relatively low numbers of lionfish in their native range which makes them unlikely to be involved in the displacement of other reef fishes in this region.

While *P. volitans* juveniles were relatively rare on Guam and seemed to be restricted to shallow nursery areas, sister species *P. antennata* and *P. radiata* were encountered more frequently at various sites around the island and depths varying between 1-30m. However, the aim of this study was to resemble the Caribbean invasion as closely as possible and hence to refrain from the use of these two sister species. Admittedly, results would have been more accurate when using a new juvenile lionfish for every trial and as such represent each size class by more than one individual, yet this proved unrealistic when merely using *P. volitans* juveniles. Similarly, catching decent-sized predatory fish species outside the MPAs of Guam was another challenge, mainly as a result of overfishing and land-based pollution (Richmond & Davis, 2002; Porter et al., 2005). Two of the snapper species that were eventually caught turned out to be relatively small, with *L. monostigma* measuring 24.7 cm in TL while adults reach an average length of 50.0 cm TL (www.fishbase.org) and *L. argenteimaculatus* of 32.3 cm TL with an average of 80.0 cm TL in adults (www.fishbase.org). Still, the size difference compared to the juvenile lionfish used in this study should be large enough to allow for natural predation to occur (predation did occur on *Gambusia* that were a few cm smaller), so snapper body length is not regarded as a strong limitation of this study.

While the three snapper species used in this study do not occur naturally in the WA and Caribbean, the genus *Lutjanus* is well represented in this region. As such, the results from this study imply that, since snapper species from the Western Pacific do not readily prey upon lionfish, it is unlikely that their congeneric sister species from the Caribbean will develop strategies to prey upon lionfish there.

Other predatory species found on Guam were considered for use in this study, and multiple test trials were performed with honeycomb groupers (*Epinephelus merra*), thumbprint emperors (*Lethrinus harak*, Lethrinidae) and yellowlip emperors (*L. xanthochilus*). Unfortunately, these species did not acclimate well enough in the experimental tank, did not feed upon natural prey species

provided to them and displayed strong signs of stress in their body colouration and behaviour. In the field, emperor snappers were often observed foraging in the same shallow nursery area from which the juvenile lionfish were caught, raising the possibility that natural predation upon juvenile lionfish could occur in these areas. While this hypothesis could not be tested in this study, the morphology and feeding behaviour of emperors closely resembles that of snappers which suggests that if predation takes place, it is most likely towards the smallest juveniles or post-larvae.

The movement of prey species while being kept inside the tubes could possibly have influenced the behaviour of the snappers. Similar to the no-tube experiment, lionfish were also observed to be either stationary or hovering just above the bottom of the tank whilst inside the tube. In contrast, *Gambusia* were often swimming vertically through the water column and generally showed a much higher swimming activity. Naturally, this behaviour would imply a higher degree of visual cues being received from the actively swimming *Gambusia* compared to the stationary lionfish, which is of course exactly the strategy of a cryptic bottom-dweller. Still, during the majority of predation trials snappers were observed to be active near the bottom often closely approaching the lionfish for brief periods and only swimming to the surface to attempt strikes on *Gambusia*. As such, it is reasonable to assume that the snappers received a sufficient amount of visual cues from the lionfish as well, which was never stationary for the entire trial but was also observed to be hovering large proportions of the time.

The choice of conducting the three experiments consecutively may also have slightly influenced the outcome by unexpected side bias or conditioning in snappers. For example, a snapper might still be biased for one side of the tank in the second experiment as a result of a specific setup in the first experiment, or a snapper might attempt fewer strikes at the tubes during the second experiment after failing to succeed in the first one. These effects were tried to be kept to a minimum by allowing for an additional ten minute acclimation period after each experiment in order to let the snapper adapt to the new setup and decrease the risk of biased, unnatural behaviour. Also, snappers were never attempting any strikes when presented with lionfish only during the first experiment, which implies no conditioning could have taken place going into the second experiment. This particular setup did however provide an advantage as well, since snapper behaviour could accurately be compared between a single prey-choice setup (lionfish only) versus a double prey-choice setup (lionfish and *Gambusia*), instead of comparing between experiments from different days and individual snappers.

Finally, the experimental tank, located in the outdoor facilities of the Marine Laboratory, had a negative influence on a few trials. Whereas incoming sunlight could be blocked using tarps and shade cloths, the background noise of the surrounding area occasionally interfered with the conduct of an experiment. A small beach area close to the Marine Laboratory was open to the public during both day and night, and a shower was located next to the experimental tank that sometimes attracted a gathering of people that would create loud background noises which noticeably influenced the behaviour of the snappers. The negative effects were minimized by performing trials during late afternoon and early evening instead of earlier in the day. Still, a few trials were aborted as a consequence (one for *L. fulvus* and two for *L. argentimaculatus*) and one outlier was also eventually removed from the data for *L. monostigma* since the snapper was not behaving naturally while people were using the shower facilities.

This study has revealed the challenges related to studying predator-prey interactions with relatively large predators in a confined space (i.e. an experimental tank). Similar future studies with the aim to examine interactions between lionfish and potential predators should avoid using tank experiments and instead identify accessible sites to perform *in-situ* studies when possible. Potential predators might share the same cryptic lifestyle (e.g. larger members of Scorpaenidae and Muraenidae family)

and thus be unrealistic candidates for predation trials in experimental tanks. Studying the interactions between these potential predators and juvenile lionfish in the field however may be even more challenging, since juveniles are mostly found in low visibility areas (Cure et al., 2014) and such interactions will mainly take place in holes, crevasses and similar poorly accessible locales.

Still, future research on lionfish in their native Indo-Pacific range is encouraged in order to better understand the dynamics of the invasion in the Caribbean. It is recommended that studies in this area shift the focus more towards smaller juveniles or perhaps even larval lionfish if possible. From this study and the few other predation studies and general observations it seems reasonable to assume that if significant predation on lionfish occurs at all, it is most likely directed towards larvae, newly settled post-larvae and small juveniles. Therefore, the morphological armament of larvae and venomology of juvenile lionfish should be studied in greater detail so as to predict the potential of predatory species to develop feeding strategies effective against the early life stages of lionfish.

Realistically, at this stage of the Western Atlantic lionfish invasion we have moved past the point of possible eradication and our focus should be towards mitigating negative effects, especially in and around marine protected areas to protect critically endangered species. While culling efforts have been carried out in the Western Atlantic for nearly a decade and are shown to have some effect, e.g. up to 90% reduction of lionfish numbers in fewer than 2.5 dive hours per 1.000m² (Usseglio et al., 2017), they involve a lot of long-term uncertainty in relation to post-culling recolonization rates of lionfish and the sustainability of such efforts. Additionally, scientists have been attempting to create incentives to increase lionfish exploitation, both through the culinary pathway of promoting lionfish meat as a healthy food source (Gallagher, 2013), and for the pharmaceutical industries by suggesting that lionfish venom may have the potential to reduce cancer growth in humans (Sri Balasubashini et al., 2006). While such incentives, if successful, can potentially increase lionfish exploitation in the Western Atlantic, caution should be warranted. Creating a lionfish-dependent industry in an already over-exploited region seems counterproductive and could lead to substantial amounts of bycatch and other unwanted side effects, as well as the risk of lionfish fisheries outside the Caribbean where lionfish are already rare.

It seems that other than preserving the populations of native apex predators by enforcing fishing restrictions and maintaining MPAs, human interference will have minimal effects and the lionfish invasion is likely going to run its course. Ultimately, lionfish may limit their own numbers in the Western Atlantic by exceeding the carrying capacity of the native system and possibly resort to cannibalism which has been observed before (Valdez-Moreno et al., 2012; Côté et al., 2013). However, the low frequency of intraspecific predation in lionfish makes it an unlikely form of biocontrol which will presumably only increase under the worst case scenario of lionfish starvation as a result of food depletion in the Western Atlantic.

5. Conclusion

This study aimed to help fill the large knowledge gap about the ecology of lionfish in their native range by showing that native meso-predators, snappers, do not readily prey upon juvenile lionfish. Previous suggestions that snappers could provide a possible biological control on lionfish populations have been based on stomach analyses of mutton snappers (*Lutjanus analis*) indicating a diet which included similar venomous scorpaenids (Randall, 1967; Muñoz et al., 2011). While lionfish may occasionally be consumed by large snappers, this is unlikely to occur at a sufficiently large scale to effectively control lionfish populations in either their native or invasive range.

Additionally, while juvenile lionfish did make use of the provided shelter (with the smallest juvenile spending the most time in hiding), none of the juveniles seemed to show any response (e.g. fleeing towards shelter) when a snapper was swimming nearby. These observations either indicate that

lionfish do not recognize snappers as a potential threat or they rely heavily upon their venomous defensive mechanism and aposematic warning colouration to deter any predator.

Results from this study indicate that it is highly unlikely that snapper species will develop the necessary feeding behaviour to effectively provide a biocontrol for lionfish in the near future. Still, future research should focus more on lionfish behaviour and ecology in their native range, especially on venomology of juveniles and their interactions with other reef inhabitants in their natural habitat, to be able to understand what factors limit lionfish populations here. The possibility remains that native Western Atlantic species can provide some sort of biotic resistance, either through competition, pathogens and/or predation which would be highly preferable to the alternative scenario of stabilization through food depletion or habitat degradation.

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Appendix A

Table A1 – Species used. The number of individuals, number of trials and repeats per individual are shown. TL: total length, SD: standard deviation; images on the left-hand side are scaled to relative size.

Predator species	Common name	No. of individuals	No. of trials	TL (mean ± SD)	Number of trials per individual
 <i>Lutjanus argentimaculatus</i>	Mangrove snapper	3	6	32.3 ± 5.0 cm	* LA1: 2 * LA2: 2 * LA3: 2
 <i>Lutjanus fulvus</i>	Blacktail snapper	4	6	26.5 ± 1.7 cm	* LF1: 1 * LF2: 2 * LF3: 2 * LF4: 1
 <i>Lutjanus monostigma</i>	One-spot snapper	1	4	24.7 cm	4
Prey species					
 <i>Pterois volitans</i>	Red lionfish (juvenile)	3	16	6.5 ± 2.1 cm	* PV1: 4 * PV2: 6 * PV3: 6
 <i>Gambusia sp.</i>	Mosquitofish (natural prey species)	16	16	4.2 ± 0.3 cm	1

Table A2 – Summary of measured variables. Results from both controls, the single prey-choice and double prey-choice experiment are shown expressed in mean ± SD. Both strike attempts and PZ visits represent counts whereas the time inside each PZ is measured in seconds. Images on top are scaled to relative size.

Control Exp. Tank	<i>L. argentimaculatus</i> 		<i>L. fulvus</i> 		<i>L. monostigma</i> 	
	Left	Right	Left	Right	Left	Right
Time (s) in PZ (mean±SD)	12.95 ± 7.77	12.82 ± 7.64	8.74 ± 5.19	9.13 ± 7.08	21.11 ± 10.45	19.65 ± 8.33
Control Exp. Tubes	Gambusia	Empty	Gambusia	Empty	Gambusia	Empty
Time (s) in PZ (mean±SD)	69.68 ± 36.55	14.78 ± 17.38	53.92 ± 25.62	19.08 ± 8.91	75.95 ± 35.74	15.58 ± 10.21
Single Prey-Choice	Lionfish	Empty	Lionfish	Empty	Lionfish	Empty
Time (s) in PZ (mean±SD)	14.4 ± 7.28	17.34 ± 6.26	12.94 ± 12.31	21.82 ± 13.37	23.77 ± 7.33	39.43 ± 7.16
Strike attempts (mean±SD)	0	NA	0	NA	0	NA
PZ visits (mean±SD)	7.25 ± 3.40	7.25 ± 3.86	8.6 ± 5.98	11.4 ± 4.16	14 ± 9.56	19 ± 12.03
Double Prey-Choice	Lionfish	Gambusia	Lionfish	Gambusia	Lionfish	Gambusia
time (s) in PZ (mean±SD)	18.15 ± 12.23	80.7 ± 35.16	24.84 ± 11.44	77.46 ± 38.77	26.51 ± 15.38	54.2 ± 30.63
Strike attempts (mean±SD)	0	6.25 ± 2.5	0	4.6 ± 3.36	0	10.67 ± 0.58
PZ visits (mean±SD)	7 ± 4.32	11.5 ± 5.20	12.75 ± 3.86	21.75 ± 3.40	20.5 ± 11.47	22 ± 16.08

Appendix B

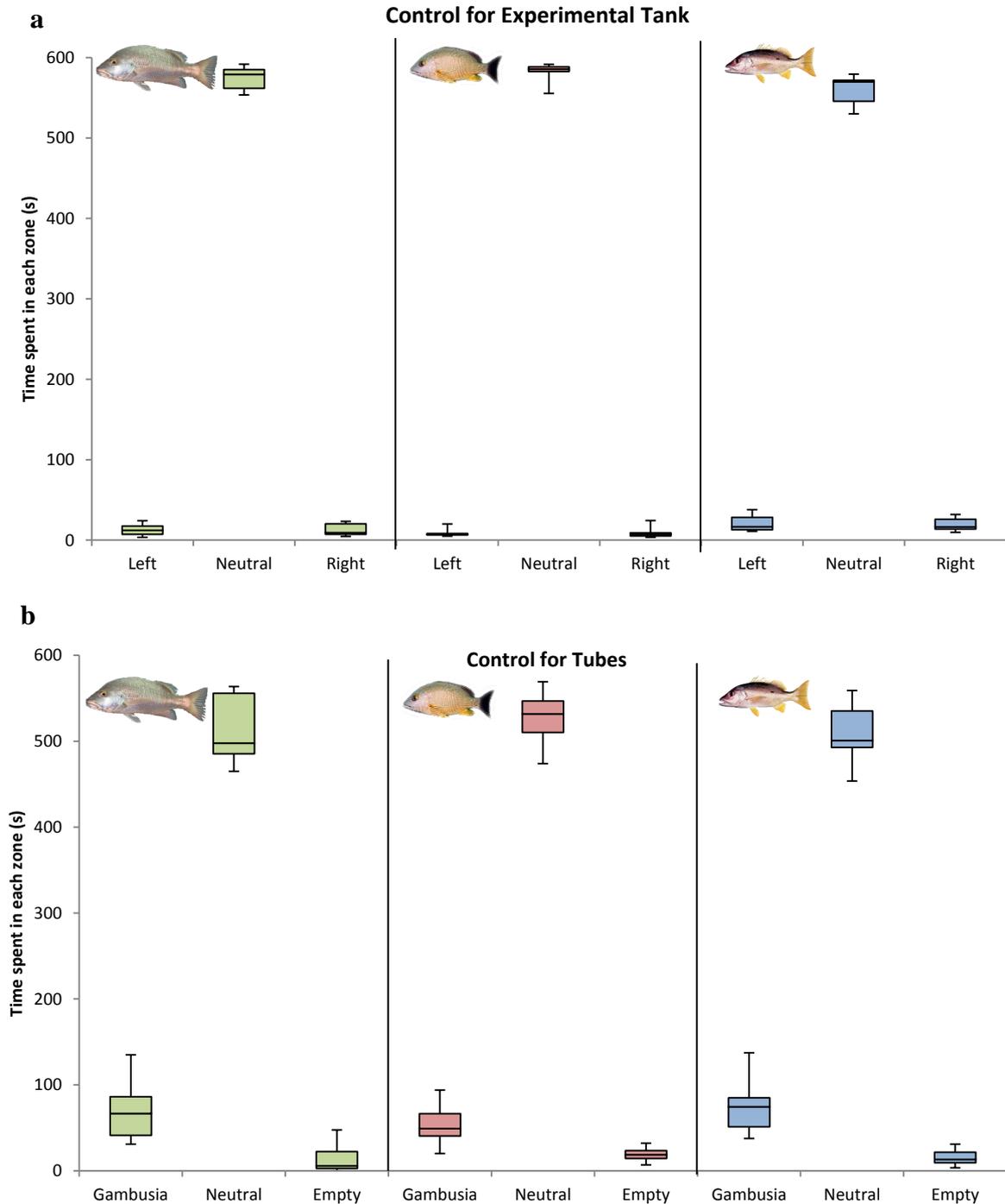


Fig. B1 – Total time budget for control experiments. Boxplot showing results from both control experiments, with time spent in each zone for all three snapper species. A third zone named the ‘neutral’ zone is included here to give a better indication of the position of the snapper for the entire duration of a trial (600s per trial), with *L. argentimaculatus* in green, *L. fulvus* in red and *L. monostigma* in blue. The neutral zone is excluded from the regular result graphs in section 3.1, to better visualize differences between the other two zones on which this study focuses. a) Results for the Tank Control Experiment b) Result for the Tubes Control Experiment.

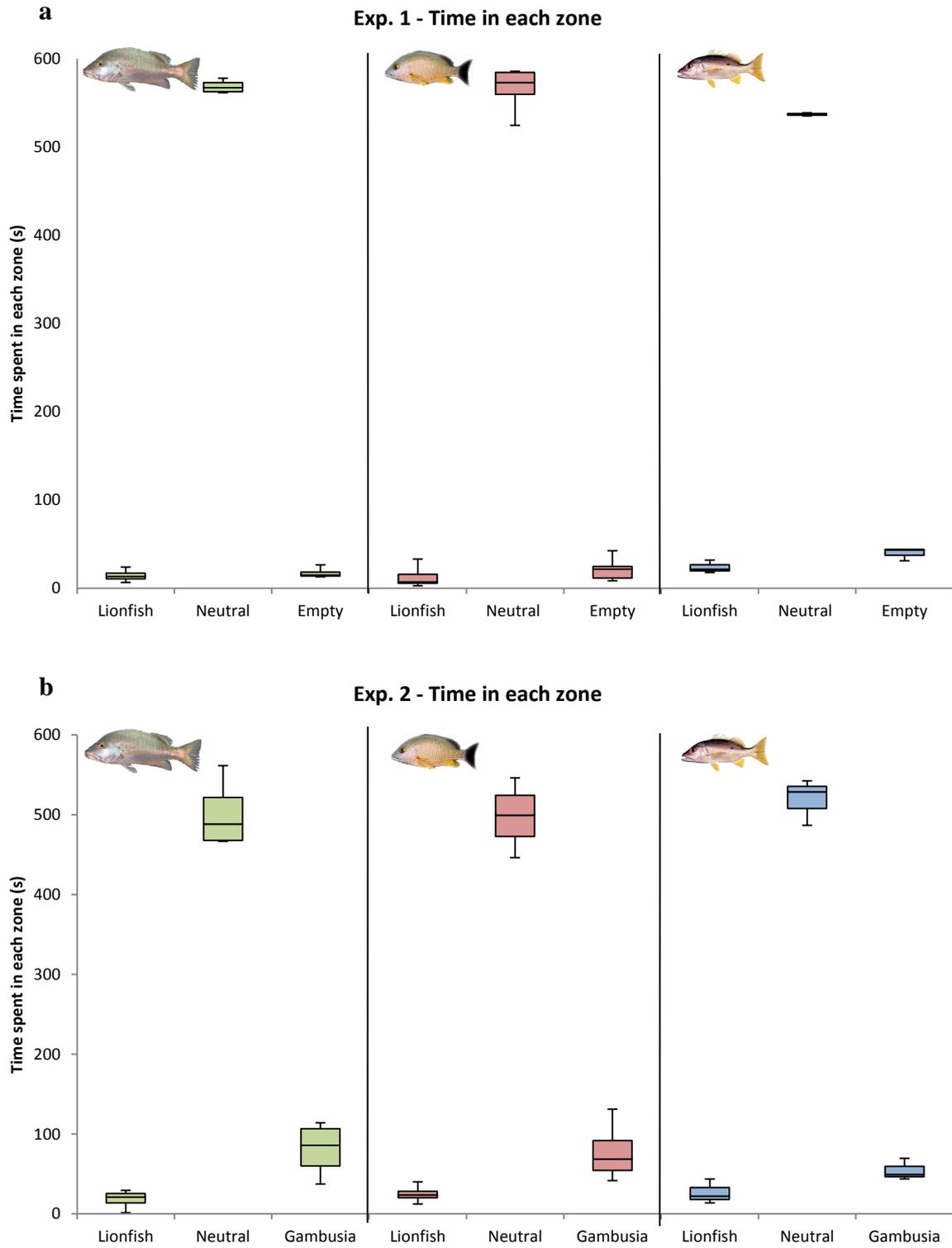


Fig. B2 – Total time budget for experiment 1&2. Box plot showing the amount of time (in seconds) spent in each zone during both the single- (a, Exp. 1) and double prey-choice (b, Exp. 2) experiments. A third zone named the 'neutral' zone is included here representing the amount of time a snapper spent outside the two PZs, with *L. argentimaculatus* in green, *L. fulvus* in red and *L. monostigma* in blue.

Appendix C

Topic	Focus	Literature from Invaded Range + Findings	Literature from Native Range or Comparison + Findings	Topic	Focus	Literature from Invaded Range + Findings	Literature from Native Range or Comparison + Findings		
Life History	* Larval Stage	* Ahrenholz & Morris (2010) : Settlement age between 20 and 35 days, low compared to other reef fishes	* Hammer & Yabe (1996) : Larval stage described from Australia and vertebral counts	Lionfish as Predator	* Diet	* Morris & Akin (2009) : 41 species of 21 families of teleost fish represented in lionfish diet, proportion of crustaceans in diet inversely related with lionfish TL	* Care et al. (2012) : Lionfish mainly targeted 6 fish families in invaded range vs only two in native range (Pomacentridae and Trichonotidae)		
	* Juvenile vs Adult	* Biggs & Olden : Aggregate and patch reef habitats contain adults whereas sea grass habitats contain juveniles	* Fishelson (1997) : Exponential increase in body weight found with 5.5-13.5g per day food intake for juveniles vs 14.6g per day for adults		* Impact on native species	* Barbour et al. (2010) : Lionfish hunt on gobies, parrotfish and goatfish in mangroves	* Green et al. (2011) : Majority of strikes on fishes, 12 species successfully captured with mean length of 4 ± 1 cm		
	* Growth Rates	* Benkwitt (2013) : Lionfish grow slower at higher densities, length declines linearly with increasing lionfish density, while mass declines exponentially	* Fishelson (1997) : Parallel to D. brachypterus until 20-25g weight, then exponential until 70-80g body weight		* Darius et al. (2011) : Higher density with larger body size in Bahamas vs Kenya		* Albins & Hixon (2008) : 79% reduction in recruitment of native fishes in field experiment	* Ellis & Faletti (2016) : See above	
			* Care et al. (2012) : Atl. prey twice as large as Pac. prey, so greater nutritional input		* Pasack et al. (2016) : Lionfish in Atl. grow 1.25-2.25 times faster than in Pacific		* Albins (2013, 2015) : Reduction in total density, species richness and biomass of native species	* Ingram (2016) : Lionfish caused mortality rates of >50% in 9 out of 14 studied basket populations resulting in local extinction of 2 populations	
	* Reproduction Biology	* Gardner et al. (2015) : Mature lionfish (from 190mm TL) have potential to spawn every 2-3 days, ovaries contained 1,800-41,945 oocytes with greater number in larger females	* Fishelson (1975) : Courtship includes circling, side winding and following and starts shortly before dark. Female releases 2 buoyant egg masses which male fertilizes		* Fogg et al. (2017) : Fecundity highest in August, max. potential 2,332,490 eggs for age sized female (188.6g).	* Prey Naïveté	* Marsh-Hookin & Goehfeld (2013) : Two gobies responded to lionfish via visual cues	* Black et al. (2014) : Damselfish failed to adjust courtship behaviour when near lionfish	* Limstich & McCormick (2013) : Lionfish virtually undetectable by prey in native range
	* Genetics	* Hammer (2005), Hammer et al. (2007) : Strong founder effect resulted in large decrease in genetic diversity compared to native range	* Kochzius et al. (2003), Kochzius & Blohm (2005) : siblings P. miles and P. volitans are clearly separated, allopatric distribution confirmed in Indian and Pacific Ocean		* Fashbender et al. (2009) : Source of invasion is egg and larval dispersal from East Coast	* Hunting Techniques	* Kindinger (2014) : response of damselfish to lionfish not sig. different from empty bottles	* Albins & Lyons (2012) : Lionfish blow direct jets of water towards prey to confuse or distract them	* Anton et al. (2016) : 3 families of small fish maintain greater distance from lionfish in Pacific compared to Atlantic, prey naïveté found in at least 8 species of Atlantic fish
	* Venomology	No studies	* Halstead et al. (1955) : Each spine (except caudal spines) is venomous, including 13 dorsal spines, 3 anal spines and 2 pelvic spines		* Cohen & Olek (1989) : Venom contains toxin that affects neuromuscular transmission	* Side effects	* Lesser & Slattery (2011) : Phase shift to algal-dominated community at mesophotic depths	* Kindinger & Albins (2017) : 66-80% less algae removed on high-density lionfish reefs	* Kendall (1990) : Observed to hunt in conspecific groups
			* Balshubani (2006) : Lionfish venom reverses negative effects from EAC tumor cells in mice, might increase pharmaceutical demand for lionfish venom		* Donaldson (2010) : Lionfish observed in small social groups on Guam, sharing shelter such as coral pillars, holes and artificial structures		* Maljkovic & van Leeuwen (2008) : Lionfish found in stomach of tiger & Nassau grouper	* Pimiento et al. (2013) : Spotted moray consumed living (but separated) lionfish	* Bernatsky & Goulet (1991) : P. miles found in cornetfish stomach in Red Sea
			* Smith (2010) : Artificial structures facilitate colonization of sand bottoms		* Kulbicki et al. (2012) : Densities higher in continental areas than around islands. Not only found on Indo-Pacific reefs, but also in mangroves and estuaries	* Stomach Content	* Munby et al. (2011, 2013) : Inverse relation between lionfish and large grouper biomass	* Marcis (2009) : Grouper and black sea bass show no pred. behav towards juvenile lionfish	* Care et al. (2014) : No predators observed during many field expeditions
	Behaviour	* Habitat Preference	* Barbour et al. (2010), Biggs & Olden (2011), Schofield et al. (2014) : lionfish primarily found on coral reefs but sometimes in mangroves and sea grass areas		* Care et al. (2012) : Crepuscular pattern in activity, peaks at sunrise and/or sunset	Lionfish as Prey	* Anecdotal Evidence / Field Obs.	* Anton et al. (2014) : Density & biomass more than 20 and 120 times greater respectively at sheltered areas compared to wave-exposed environments	
		* Smith (2010) : Artificial structures facilitate colonization of sand bottoms	* Claydon et al. (2012) : Larger lionfish on deep reefs, they preferentially settle in shallow habitats before moving to deeper parts	* Field Studies	* Hackerott et al. (2013) : No relation between grouper and lionfish density on 71 reefs				
		* Benkwitt (2016) : Increased foraging movements between coral reef patches and seagrass habitats at higher lionfish densities	* Care et al. (2012) : Lionfish are habitat generalists in native range, occupying various environments including areas with low salinity and high sediment loads	* Care et al. (2012) : Lionfish are habitat generalists in native range, occupying various environments including areas with low salinity and high sediment loads			* Munby et al. (2011, 2013) : Inverse relation between lionfish and large grouper biomass		
* Diel Activity		* Morris & Akin (2009) : Diurnal feeders with highest activity in morning (8-11 AM)	* Claydon et al. (2012), Benkwitt (2016) : See above	* Tamburello & Côté (2015) : Max movement of 1.35km in 15 days of 79 tagged lionfish	* Tank Experiments		* Kimball et al. (2004) : Feeding cessation at 16.1 °C and lethal minimum at 10 degrees		
* Movement Ecology		* Munby et al. (2011) : Lionfish biomass significantly negatively related to that of grouper	* Curtis-Quick et al. (2014) : Lobster displaced from shelter in presence of lionfish	* Raymond et al. (2015) : Grouper use more shelter when with similar size lionfish, competition for shelter may be size dependent			* Hackerott et al. (2013) : Lionfish densities significantly lower on windward sites		
* Competition with Native Species		* Ellis & Faletti (2016) : Lionfish diet shifted from mostly teleost fishes when alone, to mostly crustaceans when in presence of red grouper	* Kindinger & Anderson (2016) : Lionfish able to switch to less preferred prey of grayshby	* Shwigel & Fishelson (1991) : The removal of <i>Cephalophis spp.</i> resulted in an increase of the lionfish population in Red Sea	* Abiotic Stress Factors / Limitations		* Anton et al. (2014) : Density & biomass more than 20 and 120 times greater respectively at sheltered areas compared to wave-exposed environments		
					* Parasitology		* Sellers et al. (2015) : Limited direct effect of parasites on lionfish, parasite species richness and abundance sig. higher in native grayshby grouper and lizardfish		* Sikkel et al. (2014) : Lionfish not highly susceptible to ectoparasite in both ranges
					* Management & Control		* Smith et al. (2010) : Removal and prevention of dump may slow lionfish spread		* Tittle et al. (2016) : Fewer lionfish parasitized in Atl. than in Pac range, when compared to native fishes in Atl. 18-40 times likely to host parasite
							* Morris et al. (2011) : Lionfish meat contains healthy fatty acids, meets customer threshold		
							* Adas-Gonzalez et al. (2011) : When exploiting adults, fishing pressure has to be kept high		
					* Albins & Hixon (2013) : Marine reserves protecting potential predators of lionfish needed				
					* Schwartz et al. (2015) : Emphasis on colonization of deep habitats in efforts to control lionfish populations				
					* Tamburello & Côté : Lionfish movement patterns provided to aid invasion management				
					* Hanky (2017) : Sig. increase in biomass of native fish in lionfish culling areas				
					* Prakash et al. (2012) : 184 individuals captured from the Vellar estuary, southeast India				
					* Bariche et al. (2013), Kletou et al. (2016), Turan et al. (2017) : Invasion began in Med. Sea with almost complete colonization of Cyprus coast and groups of lionfish observed mating				
					* Barbour et al. (2011) : Eradication through fishing unlikely, may only be feasible in localized areas where annual exploitation can be intense over multiple years				
					* Albins & Hixon (2013) : Invasion will either be limited by starvation or a combination of pathogens, parasites, predators and competitors				

Table C1 – Literature summary. This table gives an overview of the different studies that have been done over the past years regarding the lionfish invasion, together with their main findings. Studies are sorted on topic and their focus, with the exception of the hundred odd reports on new sightings of lionfish throughout the Caribbean and NW Atlantic as they are mainly range descriptions. A distinction has been made between studies carried out in the invaded and the native range of the lionfish. Studies investigating lionfish as a potential prey to test for a possible biocontrol have been highlighted in yellow, which is also the category to which this study belongs.