Aquatic Invasions (2015) Volume 10, Issue 3: 333–337
doi: http://dx.doi.org/10.3391/ai.2015.10.3.08

Research Article

Survival and growth of invasive Indo-Pacific lionfish at low salinities

Pamela J. Schofield[1]*, Dane H. Huge[1,2], Troy C. Rezek[3], Daniel H. Slone[1] and James A. Morris Jr.[3]

[1]*US Geological Survey, 7920 NW 71st Street Gainesville, Florida 32653, USA*
[2]*University of Florida, School of Natural Resources and Environment, 103 Black Hall, Gainesville, Florida 32611, USA*
[3]*National Oceanic and Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Science, 101 Pivers Island Road, Beaufort, North Carolina 28516, USA*

**Corresponding author*

E-mail: lionfish@usgs.gov

Received: 2 May 2014 / Accepted: 24 April 2015 / Published online: 7 May 2015

Handling editor: Vadim Panov

Abstract

Invasive Indo-Pacific lionfish [*Pterois volitans* (Linnaeus, 1758) and *P. miles* (Bennett, 1828)] are now established throughout the Western North Atlantic. Several studies have documented negative effects of lionfish on marine fauna including significant changes to reef fish community composition. Established populations of lionfish have been documented in several estuaries, and there is concern that the species may invade other low-salinity environments where they could potentially affect native fauna. To gain a better understanding of their low-salinity tolerance, we exposed lionfish to four salinities [5, 10, 20 and 34 (control)]. No lionfish mortality was observed at salinities of 34, 20 or 10, but all fish died at salinity = 5 within 12 days. Lionfish survived for at least a month at a salinity of 10 and an average of about a week at 5. Fish started the experiment at an average mass of 127.9 g, which increased at a rate of 0.55 g per day while they were alive, regardless of salinity treatment. Our research indicated lionfish can survive salinities down to 5 for short periods and thus may penetrate and persist in a variety of estuarine habitats. Further study is needed on effects of salinity levels on early life stages (eggs, larvae).

Key words: growth, *Pterois volitans/miles*, salinity tolerance, survival

Introduction

Empirical derivation of physiological tolerances can document absolute range limits for survival as well as provide information on sub-lethal effects of environmental variables. For example, species living in favourable environmental conditions (e.g., optimal salinity or temperature) may have increased growth rates or fecundity compared to those living in suboptimal conditions. For marine and estuarine fishes, salinity is one of the most important environmental variables. Salinity affects many aspects of fish lifehistory, including: growth (Boeuf and Payan 2001); endocrinology of developing larvae (Hiroi et al. 1997); egg and larval survival (Holliday and Jones 1967); and others. Information on growth and survival of marine species along a salinity gradient can be used in models that forecast range expansions and population densities. These kinds of predictions are especially useful when applied to non-native species, whose spread across coastal habitats creates challenges for management.

This study focussed on lionfish [*Pterois volitans* (Linnaeus, 1758) and *P. miles* (Bennett, 1828)] growth and survival responses to a range of salinities. Lionfish were first found in Florida waters in the 1980s and have since spread along the Atlantic coast of the USA, throughout the Caribbean Sea, and in the Gulf of Mexico (Schofield 2009, 2010; Schofield et al. 2014a). The lionfish invasion is relatively recent; yet some studies document its negative effect on native fauna (e.g., Albins 2012; Green et al. 2012). While lionfish are typically considered a coral-reef species, they also occupy habitats such as seagrass meadows, mangrove habitats, and artificial habitats such as docks, artificial reefs, and bridge pilings (Barbour et al. 2010; Claydon et al. 2012; Schofield et al. 2014b). Recent articles have documented lionfish in low-salinity estuarine habitats in Florida (Jud et al. 2011; Jud and Layman 2012; Schofield et al. 2014a) and have suggested a broad salinity tolerance (Jud et al. 2014). Understanding how low salinities affect lionfish is an important part of understanding the species' ecology in estuarine habitats, as well as predicting potential negative effects on the native fauna.

A recent report provided field and laboratory data on lionfish low-salinity tolerance (Jud et al. 2014). In this study, we follow-up and extend those salinity tolerance trials by providing growth and survival rates for larger fish over an extended period of time and with more salinity treatments.

Methods

Fish collection and experimental setup

Lionfish were collected during the summer of 2012 from the Florida Keys and off the North Carolina coast. Lionfish collected from North Carolina waters were transported directly to the National Oceanic and Atmospheric Administration (NOAA), Center for Coastal Fisheries and Habitat Research (CCFHR) in Beaufort, North Carolina. Lionfish collected from the Florida Keys were held in a flow-through aquaculture system before being shipped overnight to CCFHR. All fish were placed in a 5,000 L recirculating aquaculture system where they were prophylactically treated for 24 hours with 30 ppm formalin then for 10 days with $CuSO_4$ (0.15 ppm) to eliminate potential protozoan parasites (e.g. *Cryptocaryon irritans* and *Amyloodinium ocellatum*).

The experimental system used in this study consisted of four independent recirculating systems ("units"), each set at a different salinity. Each of the four units consisted of four 100 L cylindro-concial tanks (56 × 42 cm) and a 170 L sump for a total system volume of 570 L. Each unit was equipped with a one cubic-foot bubble-washed bead filter (Aquaculture Systems Technology LLC, LA USA) for biological and mechanical filtration, a 25 watt UV sterilizer (Emperor Aquatics Inc., PA, USA), and a magnetic drive water pump operating at 1200 gallons per hour (Danner Manufacturing Inc., NY, USA). Ambient lighting was provided by fluorescent lights on a timer with a photoperiod of 12L:12D (Biswas et al. 2005). Temperature was maintained by ambient room temperature and a 50 watt heater placed in the sump of each system. Aeration was provided to each tank by a linear diaphragm air pump (Pentair AES, FL, USA) through a single fine-pore air diffuser (2.5 cm).

Temperature, pH, dissolved oxygen (DO), and salinity were measured daily (one tank per day for each treatment, rotating tank numbers each day) with a YSI Pro 230 (YSI Inc., OH, USA).

Salinity challenge

The four independently-recirculating units provided 16 tanks for the experiment. Salinity for all four units was initially set at 34 and one fish was placed in each tank (n = 16 total fish). Fish were acclimated to the experimental tanks at 34 for 12 days, during which time they appeared healthy and readily consumed food [dead *Menidia menidia* (Linnaeus, 1766)] offered daily. After the equilibration period, each unit was gradually shifted (about 1.0 salinity unit per day) to one of four salinities [5, 10, 20 and 34 (control)]. Thus, each treatment was replicated on four fish.

Fish were initially placed in tanks on 30 January 2013, and we began to lower salinities on 12 February. All fish were measured (standard length [SL], total length [TL] ± 0.5 cm and mass ± 0.5 g) on 31 January, 1 March, 14 March, 1 April, and 15 April. Fish that died were measured the day they expired. To measure live fish, individuals were collected from each tank and anesthetized using Tricaine-S (Western Chemical Inc., WA USA) at a concentration of 150 ppm. After measurements were taken, fish were immediately returned to their tanks where they began feeding within eight hours.

One-way analysis of variance (ANOVA) was used in SPSS version 13.0 to determine whether lionfish differed in initial mass, as well as whether environmental conditions (DO, temperature, pH) differed between treatments. Levene's test was used to check for heteroscedasticity. When variances were homogenous, we used Scheffé's *post-hoc* test to compare treatments, and when variances were not homogenous we used Dunnett's T3 test. A Kaplan-Meier survival estimator was used to estimate life expectancy (Kaplan and Meier 1958). Growth (grams per day increase in mass and centimeters per day increase in SL) of the fish was analyzed with a joint linear mixed model using Jointlcmm (package lcmm; Proust-Lima et al. 2015) in R (version 3.1.0; R core team 2014). Candidate models included time (day) and salinity as linear predictors for growth, and salinity treatment was the predictor for survival.

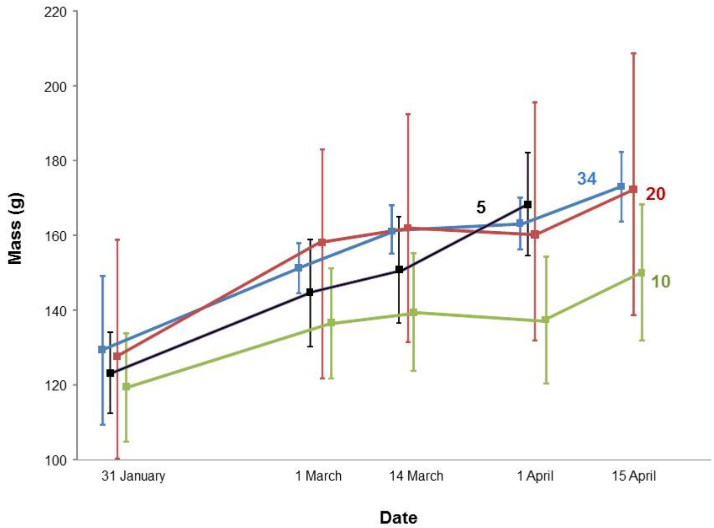

Figure 1. Mass of lionfish during the salinity-tolerance experiment. Each line denotes a specific treatment (5, 10, 20 and 34). Each marker is the mean value for the four fish in that treatment. Error bars are ± 1 SE. While all fish were measured on the same day, markers are slightly offset to better visualise error bars.

A subject indicator was included in all models to control for repeated measurements. Models were fit with maximum likelihood estimators, and the best overall model for each growth variable was selected with AIC (Akaike 1974). The time parameter from the final model was used to determine unbiased estimates of the gain in mass and length of the fish per day (mean ± SE) that accounted for mortality.

Results and discussion

Lionfish mass did not differ (ANOVA, $P = 0.56$) across treatments at the beginning of the experiment (mean ±SD = 131.2±34.2 g; range 58.5–202.0 g). Fish used in the experiment ranged from 14.0–18.5 cm SL (mean ± SD =15.6±1.3 cm) which is equivalent to 19.0–25.0 cm TL (mean ± SD = 21.3±1.5 cm) at the beginning of the experiment. Dissolved oxygen, temperature and pH all varied significantly among treatments. However, these parameters were maintained within acceptable ranges (Hoff 1996; Timmons 2002): DO never dropped below 6.5 mg L^{-1}; temperature only varied 3.5 °C over the course of the experiment (range 22.2 to 25.8 °C); and the mean ± SD pH was 8.2 ± 0.29.

All individuals in salinity treatments of 10, 20 and 34 were alive and feeding at the completion of the experiment. The overall experiment ran for a fixed length of time (62 days, not counting the equilibration period), but because lower salinity treatments required longer acclimation periods, the "time at salinity" was shorter at lower salinities. Thus, time for fish at each salinity was: 62 days for the fish at 34; 46 days for fish at 20; and 32 days for fish at 10. All lionfish in the salinity = 5 treatment died at 4, 5, 5 and 12 days after reaching the target salinity. The Kaplan-Meier estimator for survival at salinity 5 was 6.5 days (95% confidence interval = 2.9–10.1 days).

The fish in our study were significantly larger than those used by Jud et al. (2014); however, our results are comparable. Jud et al. (2014) documented survival of lionfish (7.7–18.8 cm TL) for 28 days at a salinity of 7, and we showed that larger lionfish (19.0–25.0 cm TL) could survive for at least one month at salinity of 10 but died in about a week when salinities were reduced to 5. Methodologies of the two studies (ours and Jud et al. 2014) were slightly different. In Jud et al. (2014), salinities were slowly lowered from 7 to 4 (the lowest salinity they could tolerate before losing equilibrium or dying) by decreasing salinities by 1 every 48 hrs. For our experiment, salinity was lowered by about 1 every 24 hrs until the target salinity was reached. After that, fish were maintained at target salinities. Both showed lionfish exhibit tolerance to low-salinity waters.

All individuals gained weight during our experiment (Figure 1) and at a higher rate than those of Jud et al. (2014; mean = 0.55 g per day vs. 0.03 or 0.10 g per day). Moreover, our fish continued to grow throughout the experiment while fish in the first study lost mass during the final two weeks (Jud et al. 2014), probably due to reduction in feeding during that time. All of our individuals also either stayed the same length or grew up to 2.5 cm SL during the experiment (mean ± SE = 0.016 ± 0.003 cm SL day^{-1}). Thus, while we are able to say that fish grew at salinity of 10 for at least a month (this study), it is unclear whether this threshold could be extended to apply to fish maintained at a salinity of 7 with our larger fish. The difference between 7 and 10 may be biologically insignificant, as tidal fluctuation can create salinity differentials of 1 to 35 within estuaries over a few hours. Additionally, both studies showed that lionfish are capable of short-term survival at salinities of 5 (this study) and 4 (Jud et al. 2014). In the field, these short time-frames may be sufficient to allow lionfish the opportunity to relocate to an area with a more tolerable salinity regime or withstand tidal fluctuations without moving.

In some marine fishes, intermediate salinities (8–20) have positive effects on growth. Reduced salinities are often correlated with lower metabolic rates (due to less osmoregulatory demand) and increase food conversion rates (Lambert et al. 1994; Boeuf and Payan 2001; Laiz-Carrión et al. 2005). However, when salinities are reduced too far, conditions can become stressful as more energy must be spent on osmoregulation, and growth rates decrease (e.g., Sampaio and Bianchini 2002). In our study, growth rates were similar across all treatments (salinity was non-significant when included in growth model, and AIC for model without salinity was better), showing that neither reduced growth rates from stress nor increased growth rates from better feed conversion ratios were measurable in lionfish. Similar results were found for rabbitfish *Siganus rivulatus*, that grew equally well over a range of salinities from 15 to 40 (Saoud et al. 2007).

It is now clear that lionfish from 7.7–25.0 cm TL are capable of tolerating low salinities for prolonged periods when salinities are slowly shifted over time (Jud et al. 2014; this study). This corroborates with field evidence (Jud et al. 2011) and leads to increased concern over their colonization of low-salinity habitats. To obtain a complete assessment of the ability of lionfish to use estuarine habitats throughout their life cycle, further research on the effects of salinity on early life stages (eggs, larvae) is necessary.

Acknowledgements

Support for this study was provided by the US Geological Survey's Invasive Species Program, Southeast Ecological Science Center and the Southeastern Region. Support was also provided through the National Oceanic and Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Science. We thank the staff at the NOAA Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina for laboratory support in performing the salinity tolerance experiments. This research was conducted under Institutional Animal Care and Use Committee permit USGS/SESC 2011-07 (2013 extension). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

References

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723, http://dx.doi.org/10.1109/TAC.1974.1100705

Albins MA (2012) Effects of invasive Pacific red lionfish *Pterois volitans* versus a native predator on Bahamian coral-reef fish communities. *Biological Invasions* 15: 29–43, http://dx.doi.org/10.1007/s10530-012-0266-1

Barbour AB, Montgomery ML, Adamson AA, Diaz-Ferguson E, Silliman BR (2010) Mangrove use by the invasive lionfish, *Pterois volitans*. *Marine Ecology Progress Series* 401: 291–294, http://dx.doi.org/10.3354/meps08373

Biswas AK, Seoka M, Inoue Y, Takii K, Kumai H (2005) Photo-period influences growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*). *Aquaculture* 250: 666–673, http://dx.doi.org/10.1016/j.aquaculture.2005.04.047

Boeuf G, Payan P (2001) How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part* C 130: 411–423

Claydon JAB, Calosso MC, Traiger SB (2012) Progression of lionfish invasion in seagrass, mangrove and reef habitats. *Marine Ecology Progress Series* 448: 119–129, http://dx.doi.org/10.3354/meps09534

Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive lionfish drive Atlantic coral reef fish declines. *PLoS ONE* 7: e32596, http://dx.doi.org/10.1371/journal.pone.0032596

Hiroi J, Sakakura Y, Tagawa M, Seikai T, Tanaka M (1997) Developmental changes in low-salinity tolerance and responses of prolactin, cortisol and thyroid hormones to low-salinity environment in larvae and juveniles of Japanese flounder *Paralichthys olivaceus*. *Zoological Science* 14: 987–992, http://dx.doi.org/10.2108/zsj.14.987

Hoff FH (1996) Conditioning, Spawning and Rearing of Fish with Emphasis on Marine Clownfish. Aquaculture Consultants Inc, Dade City, Florida, 212 pp

Holliday FGT, Jones MPJ (1967) Some effects of salinity on the developing eggs and larvae of the plaice (*Pleuronectes platessa*). *Journal of the Marine Biological Association of the United Kingdom* 47: 39–48, http://dx.doi.org/10.1017/S0025315400033543

Jud ZR, Layman CA, Lee LA, Arrington DA (2011) Recent invasion of a Florida (USA) estuarine system by lionfish *Pterois volitans/miles*. *Aquatic Biology* 13: 21–26, http://dx.doi.org/10.3354/ab00351

Jud ZR, Layman CA (2012) Site fidelity and movement patterns of invasive lionfish, *Pterois* spp., in a Florida estuary. *Journal of Experimental Marine Biology and Ecology* 414–415: 74, http://dx.doi.org/10.1016/j.jembe.2012.01.015

Jud ZR, Nichols PK, Layman CA (2014) Broad salinity tolerance in the invasive lionfish *Pterois* spp. may facilitate estuarine colonization. *Environmental Biology of Fishes* 98: 135–143, http://dx.doi.org/10.1007/s10641-014-0242-y

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* 53: 188–193, http://dx.doi.org/10.1080/01621459.1958.10501452

Laiz-Carrión R, Sangiao-Alvarellos S, Guzmán JM, Martín del Río MP, Soengas JL, Mancera JM (2005) Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: Implications for osmoregulation and energy metabolism. *Aquaculture* 250: 849–861, http://dx.doi.org/10.1016/j.aquaculture.2005.05.021

Lambert Y, Dutil J-D, Munro J (1994) Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 51: 1569–1576, http://dx.doi.org/10.1139/f94-155

Proust-Lima C, Philipps V, Liquet B (2015) Estimation of extended mixed models using latent classes and latent processes: the R package lcmm. arXiv:1503.00890 [stat]. http://arxiv.org/abs/1503.00890 (Accessed 16 Mar 2015)

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Saoud IP, Kreydiyyeh S, Chalfoun A, Fakih M (2007) Influence of salinity on survival, growth, plasma osmolality and gill Na+-K+-ATPase activity in the rabbitfish *Signaus rivulatus*. *Journal of Experimental Marine Biology and Ecology* 348: 183–190, http://dx.doi.org/10.1016/j.jembe.2007.05.005

Sampaio LA, Bianchini A (2002) Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. *Journal of Experimental Marine Biology and Ecology* 269: 187–196, http://dx.doi.org/10.1016/S0022-0981(01)00395-1

Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828] in the Western North Atlantic and Caribbean Sea. *Aquatic Invasions* 4: 473–479, http://dx.doi.org/10.3391/ai.2009.4.3.5

Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans/miles*) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. *Aquatic Invasions* 5: 117–122, http://dx.doi.org/10.3391/ai.2010.5.S1.024

Schofield PJ, Langston JN, Morris JA, Jr, Fuller P (2014a) *Pterois volitans/miles* FactSheet. USGS Nonindigenous Aquatic Species Database, Gainesville, FL, http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=963 (Accessed 16 Sept. 2014)

Schofield PJ, Akins L, Gregoire-Lucente DR, Pawlitz RJ (2014b) Invasive lionfish use a diversity of habitats in Florida. U.S. Geological Survey Fact Sheet 2014–3032, http://dx.doi.org/10.3133/fs20143032

Timmons MB, Ebeling JM, Wheaton FW, Summerfelt ST, Vinci BJ (2002) Recirculating Aquaculture Systems. Cayuga Aqua Ventures, Ithaca, New York, 769 pp