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# Low salinity negatively affects metabolic rate, food consumption, digestion and growth in invasive lionfish *Pterois* spp.

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ABSTRACT: The establishment of the piscivorous lionfish *Pterois* spp. in the Western Atlantic and wider Caribbean is a well-documented example of a successful marine invasion. Recently, lionfish have been shown to colonise a wide range of ecosystems and tolerate a wider range of salinities than previously thought. In the present study, lionfish were maintained in aquaria under differing salinity treatments (10, 20 and 37 psu) similar to those they might experience in an estuarine ecosystem. The effects of long-term hyposaline exposure on growth, metabolic rate, maximum food consumption and digestion were examined. Consistent with previous studies, lionfish were able to survive in hyposaline conditions for extended periods of time. However, lionfish in the most hyposaline treatment (10 psu) exhibited reduced growth under low food conditions, lower maximum metabolic rate, lower aerobic scope, lower maximum food consumption, took longer to digest a standardized meal size and occupied a greater percentage of their aerobic scope during digestion. Results suggest that (1) given the ability of lionfish to tolerate low salinity, updated range expansion models should incorporate salinity data to improve accuracy of predicted range expansion and (2) the invasion of lionfish into low salinity ecosystems, although a serious concern, will not likely lead to the same level of population increase observed for coral reef habitats due to the physiological costs associated with living in low salinities.

KEY WORDS: Back-reef ecosystems · Invasive species · Mangroves · *Pterois miles* · *Pterois volitans* · Salinity



## 1. INTRODUCTION

The establishment of the piscivorous lionfish (*Pterois miles* and *Pterois volitans*, Wilcox et al. 2018; hereafter referred to simply as lionfish, *Pterois* spp.) in the Western Atlantic and wider Caribbean (Bax et al. 2003, Whitfield & Hare 2003, Snyder & Burgess 2007) is a well-documented example of a successful marine invasion (Côté & Smith 2018). The introduction is thought to be the result of intentional (human mediated) releases, with the first confirmed sightings of lionfish in the Western Atlantic in Florida, USA, in 1985 (Whitfield & Hare 2003, Morris & Akins 2009). Thereafter, the spread was likely facilitated naturally through larval dispersal, with lionfish reaching the Bahamian archipelago in 2004 (Schofield 2009). Since then, lionfish have become established in the waters of every island nation in the wider Caribbean and much of the Central and South American coasts (Schofield 2009, Côté et al. 2013), with individuals recorded as far south as the Atlantic coast of Brazil (Ferreira et al. 2015).

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Lionfish in their invaded range can be found at densities and body sizes that far surpass that of their native range counterparts in the Indian Ocean and Red Sea (Côté et al. 2013), due to a lack of predators and higher survival rates from egg to adult. Paired with voracious feeding habits (Côté et al. 2013) and the ability to exploit the naivety of native reef fish (McCormick & Allan 2016), these efficient predators significantly reduce the recruitment and biomass of native reef fish and invertebrates (Albins & Hixon 2008, Green et al. 2012). In a controlled field experiment on patch reefs in The Bahamas, Albins & Hixon (2008) found that lionfish at high densities were responsible, on average, for a 79% reduction in the recruitment of native fish and invertebrate species in just 5 wk. In a similar study but over a larger spatial scale, Albins (2015) found a 46% reduction in total native reef fish densities.

Recently, it has become evident that lionfish are able to colonise a wide variety of habitats including mesophotic reefs at depths >300 m (Albins & Hixon 2013), sea grass beds <1 m (Albins & Hixon 2013) and even hyposaline ecosystems (Barbour et al. 2010, Jud & Layman 2012, Jud et al. 2015). Hyposaline ecosystems such as coastal estuaries are often critical nursery and developmental grounds for fish and invertebrates, and provide a net export of economically important species, regionally and internationally (Faunce & Serafy 2006). For example, spiny lobster *Panulirus argus* and stone crab *Menippe mercenaria* larvae settle from free-floating plankton stages into coastal estuaries and reside there until they mature before migrating out onto the reefs (Dahlgren & Marr 2004). The critically threatened and culturally and commercially important queen conch *Lobatus gigas* and juvenile Nassau grouper *Epinephelus striatus* rely heavily on estuarine habitats for food and shelter (Dahlgren & Marr 2004). Therefore, any change in the trophic balance of these ecosystems could cause significant declines in many culturally, economically, recreationally and ecologically important species.

Lionfish have been found to tolerate a wider range of salinities than originally thought (Jud et al. 2015), indicating that they may have a greater capacity for range expansion than previously anticipated. Jud et al. (2011) were among the first to observe the presence of lionfish in hyposaline environments as far as 6.6 km from the ocean, and experimentally demonstrated the salinity tolerance of lionfish — with 15 of 16 individuals surviving for more than 28 d in a salinity of 7 psu, whereas salinities in typical marine reef environments in the Caribbean range from 35–37 psu (Jud et al. 2015).

It might be expected that fish in brackish waters would have a lower energetic cost associated with osmoregulation compared with those in salt or freshwater because the osmolality of the body fluids are closer to that of the external environment (Boeuf & Payan 2001). In many euryhaline fish species it has been suggested that a period of 3–12 d is needed to acclimate from salt to freshwater (Ferraris et al. 1988, Nonnotte & Truchot 1990, Jensen et al. 2002, Sampaio & Bianchini 2002). Therefore, a species' success in utilizing variable salinity habitats is dependent on the timing and magnitude of salinity change and the ability to balance energetic needs with biological function.

While lionfish have been observed to survive hyposaline environments, the bioenergetic costs (or benefits) of surviving these conditions has not been well explored. The measurement of metabolic rate — standard metabolic rate (SMR), the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state (Chabot et al. 2016); maximal metabolic rate (MMR), the rate of oxygen consumption during the maximum sustainable rate of exercise; and aerobic scope (AS), the difference between an animal's SMR and MMR, thus defining the capacity of an animal to increase its rate of aerobic metabolism (Norin et al. 2014) — and changes thereof, are important processes to assess the energetic reactions of organisms. Together, these metabolic traits (SMR, MMR and AS) make up what is termed the metabolic phenotype of an individual, and are important factors associated with organism homeostasis, food consumption, digestion (specific dynamic action [SDA]: the peak in oxygen consumption following a meal, so that SDA defines the feeding capacity of an animal) and the anabolism and catabolism of tissues (growth) (Metcalfe et al. 2016).

The effect of salinity on the growth of juvenile and adult fish has been studied in marine (e.g. Atlantic cod *Gadus morhua*; Boeuf & Payan 2001) and freshwater species (e.g. naked carp *Gymnocypris przewalskii*; Wood et al. 2007). As growth is continuous in fish, it is predicted to be one of the first processes affected during stressful environmental situations (Boeuf & Payan 2001). Higher growth rate is often observed at intermediate salinity (Boeuf & Payan 2001). In most cases this occurs because of a decreased osmotic gradient, subsequently reducing the metabolic cost of osmoregulation, and potentially an increased scope for digestive capacity. Even species that are considered 'true' marine species, such as *G. morhua* or turbot *Scophthalmus maximus*, increase growth rates significantly at intermediate salinity conditions of 12–

19 psu (Lambert et al. 1994, Gaumet et al. 1995, Dutil et al. 1997, Boeuf & Payan 2001, Imsland et al. 2001). As lionfishes are typically considered true marine species, understanding their growth and physiological capacity in hyposaline conditions, such as those often experienced in estuarine ecosystems, is pivotal to understanding impacts of lionfish in these ecosystems.

The present study used a series of laboratory experiments to investigate the effect of lowered salinity on lionfish growth, metabolic rate, maximum food consumption and digestion. As previous research has indicated that lionfish are able to survive in low salinities for extended periods, our objectives were 3-fold: (1) to calculate the daily growth rates of lionfish acclimated to different salinity treatments during a period of both low and high food availability; (2) to determine how metabolic rate (SMR, MMR and AS) varied under different salinity treatments; and (3) to examine how food consumption and digestion varied under different salinity treatments.

## 2. MATERIALS AND METHODS

### 2.1. Animal capture and transport

Lionfish (n = 66, 11 per aquarium; mean ± SD mass: 110.2 ± 76.6 g; standard length: 149.6 ± 28.5 mm) were collected by divers on SCUBA using clear plastic hand nets from patch reefs (~3 m depth) located adjacent to the Cape Eleuthera Institute (CEI), The Bahamas (24° 50′ N, 76° 20′ W). Once captured, lionfish were transported by boat in large coolers filled with seawater to aquarium holding facilities at CEI. An airstone was placed in the cooler and the seawater was changed periodically to ensure adequate dissolved oxygen during transport. All fish were anaesthetised using clove oil (20 mg $l^{-1}$) (NRC 2010), measured (standard length to the nearest mm), weighed (to the nearest 0.1 g) and externally tagged (T-bar anchor tag, model no. FD-68B FF; Floy tag) in the dorsal musculature so that fish could be individually identified.

### 2.2. Lionfish husbandry and laboratory settling

Lionfish (n = 66, 11 per aquarium) were equally divided across six 750 l aquaria (160 cm diameter × 60 cm depth), shaded under a large canopy and supplied with flow-through seawater at ambient temperature (25.0 ± 2.7°C) and salinity (37.0 ± 2.3 psu) and maintained on a natural day/night cycle (Fig. 1). Aquarium temperature, salinity and water flow were checked daily. Aquaria were cleaned using a vacuum siphon and scrub brush to remove any debris accumulating on the bottom. All lionfish were left for 1 wk after capture and tagging to allow for settling and recovery.

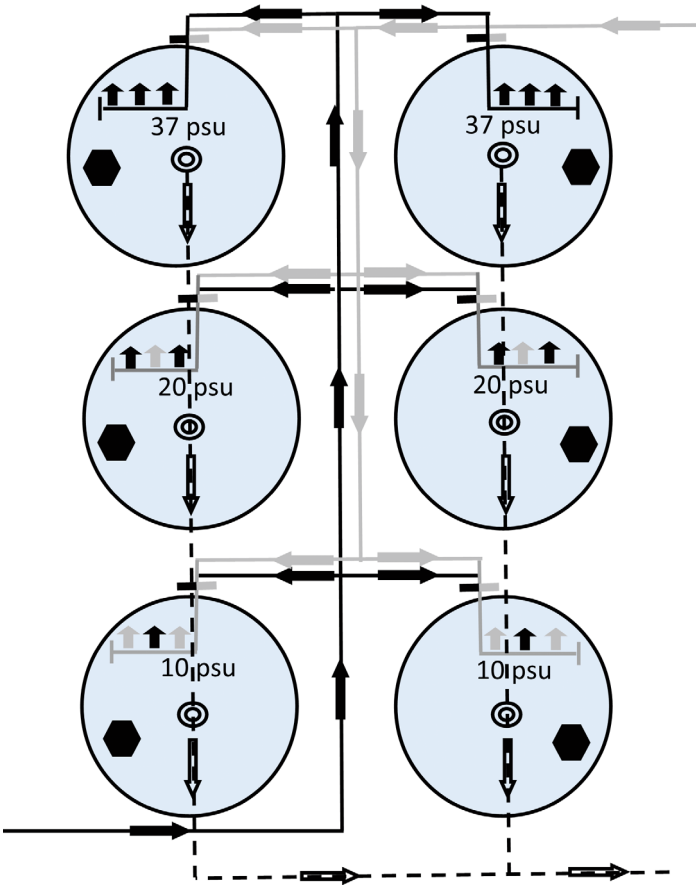


Fig. 1. Experimental setup used to maintain lionfish (n = 66; 11 per aquarium) at 3 salinity treatments (37, 20 and 10 psu). Aquaria (750 l; large blue circles) contained a standpipe (small open circles) to which waste water flowed during water changes. Lines and arrows: piping and direction of flow for fresh water (light grey), salt water (black) and waste water (dashed line, open arrow). Fresh water was pumped from an artesian well; salt water was pumped directly from Exuma Sound, adjacent to The Cape Eleuthera Institute. Salinity treatments were achieved by adjusting 2 valves on each aquarium (small black/grey bars); one that supplied salt water and one that supplied fresh water. Continuous aeration (solid black hexagon) was added to ensure oxygen saturation and to further ensure constant mixing

### 2.3. Salinity treatments

Following the settling and recovery period in ambient seawater (37 psu), lionfish were acclimated for 1 mo prior to experimentation to 3 salinity treatments

(low: 10 psu; intermediate: 20 psu; control: 37 psu), in the same aquaria as those described in Section 2.2 (Fig. 1). Due to a shortage of fresh water, all aquaria (2 per treatment; n = 6) were static (as opposed to continuous flow-through). However, daily flow-through water changes, using water of the desired treatment salinity and temperature, were conducted to maintain water quality. Salinities of the treatment aquaria were lowered over 5 d (by 5 psu every 24 h between 27 February and 3 March 2017) by gradual addition of fresh water throughout the day until the desired salinities were achieved (Fig. 1). Thereafter, water in the aquaria was maintained at the correct salinities for the remainder of the experiment. Adequate mixing was achieved using an air-stone and by ensuring the fresh water and salt water input was located adjacent to the bottom of the aquaria (Fig. 1). The salinity and temperature of the in-flowing seawater and fresh water were measured prior to water changes to ensure minimal disturbance. Waste water flowed out through a standpipe located in each aquarium (Fig. 1). Salinity and temperature were checked daily using a refractometer calibrated using distilled water and a digital thermometer located in each aquarium, respectively.

### 2.4. Lionfish feeding

All lionfish were first fed live Atlantic silversides *Menidia menidia* at (1) low food availability (1.5% of individual lionfish's body mass every 4 d from 17 March 2017 to 10 April 2017 [24 d]; 'low-food growth' portion of the experiment) and then (2) high food availability (fed *ad libitum* daily from 7 May to 24 May 2017 (17 d); 'high-food growth' portion of the experiment). Each lionfish was used as a repeated measure for both feeding trials due to the length of time required for acclimation to salinity treatments and captive feeding. During feeding, individual lionfish were removed from their holding aquarium using plastic hand nets, identified by their external tag and placed in a feeding arena (63 × 40 × 35 cm plastic container) floating within the main aquarium. Individuals from each of the 6 holding aquaria were fed individually. This ensured that all lionfish consumed their allocated ration (1.5% body mass) without competition and disturbance from conspecifics during the low food availability feeding. Although netting disturbance was not specifically tested as an effect on lionfish feeding, individual fish at each treatment level were handled in a similar manner, thus standardizing handling across all 3 treatments. Also, given that all fish ate consistently throughout the duration of the experiment, we assumed that any disturbances were minimal, as feeding and activity often ceases in stressed fish (Schreck et al. 1997, Wendelaar Bonga 1997, Santos et al. 2010). Once a lionfish consumed their allocated ration, it was removed and placed in a temporary post-feeding arena (63 × 40 × 35 cm plastic container with holes, floating in their respective treatment aquarium) to further minimize repeat handling. After feeding, all lionfish were released from the temporary post-feeding arena. The time taken for each lionfish to consume all prey offered (in min) and the amount of prey consumed (in g) were recorded. Preliminary experiments determined that lionfish would normally consume all prey offered during the low-food feeding within a 5 min period. Maximum food consumption was determined using the same methods, although there was no time limit and prey were introduced first at 5% of the fish's body mass. Thereafter, silverside prey were subsequently added until signs of satiation were observed (food was regurgitated, lionfish showed no predatory behavioural displays [e.g. fin display] or appeared uninterested for a continuous 5 min period). Once lionfish were satiated, any remaining prey were removed from the arena, blotted dry, weighed, and subtracted from the total mass of prey given so that maximum food consumption could be calculated.

### 2.5. Oxygen consumption measurements

After at least 1 mo of acclimation in their respective salinity conditions and immediately after the low-food growth trials, oxygen consumption rates of the lionfish were measured (see metabolism and digestion-related parameters for specific measurements; Sections 2.6–2.8). Fish used in the respirometry experiments were fasted for at least 48 h prior to measurements, to ensure sufficient time for gut clearance and to abolish any effects associated with assimilation of a previous meal. A total of 8 separate plastic respirometry chambers (23 × 19 × 22 cm; chamber volume: 9614 ml) were submersed in a water bath maintained at the respective acclimation salinity and temperature. Therefore, respirometry could be conducted concurrently on 8 lionfish per salinity treatment. An air-stone in the water bath ensured adequate dissolved oxygen levels. Oxygen consumption was measured using intermittent flow-through respirometry (Steffensen 1989) and AutoResp software (AutoResp; Loligo Systems). Briefly, this fully auto-

mated system is equipped with 2 pumps; the first pump continually flushes seawater through the chamber to ensure adequate water saturation during the non-measurement phase. For measurements, the flush pump is turned off using the AutoResp software, and the chamber is sealed while a second pump re-circulates the water through the chamber ensuring that oxygen gradients do not build up within the chamber. Oxygen consumption ($\dot{M}O_2$) was calculated during the decline in oxygen levels caused by the lionfish respiring (oxygen saturation in the chambers was monitored and never dropped below 90% $O_2$) while the chamber was sealed, then the chamber was continuously flushed between readings. Oxygen concentration in the chamber was measured using one of 8 fibre-optic oxygen probes (Witrox 1; Loligo Systems) every 1 s for 20 min. Oxygen probes were calibrated using a sodium sulphite solution (0% $O_2$) and air-saturated water (100% $O_2$). Baseline oxygen concentration was corrected for ambient temperature, salinity and barometric pressure, which were inputted into the software prior to the respirometry trial. The rate of oxygen consumption was determined using the following equation (Ege & Krogh 1914):

$$\dot{M}O_2 = \frac{V_w \cdot \Delta C_w O_2}{\Delta t} \quad (1)$$

where $V_w$ is the volume of water in the respirometer, $\Delta C_w O_2$ is the change in oxygen concentration of the water and $\Delta t$ is the time period associated with the drop in oxygen concentration in the respirometer (Steffensen 1989). The coefficients of determination ($r^2$) for all slope measurements (oxygen concentration vs. time) were greater than 0.95. The effect of background levels of oxygen consumption (e.g. by bacteria in the water) for each specific fish and chamber were corrected by measuring the temporal change in oxygen concentration over 20 min, prior to the addition of fish at the beginning and end of each trial.

### 2.6. Measuring MMR

To determine MMR, individual fish (n = 11–16; see Table 1) were haphazardly captured from their treatment aquaria and sequentially subjected to an exhaustive chase protocol (Reidy et al. 1995, Killen et al. 2010, Norin et al. 2014), whereby a single fish was introduced into a rectangular arena (60 l) and hand-chased with a small net to exhaustion. Exhaustion behaviour involved the fish having ceased swimming when touched and generally occurred after 4 min. Once exhausted, individual lionfish were immediately placed into a plastic respirometry chamber and their oxygen consumption measured as detailed in Section 2.5.

### 2.7. SMR and determination of AS

Once MMR measurements had been completed, the same fish were left to settle in respirometry chambers for 24 h to allow for measurements of oxygen consumption (as detailed in Section 2.5) at rest (i.e. SMR). A plastic divider was placed between each respirometer to prevent visual contact between individual fish during measurements. Human disturbance through noise and entry to the respirometry laboratory was kept to a minimum. Whole-animal SMRs (mg $O_2$ $h^{-1}$) were calculated as the $10^{th}$ percentile of all $\dot{M}O_2$ measures taken throughout the 24 h measurement period (Steffensen 1989, Chabot et al. 2016). AS was calculated post experiment as the difference between MMR and SMR.

### 2.8. SDA

Once MMR and SMR measurements had been completed, lionfish (n = 11–16; see Table 1) were fed 1.5% of their body mass of live silversides inside the respirometer. Live silverside prey were introduced through a porthole in the respirometer chamber, which was plugged with a rubber stopper thereafter. During feeding, the flush pump and recirculation pump were stopped to prevent silversides from entering the respirometry tubing. All meals were generally consumed within 2 min of being introduced. Lionfish that did not consume all the food offered whilst in the respirometer were excluded from the analyses (n = 2). Oxygen consumption was recorded (as described in Section 2.5) until it returned to pre-feeding levels (approximately 48 h). From the recording, 6 different parameters were calculated: (1) $SDA_{Max}$: peak oxygen consumption recorded after feeding; (2) $SDA_{Scope}$: difference between the peak in oxygen consumption following feeding and baseline oxygen consumption prior to feeding; (3) $SDA_{Total}$: the total increase in oxygen consumption above baseline, standardized to kJ using the conversion factor of 1 mg $O_2$ = 0.014 kJ (Secor 2009); (4) $SDA_{Duration}$: total time elapsed between the first increase in oxygen consumption after feeding and return of oxygen consumption to pre-feeding levels; (5) time to $SDA_{Max}$: time elapsed between the first increase in oxygen consumption after

feeding to $SDA_{Max}$ and (6) occupied digestion expenditure: $SDA_{Scope}$ / AS × 100.

### 2.9. Calculations and statistical analyses

Instantaneous growth rates of fish (% body mass $d^{-1}$) were calculated following Ricker (1975):

$$(\log_e bm_f - \log_e bm_i) \times 100 / t \quad (2)$$

where $bm_f$ is final body mass, $bm_i$ is initial body mass and $t$ is time (in days). We tested for the effects of individual fish mass, salinity treatment and individual fish mass × salinity treatment interaction on low- and high-food growth, MMR, SMR, AS, $SDA_{Max}$, $SDA_{Scope}$, $SDA_{Total}$, $SDA_{Duration}$, time to $SDA_{Max}$, occupied digestion expenditure and maximum food consumption using general linear models (see Table 1). Values were used with mass as a covariate because of the strong but somewhat predictable influence of mass on the metrics of interest and because mass varied from 37.2–292.9 g. Likelihood ratio tests comparing models with (full model) and without (reduced model) a mass × treatment interaction were used to compare model fit. If the log-likelihood ratio statistic was significant ($p < 0.05$), the full model was used; if not significant ($p > 0.05$), the interaction term was dropped and the reduced model was used. Tukey's post hoc analysis was used to assess differences among treatments. All analyses were conducted using RStudio Desktop v.3.5.2 statistical software (RStudio Team 2019).

### 2.10. Ethics

All work was carried out under the Bahamas Department of Marine Resources (permit no. MAMR/FIS/17) and gained ethical approval from the University of Exeter (reference no. 2017/1760). As lionfish are an invasive species in the Atlantic and wider Caribbean (Whitfield & Hare 2003), subjects could not be released back to the wild after experiments, and were instead euthanized using a lethal solution of water and clove oil, a widely accepted method of fish euthanasia as suggested by The Guide for the Care and Use of Laboratory Animals (NRC 2010).

## 3. RESULTS

### 3.1. Growth and maximum food consumption

There was a significant effect of salinity on growth during the low food availability portion of the experiment ($F_{2,60} = 26.62$, $p < 0.001$; Table 1, Fig. 2A). Fish from the 10 psu treatment had lower growth than fish from the 37 ($t_{60} = -5.59$, $p < 0.001$; Fig. 2A) and 20 psu treatments ($t_{60} = -6.82$, $p < 0.001$; Fig. 2A) but there was no significant difference between the 37 and 20 psu treatments ($t_{60} = -1.35$, $p = 0.38$; Fig. 2A). When fish were fed to satiation daily, there was no significant effect of salinity on growth between fish from the 10 and 37 psu treatments ($F_{1,26} = 0.19$, $p = 0.15$; Table 1, Fig. 2B). Unfortunately, due to unexpected mortalities in the 20 psu treatment, only fish from the 10 and 37 psu treatments could be compared.

There was a significant difference in the total amount of food consumed to achieve satiation ($F_{2,48} = 4.49$, $p = 0.02$; Table 1, Fig. 3D). Fish from the 20 psu treatment consumed the highest amount of food, although significant differences were only found between the 20 and 37 psu treatments ($t_{48} = 2.98$, $p = 0.01$; Table 1, Fig. 3D) and not between the 20 and 10 psu treatments ($t_{48} = 1.78$, $p = 0.18$; Table 1, Fig. 3D).

### 3.2. SMR, MMR and AS

There was no significant difference in SMR among lionfish from the 10, 20 and 37 psu treatments ($F_{2,36} = 7.07$, $p = 0.32$; Table 1, Fig. 3A). However, there was a significant difference in MMR ($F_{2,36} = 6.57$, $p = 0.004$; Table 1, Fig. 3B) and AS ($F_{2,36} = 10.65$, $p < 0.001$; Table 1, Fig. 3C). Lionfish from the 10 and 20 psu treatments had a significantly lower MMR compared with fish in the 37 psu treatment ($t_{36} = -3.49$, $p = 0.004$, $t_{36} = -2.56$, $p = 0.04$, respectively; Table 1, Fig. 3B). Similarly, lionfish from the 10 and 20 psu treatments had a lower AS than fish from the 37 psu treatment, although statistically significant differences were only found between 10 and 37 psu treatments ($t_{36} = -4.62$, $p < 0.01$; 20 and 37 psu, $t_{36} = -1.96$, $p = 0.07$; Table 1, Fig. 3C).

### 3.3. SDA

There was a significant interaction between mass and salinity for $SDA_{Max}$ ($F_{2,32} = 7.56$, $p = 0.002$; Table 1, Fig. 4A), $SDA_{Scope}$ ($F_{2,32} = 12.51$, $p < 0.001$; Table 1, Fig. 4B) and $SDA_{Total}$ ($F_{2,32} = 12.20$, $p < 0.001$; Table 1, Fig. 4E).

There was a significant effect of salinity on $SDA_{Scope}$ ($F_{2,32} = 4.43$, $p = 0.02$; Table 1, Fig. 4B) and $SDA_{Duration}$ ($F_{2,32} = 7.78$, $p = 0.002$; Table 1, Fig. 4C) and a marginally significant effect on percentage of

Table 1. Measurement order and statistical summary of the various dependent variables used in general linear models to test for the effect of salinity, mass, and salinity × mass interaction on lionfish acclimated to 3 salinity treatments (low: 10 psu; intermediate: 20 psu; control: 37 psu). Measurements with the same order number were completed within the same experiment. Likelihood ratio tests comparing models with (full model) and without the mass × treatment interaction (reduced model) were used to compare model fit. Tukey's post hoc analysis was used to assess statistical differences ($p < 0.05$) among treatments, with letters in parentheses indicating differences (A) between 20 and 37 psu; (B) between 10 and 37 psu; (C) between 20 and 10 psu. Due to unexpected mortalities during the high-food growth portion of the experiment, only growth data from the 10 and 37 psu treatments were available for comparison. See Section 2.8 for explanations of dependent variables; bm: body mass

| Measurement order | Dependent variable | Model | Fixed effects | Mass ± SD (g) | Sample size | Estimate | SE | *t*-value | p-value | Model selection ($\chi^2$ p-value) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Low food growth (% bm $d^{-1}$) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | −0.06 | 0.03 | −1.72 | 0.09 | 0.90 |
| | | | Mass | | | 0.00 | 0.00 | −0.51 | 0.62 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 102.9 ± 70.9 | 22 | | | | | |
| | | | 10 | 114.7 ± 91.0 | 22 | −0.21 | 0.04 | −5.59 | <0.001(B) | |
| | | | 20 | 108.9 ± 69.0 | 20 | 0.05 | 0.04 | 1.35 | 0.18 (C) | |
| 2 | MMR (mg $O_2$ $h^{-1}$) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | 3.16 | 1.07 | 2.94 | 0.01 | 0.73 |
| | | | Mass | | | 0.17 | 0.01 | 22.71 | <0.001 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 13 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 16 | −3.63 | 1.04 | −3.49 | 0.001(B) | |
| | | | 20 | 106.8 ± 75.5 | 11 | −2.88 | 1.13 | −2.56 | 0.02 (A) | |
| 2 | SMR (mg $O_2$ $h^{-1}$) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | 0.60 | 0.68 | 0.89 | 0.38 | 0.19 |
| | | | Mass | | | 0.07 | 0.00 | 14.09 | <0.001 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 13 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 16 | 0.06 | 0.66 | 0.09 | 0.93 | |
| | | | 20 | 106.8 ± 75.5 | 11 | −0.92 | 0.71 | −1.30 | 0.20 | |
| 2 | AS (mg $O_2$ $h^{-1}$) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | 2.56 | 0.82 | 3.10 | <0.01 | 0.41 |
| | | | Mass | | | 0.10 | 0.01 | 18.00 | <0.001 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 13 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 16 | −3.68 | 0.80 | −4.62 | <0.001 (B) | |
| | | | 20 | 106.8 ± 75.5 | 11 | −1.96 | 0.86 | −2.27 | 0.03 | |
| 3 | $SDA_{Max}$ (mg $O_2$ $h^{-1}$) | Mass + salinity | | | | | | | | |
| | | Mass + salinity + mass × salinity | Intercept | | | −0.04 | 1.89 | −0.02 | 0.98 | <0.001 |
| | | | Mass | | | 0.13 | 0.02 | 7.86 | <0.001 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 11 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 15 | −3.94 | 2.56 | −1.54 | 0.13 | |

Table continued on next page

Table 1 (continued)

| Measure-ment order | Dependent variable | Model | Fixed | Mass ± SD (g) | Sample size | Estimate | SE effects | *t*-value | p-value | Model selection ($\chi^2$ p-value) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 20 | 106.8 ± 75.5 | 10 | 0.60 | 2.58 | 0.23 | 0.82 | |
| | | | Mass × salinity | | | | | | | |
| | | | 37 | | | | | | | |
| | | | 10 | | | 0.06 | 0.03 | 2.39 | 0.02 | |
| | | | 20 | | | −0.03 | 0.02 | −1.48 | 0.15 | |
| 3 | $SDA_{Scope}$ (mg $O_2$ $h^{-1}$) | Mass + salinity | | | | | | | | |
| | | Mass + salinity + mass × salinity | Intercept | | | 0.55 | 1.23 | 0.45 | 0.66 | <0.001 |
| | | | Mass | | | 0.05 | 0.01 | 4.47 | <0.001 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 11 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 15 | −4.51 | 1.66 | −2.71 | 0.01 | |
| | | | 20 | 106.8 ± 75.5 | 10 | −0.72 | 1.68 | −0.43 | 0.67 | |
| | | | Mass × salinity | | | | | | | |
| | | | 37 | | | | | | | |
| | | | 10 | | | 0.07 | 0.02 | 4.09 | <0.001 | |
| | | | 20 | | | −0.01 | 0.01 | −0.47 | 0.65 | |
| 3 | $SDA_{Total}$ (kJ) | Mass + salinity | | | | | | | | |
| | | Mass + salinity + mass × salinity | Intercept | | | 0.00 | 0.30 | 0.00 | 1.00 | <0.001 |
| | | | Mass | | | 0.01 | 0.00 | 3.47 | <0.01 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 11 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 15 | −0.93 | 0.40 | −2.32 | 0.03 | |
| | | | 20 | 106.8 ± 75.5 | 10 | −0.29 | 0.40 | −0.72 | 0.48 | |
| | | | Mass × salinity | | | | | | | |
| | | | 37 | | | | | | | |
| | | | 10 | | | 0.02 | 0.00 | 4.54 | <0.001 | |
| | | | 20 | | | 0.00 | 0.00 | 0.56 | 0.58 | |
| 3 | $SDA_{Duration}$ (h) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | 23.68 | 2.33 | 10.14 | <0.001 | 0.64 |
| | | | Mass | | | 0.02 | 0.02 | 1.39 | 0.17 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 11 | | | | | |
| | | | 10 | 77.4 ± 43.1 | 15 | 8.90 | 2.28 | 3.91 | <0.001 (B) | |
| | | | 20 | 106.8 ± 75.5 | 10 | 3.86 | 2.48 | 1.55 | 0.13 | |
| 3 | Time to $SDA_{Max}$ (h) | Mass + salinity + mass × salinity | | | | | | | | |
| | | Mass + salinity | Intercept | | | 10.29 | 2.02 | 5.09 | <0.001 | 0.84 |
| | | | Mass | | | −0.01 | 0.01 | −0.58 | 0.57 | |
| | | | Salinity | | | | | | | |
| | | | 37 | 100.3 ± 62.7 | 11 | | | | | |

Table continued on next page

Table 1 (continued)

| Measurement order | Dependent variable | Model | Fixed | | Mass ± SD (g) | Sample size | Estimate | SE | *t*-value | p-value | Model selection ($\chi^2$ p-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | | 77.4 ± 43.1 | 15 | –4.07 | 1.97 | –2.07 | 0.05 | |
| | | | 20 | | 106.8 ± 75.5 | 10 | –2.78 | 2.15 | –1.29 | 0.20 | |
| 3 | Digestion expenditure (% of AS) | Mass + salinity + mass × salinity | | | | | | | | | |
| | | Mass + salinity | Intercept | | | | 33.41 | 12.52 | 2.67 | 0.01 | 0.71 |
| | | | Mass | | | | 0.08 | 0.09 | 0.87 | 0.39 | |
| | | | Salinity | | | | | | | | |
| | | | 37 | | 100.3 ± 62.7 | 11 | | | | | |
| | | | 10 | | 77.4 ± 43.1 | 14 | 24.74 | 12.36 | 2.00 | 0.05 | |
| | | | 20 | | 106.8 ± 75.5 | 10 | –3.48 | 13.31 | –0.26 | 0.80 | |
| 4 | Maximum food consumption (% bm) | Mass + salinity + mass × salinity | | | | | | | | | |
| | | Mass + salinity | Intercept | | | | 11.36 | 0.87 | 13.10 | <0.001 | 0.28 |
| | | | Mass | | | | –0.04 | 0.01 | –7.15 | <0.001 | |
| | | | Salinity | | | | | | | | |
| | | | 37 | | 88.3 ± 49.5 | 20 | | | | | |
| | | | 10 | | 101.1 ± 81.5 | 22 | 1.45 | 0.93 | 1.56 | 0.13 | |
| | | | 20 | | 112.5 ± 75.9 | 11 | 3.50 | 1.18 | 2.98 | <0.01 (A) | |
| 5 | High food growth (% bm $d^{-1}$) | Mass + salinity + mass × salinity | | | | | | | | | |
| | | Mass + salinity | Intercept | | | | 1.67 | 0.12 | 14.12 | <0.001 | 0.13 |
| | | | Mass | | | | 0.00 | 0.00 | –3.78 | <0.001 | |
| | | | Salinity | | | | | | | | |
| | | | 37 | | 88.3 ± 49.5 | 10 | | | | | |
| | | | 10 | | 101.1 ± 81.5 | 19 | –0.17 | 0.12 | –1.49 | 0.15 | |
| | | | 20 | | 112.5 ± 75.9 | 0 | NA | NA | NA | NA | |

occupied digestion expenditure ($F_{2,31}$ = 3.10, p = 0.059; Table 1, Fig. 4F). However, there was no significant effect of salinity on time to $SDA_{Max}$ ($F_{2,32}$ = 1.02, p = 0.37; Table 1, Fig. 4D). Lionfish from the 10 psu treatment had a significantly higher $SDA_{Scope}$ than fish from the 20 psu treatment ($t_{32}$ = 3.31, p = 0.007; Table 1, Fig. 4B) and a higher percentage of occupied digestion expenditure, although this was borderline not statistically significant ($t_{31}$ = 2.21, p = 0.07; Table 1, Fig. 4F). No differences were found between the 10 and 37 psu treatments ($SDA_{Scope}$, $t_{32}$ = 1.89, p = 0.16; occupied digestion expenditure $t_{31}$ = 2.00, p = 0.13; Table 1, Fig. 4B,F) and between the 37 and 20 psu treatments ($SDA_{Scope}$, $t_{32}$ = 1.41, p = 0.35; percentage of occupied digestion expenditure, $t_{31}$ = 0.26, p = 0.96; Fig. 4B,F). Lionfish from the 10 psu treatment had a significantly longer $SDA_{Duration}$ than fish from the 37 psu treatment ($t_{32}$ = 3.92, p = 0.001; Table 1, Fig. 4C), but no differences were found between the 37 and 20 psu treatments ($t_{32}$ = 1.55, p = 0.28; Table 1, Fig. 4C) and the 10 and 20 psu treatments ($t_{32}$ = 2.14, p = 0.097; Table 1, Fig. 4C).

### 3.4. Mortalities

In total, 11 lionfish from the 20 psu treatment died between 12 and 18 April 2017. A further 11 lionfish from the 20 psu treatment and 15 lionfish from the control treatment died during the high-food growth portion of the experiment (7–24 May 2017), approximately 3 wk after the low-food growth and respirometry measures were concluded. No mortalities occurred in the 10 psu treatment.

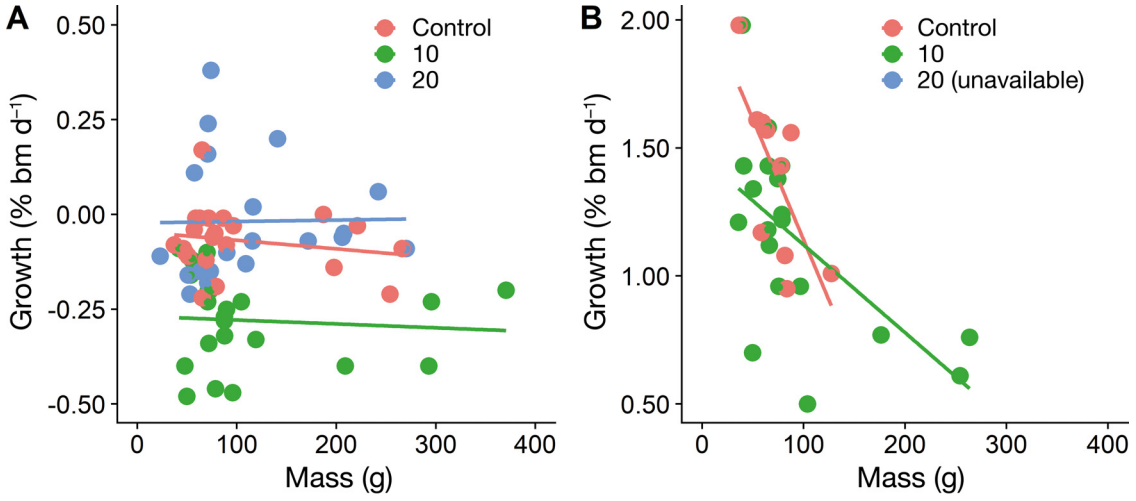


Fig. 2. Growth versus body mass (bm) of lionfish acclimated to 3 salinity treatments (low: 10 psu; intermediate: 20 psu; control: 37 psu) and fed (A) a low-food ration for 24 d or (B) a high-food ration for 17 d. Due to unexpected mortalities in the 20 psu treatment during the high food portion of the experiment, only fish from the 10 and 37 psu treatments are compared in (B)

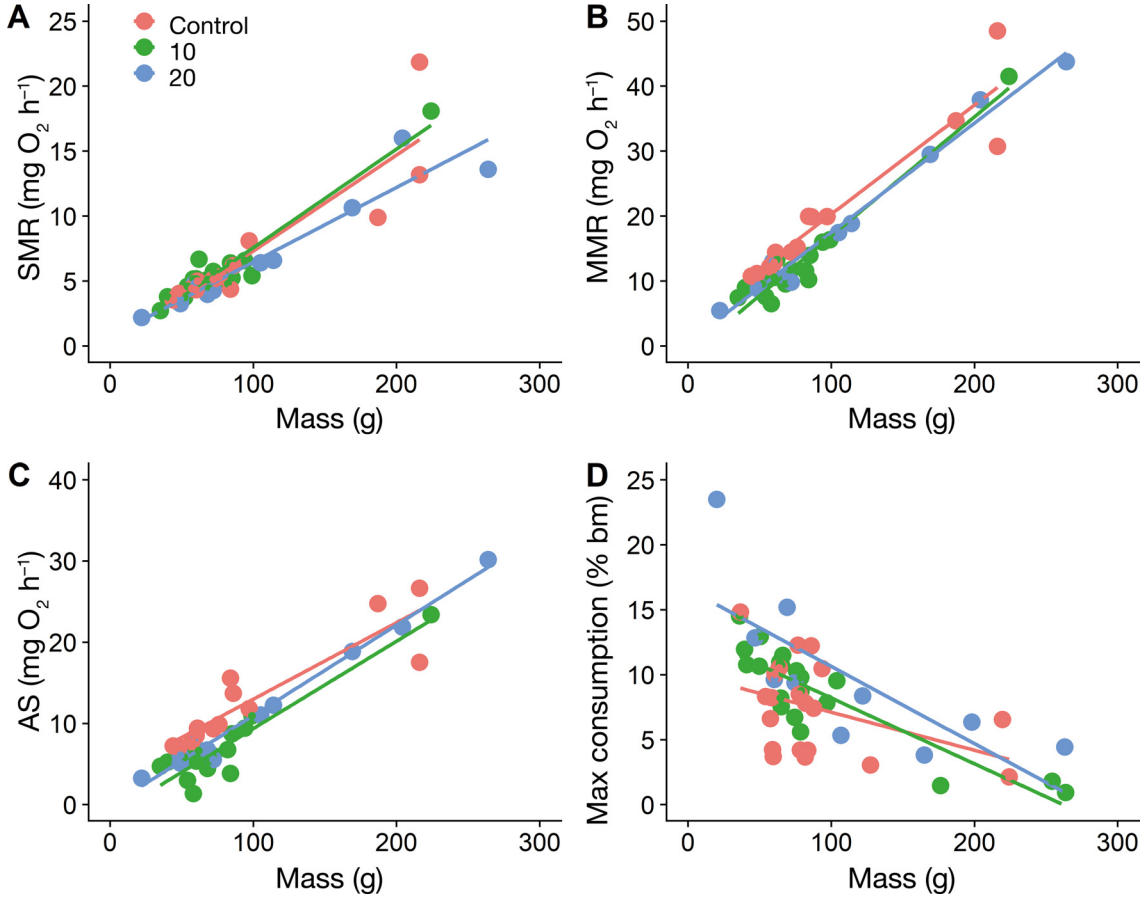


Fig. 3. Metabolism and food consumption-related parameters versus body mass (bm) for lionfish acclimated to 3 salinity treatments (low: 10 psu; intermediate: 20 psu; control: 37 psu): (A) standard metabolic rate (SMR; the minimal maintenance metabolic rate of an ectotherm in a post-absorptive and inactive state), (B) maximum metabolic rate (MMR; the rate of oxygen consumption during the maximum sustainable rate of exercise), (C) aerobic scope (AS; the difference between an animal's SMR and its MMR; defines the capacity of an animal to increase its rate of aerobic metabolism), (D) maximum food consumption (% of bm consumed to achieve satiation)

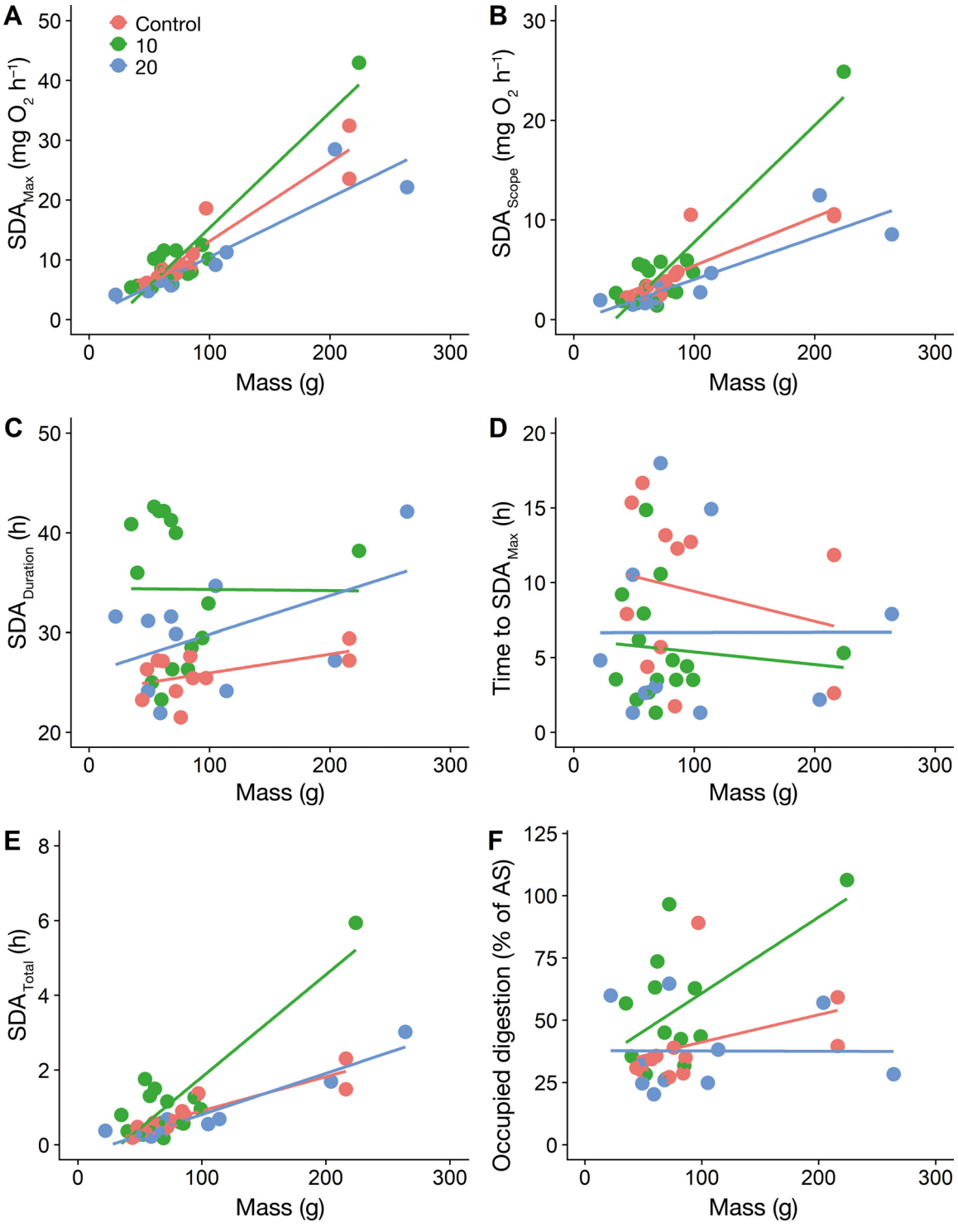


Fig. 4. Digestion-related parameters versus body mass for lionfish acclimated to 3 salinity treatments (low: 10 psu; intermediate: 20 psu: control: 37 psu): (A) peak oxygen consumption recorded after feeding ($SDA_{Max}$), (B) difference between the peak in oxygen consumption following feeding and baseline oxygen consumption prior to feeding ($SDA_{Scope}$), (C) total time elapsed between the first increase in oxygen consumption after feeding and return of oxygen consumption to pre-feeding levels ($SDA_{Duration}$), (D) time elapsed between the first increase in oxygen consumption after feeding to $SDA_{Max}$ (Time to $SDA_{Max}$), (E) total increase in oxygen consumption above baseline, standardised to kJ ($SDA_{Total}$), (F) occupied digestion (percent of aerobic scope occupied by digestion)

## 4. DISCUSSION

Consistent with Jud et al. (2015), we found that lionfish can survive in low salinity conditions (10 psu) for at least 2 mo. Coping with osmotic gradients (e.g. in hypo- and hypersaline environments) is generally regarded as being energetically expensive (Webb 1975, Stevens & Dizon 1982, Febry & Lutz 1987, Behrens et al. 2017); thus increased SMR, decreased AS and decreased growth would be expected as energy is diverted towards osmoregulatory demands to ensure biological function. We found no evidence that hyposalinity negatively affected SMR but we did find evidence that hyposalinity affected growth in lionfish at low food availability, as well as MMR and AS. Lionfish from the 37 and 20 psu treatments had higher growth at low food availability than fish from the 10 psu treatment. However, lionfish from the 10 and 20 psu treatments had a lower MMR and AS than those from the 37 psu treatment. Changes in SMR in low salinity environments have been demonstrated in other species. For example, Morgan & Iwama (1991) found that SMR increased as salinity increased in steelhead *Oncorhynchus mykiss* and Chinook salmon *O. tshawytscha* when moving from fresh to salt water, and Dalziel et al. (2012) found a significantly lower MMR and AS in three-spined sticklebacks *Gasterosteus aculeatus* in hyposaline conditions, indicating the potential for some physiological impairment (Morgan & Iwama 1991, Dalziel et al. 2012). In contrast to this, and similar to the present study, Grøtan et al. (2012) found no difference in SMR in *G. aculeatus* among salinity treatments and concluded that *G. aculeatus* may be able to move among varying salinity environments without measurable short-term metabolic costs, irrespective of their salt or freshwater environment of origin (Grøtan et al. 2012).

Differences in MMR and AS are usually discussed in relation to cardiovascular ability and swim performance, with species and/or individuals with higher MMR and AS considered more athletic (Killen et al. 2010, Metcalfe et al. 2016). In relatively sedentary species like lionfish (Jud & Layman 2012, McCallister et al. 2018) that use ambush tactics to capture prey and have few predators, the daily energetic costs allocated to movement and vigilance are likely minimal (Steell et al. 2019). Instead, lionfish appear to have the luxury of allocating a greater proportion of their AS to digestion, growth and reproduction (Steell et al. 2019). However in the present study, lowered salinity reduced MMR and AS, and consequently, lionfish in the 10 psu treatment had reduced maximum food consumption, prolonged $SDA_{Duration}$, a higher percentage of their AS allocated to digestion and decreased growth in low-food conditions compared to those from the control treatment (37 psu). Interestingly, although lionfish from the 20 psu treatment also had reduced MMR and AS, they had a much lower percentage of their AS allocated to digestion, higher food consumption and growth at low food.

After consuming a large meal, SDA can typically double or triple the maximum rate of oxygen consumption of a fish (Alsop & Wood 1997, Secor 2009). In sedentary fish species, the peak in oxygen uptake following feeding can exceed that observed during peak aerobic exercise (Fu et al. 2005, Steell et al. 2019). Interestingly, in the present study, the magnitude of SDA (i.e. $SDA_{Max}$) did not differ among treatments but did last a third longer in the lowest hyposaline treatment (i.e. $SDA_{Duration}$). Therefore, the results of our study suggest that lionfish may cope with the increase in oxygen demand brought about by feeding by extending how long digestion takes, depending on the salinity of their ambient conditions. In low salinity environments, lionfish appear to be physiologically constrained to not only eating less food but prolonging $SDA_{Duration}$, which ultimately should increase the interval between consumption of the next available meal, although this remains to be tested. However, this does not explain why digestion duration was similar between the 37 and 20 psu treatments — especially considering lionfish should have been closer to an isotonic state at 20 than 37 psu, assuming lionfish osmolality is similar to that of other marine fish species (Lambert et al. 1994). An alternative explanation could be that low salinity simply slowed gut contractions or reduced gastric activity, which has been shown previously in other species at low salinities (McGaw 2006).

The growth of Atlantic cod *Gadus morhua* has been investigated at 3 different salinities (7, 14 and 28 psu), across 2 seasons (spring and autumn) and 2 feeding levels (satiation once a week [low food] or 3 times weekly [high food]; Lambert et al. 1994). Overall, growth rates were highest for *G. morhua* in intermediate salinity conditions (14 psu) and unsurprisingly at high food levels. However, at low food, growth rates for cod in low salinity (7 psu) were greater than in high salinity (28 psu) during the spring, but similar during the autumn (Lambert et al. 1994), suggesting some level of context dependency among relationships between growth and salinity across seasons and water temperatures. Unfortunately, due to the unexpected mortalities in the 20 psu treatment during the high-food growth portion of the experiment, we were

unable to evaluate growth differences at intermediate salinity for lionfish at high food. However, we were able to evaluate maximum food consumption. Lionfish at intermediate salinities had the highest maximum food consumption. Therefore, given that lionfish at intermediate salinities had an increased capacity for food intake and coupled with the pattern of increased growth described above for intermediate salinities in other fish species, we speculate that growth for lionfish at intermediate salinities would also be highest. Interestingly, Lambert et al. (1994) attributed the pattern of higher growth at intermediate salinities in *G. morhua* to differences in food conversion efficiency and not to increased food intake, which is at odds with results of our study. Despite lionfish from the intermediate salinity having a lower AS and percentage of occupied digestion expenditure, but similar $SDA_{Duration}$ to those fish in the 37 psu treatment, the increased maximum food consumption at intermediate salinity suggests a strategy of enhanced throughput of food at intermediate salinities. It remains plausible that lionfish, which are sedentary ambush predators compared to cod, which are athletic pursuit predators, may allocate resources differently, thus managing physiological trade-offs differently across salinities than other marine fish species (Steell et al. 2019).

The results of the present study have ecological implications for modelling lionfish range expansion that suggest lionfish have considerable potential to invade hyposaline ecosystems. Given the ability of lionfish to survive in low salinity, with only minor–moderate effects on metabolism, growth and digestion, we suggest that range expansion models should begin to incorporate hyposaline ecosystems. Neglecting salinity tolerance may cause the results of range expansion models to underestimate future range expansions, although the interaction between temperature, salinity and other abiotic factors remain untested. Hyposaline ecosystems are known to be important nursery habitats for juvenile fish species (Barbier 2006, Faunce & Serafy 2006, Mateo et al. 2010, Barbier et al. 2011, Sandilyan & Kathiresan 2015). Although a serious concern, our results suggest that when occupying low salinity environments, lionfish may consume relatively less individual prey items, have slower digestive processes and decreased growth at the lowest of salinities, despite being able to survive. However, given that lionfish were able to consume more at intermediate salinities suggests that coastal ecosystems with intermediate salinities may be of increased threat. Whatever the case, because estuarine habitats are disproportionately important habitats for juvenile organisms, even physiologically compromised lionfish are likely to have some impact.

In the present experiment, there were several sudden mortalities within the 20 and 37 psu high food treatments, but no mortalities occurred within the 10 psu high food treatment. While there appears to be a pattern between salinity and mortality, the mechanisms are unknown. One possible explanation could be an association with pathogens, especially given the suddenness of the mortalities; although Tuttle et al. (2017) found that lionfish were 18 times less likely to host a parasite in The Bahamas compared with sympatric, native fishes. While it remains puzzling as to why the mortalities occurred, it is interesting that no mortalities occurred in the 10 psu treatment. It is plausible that if a pathogen caused the mortalities in lionfish, it may not have been able to survive at the lowest of salinity treatments, suggesting a hypothetical benefit for lionfish inhabiting low salinity ecosystems. Therefore, it would be beneficial for the relationship between pathogens and salinity tolerance in both lionfish and potential pathogens to be further investigated.

In some hyposaline ecosystems, lionfish would experience an influx of high salinity water (37 psu) during the flood tide, which would be replaced with low salinity water (10 psu) during the ebb tide as the flow of water changes and moves seaward. Therefore, lionfish in the upper reaches of these ecosystems may experience salinity fluctuations of ~27 psu every 6 h (Diele & Simith 2006). Therefore, the result of our study, where lionfish were acclimated to constant salinities, may not be applicable to all hyposaline ecosystems globally. Although the present study has shown that lionfish can survive in a stable hyposaline environment, a future area of research would be to investigate how lionfish cope with rapid changes in salinity and under hypersaline conditions at high food abundance. One hypothesis is that lionfish may not simply reside in hyposaline ecosystems; instead, they may be travelling in on flowing tides to feed intermittently during high tide cycles, and then moving back onto the reef habitat; although this remains unknown, it is perhaps unlikely given the sedentary nature of the lionfish (Jud & Layman 2012, McCallister et al. 2018) and the energetic cost associated with such a feeding strategy. Nevertheless, this feeding strategy would allow lionfish to exploit the high juvenile fish populations found in estuaries before returning to optimum salinity, which may allow for maximum digestion. More likely, however, is that lionfish are swept into low salinity habitats during early

stages of development, where they then grow and develop, like other juvenile reef fish. If this is the case, further research on how the acclimation of early developing lionfish larvae relates to salinity tolerance of adults would be worthwhile.

Results of this study further illustrate that lionfish have several physiological mechanisms that likely facilitate their expansion in hyposaline ecosystems. More in-depth surveys of hyposaline ecosystems for the presence of lionfish would be useful in providing further insight, as these systems remain relatively under-investigated and underreported from the perspective of the lionfish invasion (Barbour et al. 2010, Claydon et al. 2012, Pimiento et al. 2015), despite being of serious concern to Caribbean communities and their fisheries.

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