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Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization

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Abstract The ongoing invasion of non-native Indo-Pacific lionfish (*Pterois* spp.) represents a significant ecological threat throughout the Western Atlantic and Caribbean. As a generalist species, lionfish have been able to rapidly colonize a wide variety of ecosystems, including coral reefs, seagrass beds, mangroves, the sea floor at depths as great as 300 m, and even brackish estuaries. While lionfish have been encountered in a number of estuarine systems, the spatial distribution of lionfish in estuaries is likely limited by the species' ability to tolerate low salinities. Here, we experimentally identify minimum salinity tolerance in lionfish by measuring survival salinity minimum—the lowest salinity at which all individuals survive for 48 h. Additionally, we examine whether long-term exposure to low (but sublethal) salinities has negative effects on lionfish. Field observations in the Loxahatchee River estuary (Jupiter, FL) showed that lionfish can survive brief exposure to salinities as low as 1‰. At one estuarine location, fish survived exposure to salinity fluctuations of ~28‰ every 6 h for several days. In laboratory trials, survival salinity minimum for lionfish was 5‰; however, some individuals survived at 4‰ for up to 94 h before dying. Lionfish that were held at 7‰ for 28 days showed no differences in mortality, behavior or growth, when compared to control fish held at 35‰ (typical ocean salinity). This broad salinity tolerance may allow lionfish to colonize estuaries throughout their invaded range, and may facilitate dispersal across the Amazon-Orinoco plume. Because of the ecological and economic importance of estuaries, this facet of the lionfish invasion warrants further study.

Keywords Estuary · Indian River Lagoon · Invasive marine fish · Lionfish · *Pterois volitans* · Salinity tolerance

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Introduction

The rapid invasion of the Western Atlantic and Caribbean by the Indo-Pacific lionfish *Pterois volitans* and *P. miles* (morphologically indistinguishable species, hereafter referred to as lionfish) was likely facilitated by a number of behavioral and physiological traits possessed by the species (reviewed in: Albins and Hixon 2013). Lionfish are habitat generalists, having been found to occupy a variety of habitats in the invaded range, including coral reefs, seagrass beds, mangroves, human-created habitats, and the ocean floor at depths as great as 300 m (Barbour et al. 2010; Biggs and Olden 2011; Claydon et al. 2012; Côté et al. 2013a). Additionally, as dietary generalists possessing feeding behaviors novel to the region, lionfish have proven to be very efficient predators of native species (Morris and Akins 2009; Green et al. 2011; Albins and Lyons 2012; Lonnstedt and McCormick 2013). Since the presence of invasive lionfish has been linked to severe declines in fish abundance in coral reef ecosystems (Albins and Hixon 2008; Green et al. 2012), the potential ecological and economic effects of lionfish in other invaded ecosystems is of great concern.

In 2010, we identified lionfish utilizing estuarine habitats in the Loxahatchee River, near Jupiter, FL (Jud et al. 2011; Jud and Layman 2012). Since estuaries provide critical nursery habitats for numerous ecologically and economically important species (Beck et al. 2001; Courrat et al. 2009), the presence of an invasive and highly successful generalist predator in these systems is troubling. To date, we have observed lionfish as far as 6.6 km from the ocean in the Loxahatchee River, in salinities as low as 8‰ (Z. Jud, unpubl. data). However, based on limited field observations, we were unable to speculate about the range of salinities that lionfish can tolerate. Additionally, the long-term effects of low (but sub-lethal) salinity on lionfish behavior, growth, and survival may ultimately determine the distribution of lionfish in estuarine systems. While predictions of future range expansion in lionfish have been based primarily on thermal tolerance (Kimball et al. 2004; Morris and Whitfield 2009), salinity tolerance may also be an important factor controlling the eventual distribution of the species. Of particular importance, salinity tolerance may determine whether the Amazon-Orinoco plume will act as a barrier to the southward spread of lionfish along the Atlantic coast of South America.

Herein, we utilize a series of laboratory experiments to determine how reduced salinities in estuarine ecosystems may affect invasive lionfish. Our objectives were twofold: First, we wanted to determine how long-term exposure to low (but sub-lethal) salinity may affect lionfish survival, growth, and behavior. Second, we wanted to identify the minimum salinity at which lionfish can survive for at least 48 h. Preliminary field observations suggested that lionfish were able to survive in low salinities, and provided an estimate of minimum salinity tolerance. We then used these preliminary values as a starting point to more thoroughly test long- and short-term salinity tolerance in the laboratory.

Methods

Field observations

Our initial observations of in situ salinity tolerance in lionfish occurred opportunistically during an unrelated caging experiment intended to assess lionfish trophic interactions (hereafter referred to as the in situ cage study). Although that study was not specifically designed to test lionfish salinity tolerance, an unexpected period of heavy precipitation provided an opportunity to document the reaction of lionfish to varying salinities in a natural setting. These observations also allowed us to choose an appropriate sub-lethal salinity level to utilize during a subsequent laboratory trial aimed at addressing our first objective.

For the in situ cage study, we selected three sites in the Loxahatchee River, located 2.4 km (downstream site), 6.2 km (midstream site), and 7.0 km (upstream site) upriver from the ocean. At each site, eight cylindrical plastic mesh cages were deployed (55 cm diameter, 45 cm tall, 13 mm mesh). We added 20 l of limestone gravel (~20 mm diameter) and one small brick to each cage to provide shelter for lionfish prey (e.g., small crabs, shrimp, and fishes, which began to colonize cages immediately following deployment). Since this study was not originally intended to assess the effects of salinity on lionfish, cage design and site location were selected based on the objectives of the trophic interaction experiment mentioned above.

Twenty-four lionfish (76–155 mm standard length) were captured in Jupiter Inlet and the lower Loxahatchee River (Jupiter, FL). Salinities at the capture sites ranged from 24 to 36‰. Fish were divided into three groups, such that each group contained approximately the same size distribution of individuals. The groups were then placed into three temporary holding cages in the river. To allow fish to acclimate to the ambient salinity of each study site, we moved the temporary holding cages upriver in a series of incremental steps. This acclimation process took 3 days for the upstream site (where salinities were lowest), 2 days for the midstream site (with intermediate salinities), and 1 day for the downstream site (with highest salinities). We staggered the start dates of the upstream movement/acclimation process by 1 day per site, so each set of fish would arrive at their respective study site on the same day. Following an additional 24 h of acclimation at the study sites, fish were added individually to the experimental cages.

At each study site, we deployed a datasonde (Hydrolab DataSonde 5X, Hach Hydromet Inc.) that recorded salinity every 15 min. Since the Loxahatchee River frequently exhibits stratified conditions, such that highest salinities occur immediately above the benthos, datasondes were mounted ~4 cm above the river bottom, among the lionfish cages. All 24 caged lionfish were visually observed one time per day using mask and snorkel (~ 1 min/cage), with observations occurring near high tide, when water clarity was greatest. Because extremely low water clarity made it difficult to see into cages, only simple behavioral observations could be made. We noted whether fish were alive and maintaining equilibrium, alive but lacking equilibrium, or dead. Although the ultimate cause of lionfish mortality during the in situ cage study was not known, we made the assumption that deaths, which were always preceded by loss of equilibrium, were a result of reduced salinity following the precipitation event. After 40 days, living lionfish were euthanized using MS-222 (tricaine methanesulfonate, 400 mg/l), weighed, and measured.

Laboratory trials

To address our first objective, identifying the long-term effects of reduced salinity on lionfish survival, growth, and behavior, we exposed fish to a salinity of 7‰ for 28 days in a laboratory setting. We chose this salinity based on our findings from the in situ cage study (above), in situ observations of wild lionfish at 8‰ (Z. Jud, unpubl. data), as well as the results of a small pilot study that showed lionfish could survive and feed at 6‰ for short periods of time (L. Arrington, unpubl. data). During the 28-day study, we looked for changes in behavior or mortality (compared to control fish housed at 35‰) that may have been caused by long-term exposure to low salinity. Additionally, we used growth rate (mm/day for standard length, g/day for mass) to assess potential physiological costs associated with living at low salinities.

In the laboratory, we set up eight pairs of 38 l glass aquaria. Each aquarium contained a sponge filter, and lighting was provided by banks of fluorescent tubes running on a 12:12 light cycle. Ambient room temperature was maintained at 25 °C. Within each pair of aquaria, one tank (the control) was filled with 35‰ saltwater (obtained from a saltwater well), and the other with 7‰ saltwater (35‰ water, diluted with tap water and aerated for 24 h to remove chlorine). Salinity was measured using a calibrated refractometer, and verified at the start of the study with a calibrated YSI Pro2030 (YSI Inc.).

Sixteen lionfish were captured in Jupiter Inlet and the lower Loxahatchee River estuary using hand nets. Salinities at the time of capture were 27–35‰. These fish were transported in 32‰ water from the field to the laboratory, where they were divided into two groups based on approximate body length, such that both groups contained approximately the same distribution of fish sizes. The two groups (which would become the control group and the low-salinity group) were temporarily placed into separate 140 l coolers equipped with electric aerators, where they were housed for a 72-h period. During this time, food was withheld from all fish. Additionally, salinity in one of the two coolers was slowly lowered from 32 to 7‰ in ~4‰ increments through the addition of dechlorinated tap water every 12 h. After 72 h of fasting (and salinity acclimation for the eventual low-salinity group), fish were sedated using MS-222 mixed with aerated seawater (100 mg/l), weighed (blotted wet weight), and measured for standard length (SL) and total length (TL). Withholding food from fish prior to weighing minimized the effects of stomach contents on body mass.

Lionfish used in the long-term laboratory salinity trials ranged in size from 54 to 142 mm SL, with TL ranging from 77 to 188 mm (Online Resource 1). There was no difference in mean SL (± standard deviation) between the control group (95±31 mm) and the group that had been acclimated to a salinity of 7‰ (96±34 mm) (2-sample t-test: $t_{(12)}=0.08$, $p=0.94$; SPSS v.16). From these two groups of fish, we created eight approximately size-matched pairs. Within each pair, one fish was placed into a tank containing the high-salinity control treatment (35‰) and the other was placed into an adjacent tank containing the low-salinity treatment (7‰). Each set of paired tanks was randomly assigned a location on a bank of aquarium racks to minimize location-based effects.

Lionfish were observed three times per day (morning, midday, evening – 5 min per observation), and all behavioral changes that may have been an indication of stress were documented (e.g., decreased feeding compared to control fish, cessation of fin movements, loss of equilibrium, death). To maintain water quality, 40 % water changes were conducted every other day. Ammonia and nitrite levels were tested daily. During the first half of the study (day 1–16), fish were fed every 3 days. Due to high ammonia and nitrite levels, feeding frequency was reduced to every 4 days during the second half of the study (day 16–28) to improve water quality. Although several different types of food were offered to lionfish during this study (e.g., feeder guppies, feeder goldfish, feeder ghost shrimp), only one type of food was provided on each feeding day. Within lionfish pairs, both individuals were given approximately the same size prey item at each feeding to assure equal food intake. Since the lionfish we utilized encompassed a wide range of body sizes, we provided prey items that were ~1/4 to 1/3 of lionfish TL. Lionfish were weighed and measured (using above protocol) on day 16 and day 28. Fish were fasted for 72 h before being weighed. Two-sample t-tests were used to compare mean growth rates (changes in length and mass per day) between treatments (SPSS v.16).

At the culmination of the initial phase of the experiment (day 28), we began to slowly lower the salinity in each of the 7‰ treatment tanks in order to address objective 2. Our goal was to identify the survival salinity minimum (SS_{min} – the lowest salinity at which all individuals survive for 48 h) for lionfish that had already been acclimated to low salinities (7‰) for an extended period of time (Jian et al. 2003; Cheng et al. 2013). Salinity was lowered by 1‰ (over a 10 min period) every 48 h (Woo and Chung 1995) through the addition of deionized water buffered to a pH of 8.3 (Marine Buffer, Seachem Laboratories Inc. 0.02 g/l). All lionfish were observed three times per day (morning, midday, evening – 1 min per observation), in order to identify when an endpoint had been reached. The endpoint we originally planned to use for SS_{min} determination was complete loss of equilibrium in individual fish, as equilibrium loss has been observed to occur immediately before lionfish death during exposure to lethal salinities (Z. Jud, unpubl. data). Fish that had completely lost equilibrium were considered to be on the verge of death, at which point they were removed from the SS_{min} determination trial, and placed back into water with a salinity of 7‰, in order to determine whether fish in this condition would recover if salinities rapidly rose (as would occur during an incoming tide in a natural system). Once SS_{min} had been exceeded (i.e., at least one fish had lost equilibrium or died), we stopped reducing salinities in the treatment aquaria in order to determine how long the remaining lionfish could survive at salinity just below SS_{min}. While equilibrium loss was our intended endpoint, since five out of seven fish were found dead during daily observation periods, we used death as an endpoint in all but two cases. Upon completion of the study, all remaining fish were euthanized using MS-222 in aerated tank water (400 mg/l).

Results

Field observations

At all three study sites, salinities fluctuated with each tidal cycle, rising with the flood tide, and falling with the ebb tide (the estuary experiences semi-diurnal tides). Approximately 3 days after we initiated the in situ lionfish caging study, the Loxahatchee River watershed experienced a 2-day period of heavy precipitation, causing salinities in the estuary to suddenly decrease. At the downstream site, salinity at low tide briefly dropped to 8–10‰ on four occasions immediately following the precipitation event (Fig. 1a). However, high-tide salinities during this period were 32–33‰. By day 10 of the study (~7 days after the start of the precipitation event) salinities had risen back to pre-rainfall levels. For the remainder of the 40-day study, salinities at the downstream site fluctuated between 20 and 36‰. We did not observe any mortality or loss of equilibrium in the fish caged at this site, even during the initial period of reduced salinity (Fig. 1a).

Compared to the downstream site, the midstream site exhibited considerable salinity variation within each tidal cycle. For the first 3 days of the study (through the first day of the precipitation event), the daily salinity range at this site was 4–30‰ depending on tidal phase (Fig. 1b). No lionfish mortality or loss of equilibrium was observed at these salinities, but most individuals gravitated to the bottom of the cages. On days 4 and 5 of the study (during and shortly after the precipitation event), low-tide salinities fell below 2‰; however, high-tide salinities were 25–30‰ (Fig. 1b). We observed no mortality or loss of equilibrium, despite brief exposure to salinities below 2‰. On day 6 of the study, low-tide salinities dropped below 1‰, and all lionfish lost equilibrium (Fig. 1b). On the following day, all lionfish were dead.

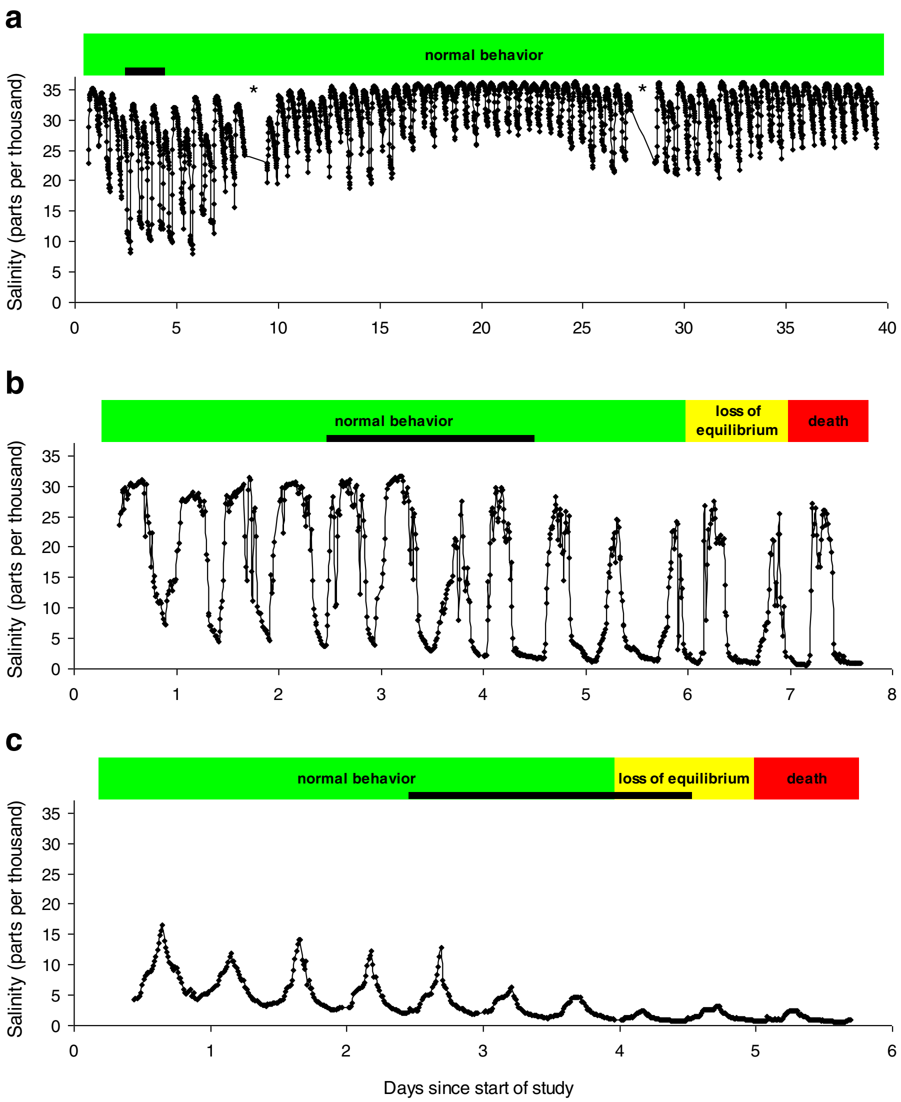

Fig. 1 Effects of fluctuating salinity on the survival of caged lionfish (*Pterois* spp.) at **a** downstream (2.4 km from ocean), **b** midstream (6.2 km from ocean), and **c** upstream (7.0 km from ocean) sites in the Loxahatchee River estuary. Salinity varied over time as a product of freshwater inflow (long term) and tidal incursion of marine water (twice daily). Estimated time of lionfish death is indicated in the bar across the top of each panel. Note that scale on the x-axis differs among panels. The period of heavy precipitation is indicated by a thick black bar at the top of each panel. *Asterisks* in (**a**) represent data gaps due to equipment malfunction

While the upstream site was in relatively close proximity to the midstream site, freshwater inflow had a greater effect at this location due to the nature of the river channel. Prior to the precipitation event (days 1 and 2 of the study), daily salinities at this site ranged from 2 to 16‰ depending on tidal phase (Fig. 1c). There was no lionfish mortality or loss of equilibrium observed during this period, although fish were primarily found in the lower portion of their cage. Salinities dropped rapidly during the third day of the study (the first day of the rain event), ranging from 6‰ at high tide to 1‰ at low tide. No mortality or equilibrium loss was observed during this 24-h period, despite salinities consistently below 6‰. By day 4 of the study, when low tide salinities fell below 1‰, all lionfish had lost equilibrium (Fig. 1c). All lionfish were dead by day 5.

Laboratory trials

During laboratory trials aimed at addressing objective 1, we demonstrated that lionfish were able to survive for extended periods of time at low salinities (7‰). Fifteen of 16 lionfish lived for the full 28-day duration of the study. One fish in the high-salinity (35‰) control treatment died on day 20. With this exception, we did not observe any behavioral changes that may have been an indication of stress in either the high-salinity (35‰) control treatment or the low-salinity (7‰) treatment (through day 25 – see below). During daily observations, all fish in both salinity treatments appeared active, either swimming around their tank, or resting on the bottom (or against the side glass) while exhibiting steady rhythmic movements of the caudal, anal, and soft dorsal fins (two behavioral patterns that we considered "normal behavior" based on numerous observations of unstressed lionfish in the wild and non-experimental aquarium settings).

Until day 25 of 28, all fish in both salinity treatments ate immediately when offered food. Prey items were typically consumed within ~5 s of being placed into the water. On day 25 (the final time food was offered during the experiment), one fish in the high-salinity control treatment, and one fish in the low-salinity treatment, did not feed. Both of these fish exhibited a reduced level of activity, and increased gill ventilation rates. Upon microscopic examination of skin smears and gill biopsies, we determined that both fish were infected by the parasitic dinoflagellate *Amyloodinium ocellatum*. It is possible that the observed changes in behavior and the failure to feed were a result of this infection. With the exception of the two fish with *A. ocellatum* infections, no other behaviors indicative of stress were observed through the culmination of the experiment on day 28. Both infected fish were euthanized on day 28.

For the first 16 days of the study, mean daily growth rate (± standard deviation) for length (SL) was identical between the high- and low-salinity treatment groups (0.13±0.06 mm/day, range 0.06–0.25 mm/day for both treatments). Additionally, there was no significant difference in mean daily growth rate for mass between the low-salinity treatment (0.10±0.15 g/day) and the high-salinity control treatment (0.03±0.06 g/day) during this time period (2-sample *t*-test: $t_{(9)}=1.33$, $p=0.22$).

Between day 16 and day 28, lionfish in both salinity treatments showed little change in length. Only three of eight fish in the low-salinity treatment and one of seven fish in the high-salinity control treatment increased in length during this period, but these length increases were very small (Online Resource 1). The four fish that increased in length were among the smallest individuals in the study. Length did not increase for the remaining 11 individuals. During this same period, 14 of the 15 remaining fish experienced a loss in mass (Online Resource 1). There was no significant difference in mean daily mass loss between the low-salinity treatment (−0.13±0.11 g/day) and the high-salinity control treatment (−0.14±0.26 g/day) during the final 12 days of the study (2-sample *t*-test: $t_{(12)}=0.08$, $p=0.94$). Fish continued to feed normally during this period (with the exception of two fish on day 25 – see above), but were fed less frequently as a means of improving water quality.

Starting on day 28, we began to slowly reduce salinities (in 1‰ increments every 48 h) in the tanks holding the seven remaining low-salinity treatment fish (fish that had been exposed to 7‰ for the previous 28 days) in order to identify the minimum salinity at which lionfish can survive for at least 48 h (objective 2). We did not observe any loss of equilibrium or death at salinities greater than 4‰. However, within 3 h of lowering salinities to 4‰, two lionfish began to exhibit a sudden and severe loss of equilibrium, a lack of response to tactile stimulation, and a reduction in the frequency of opercular movements (Fig. 2). Since these two fish had reached our predetermined endpoint for the study (i.e., they lost equilibrium), we culminated their trials, and returned them to a salinity of 7‰. Within 3 h, these fish had regained equilibrium, and were not exhibiting any behaviors indicative of stress. The remaining five lionfish did not exhibit acute signs of severe distress when salinities were lowered to 4‰. Three of these fish gradually became less active, eventually dying (without observed equilibrium loss) after 27–48 h of exposure to salinities of 4‰ (Fig. 2). The final two fish survived for 78 and 94 h at 4‰ before dying (without observed equilibrium loss). Since no loss of equilibrium or death was observed at salinities of 5‰ or greater, and all individuals reached an endpoint at 4‰, SS_{min} for lionfish appears to be ~5‰. Salinity tolerance did not appear to be affected by lionfish length within the size range we examined, as individuals of all lengths (56–146 mm SL) survived in salinities ≥5‰, but lost equilibrium or died at 4‰.

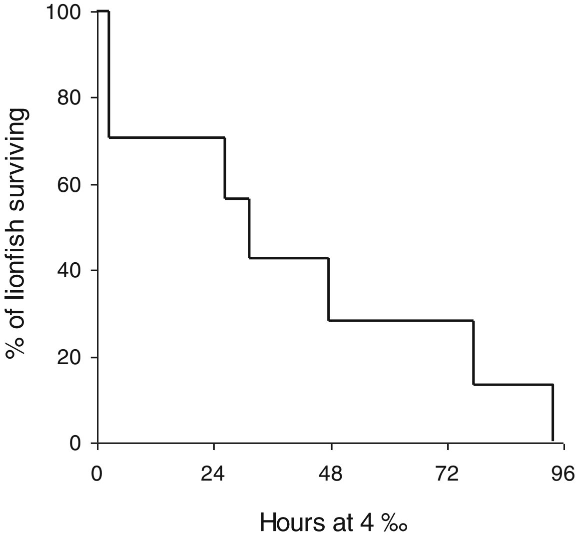

Fig. 2 Kaplan-Meier survival curve for lionfish exposed to salinities of 4‰. No mortality was observed at salinities ≥5‰, the approximate survival salinity minimum (SS_{min}) for lionfish. After 3 h at 4‰, two individuals experienced a complete loss of equilibrium (our predetermined endpoint for SS_{min} determination), combined with a lack of response to tactile stimulation, and a reduction in the frequency of opercular movements. These two individuals were included in the Kaplan-Meier curve, since their condition was an immediate precursor to death. All fish were dead after 94 h

Discussion

In addition to being habitat (Whitfield et al. 2002; Biggs and Olden 2011; Claydon et al. 2012) and dietary generalists (Albins and Hixon 2008; Morris and Akins 2009; Layman and Allgeier 2012; Valdez-Moreno et al. 2012; Côté et al. 2013b), lionfish appear to be able to tolerate a broad range of salinities. Although not typically considered a euryhaline species, our data suggest lionfish can survive at low salinities (7‰) for at least 1 month without exhibiting any obvious changes in behavior, feeding, or growth rate. While SS_{min} for lionfish appears to be ~5‰, ~70 % of the fish we tested in the laboratory survived at 4‰ for >24 h (up to 94 h for one individual). This, combined with our field observations, demonstrates the ability of lionfish to survive brief exposure to very low salinity conditions (i.e., ≤4‰). Fish that lost equilibrium at 4‰ in the laboratory recovered quickly when salinities were returned to 7‰ (similar to the salinity increases that can occur during incoming tides). In the wild, the influx of high-salinity water during the flood tide appears to allow lionfish to survive brief exposure to salinities as low as ~1‰ at low tide. At one of the estuarine sites in this study, lionfish experienced salinity fluctuations of ~28‰ every 6 h without any short-term (i.e., over several days) loss of equilibrium or mortality. These finding suggest that lionfish may be able to colonize all but the lowest-salinity sections of estuaries throughout the invaded range.

While the ability of lionfish to survive in low-salinity environments is a novel discovery, a number of marine species typically regarded as stenohaline have been shown to be able to tolerate relatively low salinities (Wu and Woo 1983; Lambert et al. 1994; Woo and Chung 1995; Jian et al. 2003; Lee et al. 2005; Cheng et al. 2013; García et al. 2013). In a study examining salinity tolerance in marine fishes, including taxa from seven coral reef-associated families, Wu and Woo (1983) found that 12 of 13 species could survive at salinities ≤10‰ for 2 weeks, with six of those species tolerating salinities ≤5‰. The emperor angelfish (*Pomacanthus imperator*), a reef-associated species that co-occurs with lionfish through much of the Indo-Pacific, can survive for a month at 7‰, and has a survival salinity minimum of 6‰ (Woo and Chung 1995), similar to our findings with lionfish in the Western Atlantic.

During the laboratory portion of this study, we failed to detect differences in growth between lionfish that had been exposed to high salinities (35‰) and those that had been exposed to low salinities (7‰), suggesting that any physiological costs associated with osmoregulation at 7‰ are insufficient to result in reduced growth (compared to 35‰). However, our ability to accurately compare growth rates between high-salinity and low-salinity treatments may have been hindered by our feeding regime in the laboratory. In particular, we were unable to feed lionfish to satiation, as this would have caused a degradation in water quality in the relatively small aquaria due to excess waste production. Our feeding regime during the first 2 weeks of the study led to growth in both salinity treatment groups, although growth rates were lower than values recorded in wild fish (Jud and Layman 2012). The losses in mass and minimal increases in length observed between day 16 and 28 (which were similar between the two salinity treatments) were likely caused by the reduced feeding frequency implemented during the second half of the experiment as a means of improving water quality. Since lionfish can survive for 3 months without food (Fishelson 1997), we were not concerned that our feeding regime would result in mortality for either group of fish (potentially confounding the effects of salinity).

The ability of lionfish to survive at low salinities may play an important role in shaping the eventual spatial extent of the invasion in the Western Hemisphere. While lionfish have spread rapidly throughout the Caribbean, Gulf of Mexico, and Northwest Atlantic, they have yet to colonize the coast of South America, south of the Amazon-Orinoco plume (AOP). The AOP has been proposed as a potential barrier to southward dispersal of lionfish (Côté et al. 2013a); however, our findings support the prediction of Luiz et al. (2013) that lionfish will eventually cross the AOP and spread along the Atlantic coast of South America. When exposed to reduced salinities in the wild, adult and post-settlement juvenile lionfish (demersal life history stages) have been observed utilizing benthic water layers (Jud et al. 2011), which typically have higher salinities than the overlying water column. The presence of a brackish layer of bottom water under the AOP would potentially allow post-settlement lionfish to traverse areas of low salinity created by the plume. However, the ability of pelagic eggs and larvae of lionfish to cross the low-salinity surface waters of the AOP is not known.

While the future establishment of lionfish south of the AOP is a likely scenario, a more pressing concern is identifying the distribution and impacts of lionfish that are currently utilizing estuarine ecosystems within the presently invaded range. Even as lionfish research has progressed at a rapid pace in other ecosystems, the inherent difficulties associated with detecting and observing lionfish in estuarine systems has hindered our understanding of this aspect of the invasion. There exists a paucity of data on habitat utilization by lionfish in their native range; however, individuals are occasionally captured in or near estuarine systems (Kulbicki et al. 2012). Prakash et al. (2012) have identified native *P. volitans* utilizing estuarine habitats in India; however, all occurrences were <2.3 km from the ocean, where salinities were >12‰. In contrast, we have demonstrated that lionfish from the invaded range can survive considerably further from the ocean, at much lower salinities. Because recreational SCUBA diving and snorkeling are not commonly carried out in estuaries (and the fact that visibility is often poor), we feel that the presence of lionfish in these ecosystems is likely being underreported. Two ecologically and economically important estuarine systems on the east coast of Florida—the Indian River Lagoon and Biscayne Bay—have already been documented to support populations of lionfish (Z. Jud, unpubl. data; E. Dark, unpubl. data). However, without increased efforts to identify lionfish in other invaded estuaries and document their effect on native estuarine organisms, we may fail to fully recognize the potential impacts of the lionfish invasion on these ecosystems.

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