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PRIMARY RESEARCH PAPER

Salinity and temperature tolerance of an emergent alien species, the Amazon fish *Astronotus ocellatus*

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Abstract *Astronotus ocellatus* (oscar), is native to the Amazon basin and, although it has been introduced to many countries, little is known regarding its tolerances for salinity and temperature. In this report, we provide data on the tolerance of *A. ocellatus* to abrupt and gradual changes in salinity, its high and low temperature tolerance, and information on how salinity, temperature, and fish size interact to affect survival. Fish were able to survive abrupt transfer to salinities as high as 16 ppt with no mortality. When salinity change was gradual (2 ppt/day), fish in the warm-temperature experiment (28°C) survived longer than fish in the cool-temperature experiment (18°C). Larger fish survived longer than smaller ones at the higher salinities when the temperature was warm, but when the temperature was cool fish size had little effect on survival. In the temperature-tolerance experiments, fish survived from 9 to 41°C for short periods of time. Overall, the species showed a wide range of temperature and salinity tolerance. Thus, in spite of the tropical freshwater origin of this species, physiological stress is not likely to hinder its dispersal to brackish waters, especially when temperatures are warm.

Keywords Cichlidae · Survival tests · Tolerance · Non-native species · Oscar

Introduction

Biological invasions are one of the most alarming problems in ecological systems, causing damage such as habitat degradation, hybridization, community collapse, spread of disease, and economic impacts (Pimentel, 2002; Gozlan, 2009; Cucherousset & Olden, 2011). Despite the concern about invasions, fishes continue escaping captivity into new waters around the world, especially through vectors of aquaculture, the ornamental-fish trade and sport fishing (Gozlan, 2008). For these reasons, fishes are some of the most frequently introduced aquatic animals in the world (Gozlan, 2008, 2009). When ornamental fishes escape from farms or are released by their owners, they may become potential invaders (Keller & Lodge, 2007; Gertzen et al., 2008; Gozlan, 2009). Even though the invasion process poses challenges to establishment, some fishes survive physiological and ecological barriers to spread successfully in new environments (Rahel & Olden, 2008; Gozlan, 2009; Capps & Flecker, 2013).

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Astronotus ocellatus (Agassiz, 1831), commonly known as oscar, is native to the Amazon basin of Peru, Colombia and Brazil (Kullander, 2003) and was moved around the world as a popular aquarium fish (Fury & Morello, 1994; Maceda-Veiga et al., 2014). It has been reported as introduced or established in the continental United States, Puerto Rico, Australia, Poland, Singapore, and southern Brazil (Ng et al., 1993; Fury & Morello, 1994; Nowak et al., 2008; Webb, 2008; Julio Junior et al., 2009; Nico et al., 2014). In its native range, the species lives in warm freshwater bodies, either Amazon basin white waters (such as the Solimoes and Amazon rivers) that are rich in suspended solids and have a neutral pH, or in acidic and ion-poor black waters of the Rio Negro (Sioli, 1984; De Pinna, 2006). *Astronotus ocellatus* is an aggressive competitor and predator, very tolerant of a wide range of physical water conditions including low pH, low dissolved oxygen concentrations (Muusze et al., 1998; Almeida-Val et al., 2000; Sloman et al., 2006), high temperatures (Val et al., 2006), and low temperatures (Shafland & Pestrak, 1982); therefore the species has the potential to invade a wide range of environments (Val et al., 2006; Nico et al., 2014). High resistance to environmental stressors makes *A. ocellatus* a successful ornamental fish but also makes it an invasive threat (Gozlan, 2009).

In the Amazon Basin, *A. ocellatus* usually encounters temperatures between 20 and 30°C in freshwater (Barletta et al., 2010). In captivity, *A. ocellatus* is found in temperatures ranging from 23 to 34°C (Silva, 2010), and in introduced places like the Paraná Basin, southern Brazil, the species faces annual water temperatures ranging from 17 to 30°C (Agostinho et al., 1995). In the southern Florida Everglades, *A. ocellatus* occurs in temperatures ranging annually from 13.2 to 35.5°C (±3°C SE; SFNRC, 2014). According to Shafland & Pestrak (1982), *A. ocellatus* does not survive below 12°C over the long term, which may limit its range in Florida, particularly during cold winters (Shafland et al., 2008). In a climate-change scenario, increases in water temperature may result in a redistribution of fish species like *A. ocellatus*, because warmer waters will aid the spread of non-native tropical fishes to higher latitudes when cold waters are no longer barriers to dispersal (Sharma et al., 2007; Rahel & Olden, 2008).

As a cichlid, *A. ocellatus* may be considered secondarily freshwater in its evolutionary history, and as a result can tolerate brackish waters for short periods (Myers, 1949; Briggs, 2003; Val et al., 2006). The use of seawater as a dispersal bridge to estuaries (via sea-level rise and storm surge) can be a potential pathway for successful fish invasions into other nearby river basins (Brown et al., 2007; Schofield et al., 2011; Gutierre et al., 2014). The response of *A. ocellatus* to abrupt and chronic exposures in salinity-tolerance experiments may help scientists and managers understand its ability to survive and disperse through saline pathways. By exposing the fish to combinations of salinity and temperature, its responses will lead to a better understanding of the tolerance of this species when different stressors act either antagonistically or synergistically. Since body size is well known to affect responses to other physiological stressors (Almeida-Val et al., 2000; Sloman et al., 2006), experiments on salinity and temperature tolerance should include more than one size class.

In this study, we tested the temperature and salinity tolerance of the cichlid *A. ocellatus* from Florida (United States) to estimate its limits of tolerance and invasiveness. We intended to answer the following questions: What are its limits of tolerance to temperature and salinity? Does fish size affect thermal and salinity tolerance in *A. ocellatus*? Are salinity and temperature limiting factors for the invasion of this species? Although *A. ocellatus* has been previously subjected to some physiological testing (low- and high-temperature tolerance, hypoxia tolerance for different sized fish), this is the first study of its salinity tolerance. Furthermore, our study is the first to provide information on how salinity, temperature, and fish size of *A. ocellatus* interact in their effects on survival.

Materials and methods

Fish collection

Fish were purchased from a fish farm (Dade City Tropicals Inc., Pasco County, Florida) in March and April 2014 and transported to the US Geological Survey laboratory in Gainesville, Florida. *Astronotus ocellatus* broodstock have been held and bred at that fish farm for at least a decade (M. Prater, Dade City Tropicals, Inc., personal communication). In the laboratory, specimens were acclimated in tanks (378 L) with well water (from Floridan aquifer, 0.2 ppt salinity hereafter termed ‘0’). For over 2 weeks after collection and transport, fish were fed twice a day with small pellets of fish food (Zeigler® High-Protein Finfish Starter Feed Pellet) while housed in tanks in which temperatures ranged from 21 to 25°C. After acclimation, the fish were transferred to new quarters for the respective experiments (see below). Two sizes of fish were used: small ($n = 135$) (total length [TL, mean ± SE] = 5.9 ± 0.05 cm, range 3.5–7.3 cm; wet mass 3.92 ± 1.11 g, range 1.6–7.1 g) and large ($n = 135$) (TL = 9.4 ± 0.06 cm, range 8.1–11.8 cm; wet mass 16.49 ± 0.34 g, range 8.2–27.6 g). Both size classes were juveniles, with the smaller fish estimated at 2 months old and the larger ones at 6 months old (M. Prater, Dade City Tropicals, Inc., personal communication).

Experimental set-up

Before experiments, fish were weighed (g), measured to total length (TL, cm), and placed individually into plastic bins (17 × 14 × 11 cm; one fish per bin) filled with 1.5 l well water. Each bin received constant aeration, was covered on three sides with opaque material (to prevent fish from seeing each other), and covered on top with a tight-fitting lid. Fish were acclimated for at least 3 days in the bins before being subject to experimental conditions. Synthetic aquarium sea salt (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD, USA) was mixed with well water for salinity treatments; well water was used as a control. Salinity was recorded with a YSI Professional Plus Multiparameter meter (YSI Inc., Yellow Springs, Ohio, USA, ±0.2 ppt). Photoperiod was 12:12 L:D during the experiments. For all experiments, fish were held individually in each bin. Death was determined when fish opercular movements ceased and there was no response when lifted from the water. Surviving fish were euthanized by submersion in ice water at the end of the experiments.

The experimental design followed Schofield et al. (2011). Salinity tolerance was determined with abrupt and chronic experiments. An initial pilot study was conducted on a few individuals to establish a general range of salinity tolerance and determine what experimental salinity levels would be used for the experiment.

Abrupt salinity-tolerance experiment

Fish were abruptly transferred from holding conditions (freshwater) to salinity treatments (0 [control], 14, 16, 18, 20 ppt). Ten fish (5 small and 5 large) were used for each salinity treatment, totaling 50 fish. Fish were checked once per hour for the first 8 h, then twice a day for 7 days or until they died. Water temperature was monitored during the experimental period and the mean value was 24.3°C (range 22–26°C). Water was not changed during this experiment and fish were not fed.

Chronic salinity experiments in warm and cold temperatures

All fish started at the control salinity of 0 ppt on the same day, and were exposed to gradual increase in water salinities (2 ppt per day) until all treatments reached their target salinity (0 [control], 14, 16, 18, 20 ppt). Thus, the fish reached their target salinities at different times; however, when the salinity was changed for one or more treatments, the water was changed for all fish to maintain similarity of handling across treatments. Experiments were performed at two temperatures: warm (28°C) and cold (18°C). Those temperatures were based on mean conditions during the summer and winter in the Everglades region (SFNRC, 2014). Ten fish (5 small and 5 large) were used for each salinity treatment at both temperatures. Thus, the total samples size was 100—10 fish per salinity treatment × 5 salinity treatments × 2 temperatures. Air temperatures were monitored daily. Fish were fed twice per week and the water was changed the day after the feeding day to maintain water quality. Once fish reached their target salinity, they were held there for 30 days or until death.

For the warm-temperature experiment, fish were placed in a climate-controlled laboratory where the air temperature was gradually changed until it reached the mean of 27.8°C (range 26.4–28.6°C; hereafter 28°C). The cold-temperature experiment took place in a walk-in environmental chamber where the air temperature was gradually changed until it reached the mean of 17.7°C (range 16.5–18.7°C; hereafter 18°C).

Lethal thermal maximum (LTmax) and minimum (LTmin) experiments

To determine the maximum (LTmax) and minimum (LTmin) temperatures tolerated by *A. ocellatus*, fish were acclimated to a set temperature (i.e., acclimation temperature) and then monitored every hour while the water temperature was either increased or decreased at a rate of 1°C/hour. During LTmax and LTmin experiments, a set of control fish maintained at the acclimation temperature was also monitored. The lethal temperature was determined when fish opercular movements ceased and there was no response when lifted from the water. The temperature-change rate of 1°C/hour was determined based on previous LT experiments (Ospina & Mora, 2004; Schofield et al., 2010) and field measurements of the rapidly cooling or warming Everglades flooded region (Schofield et al., 2010).

For the LTmax experiment, all fish (experimental and control) housed in individual plastic boxes were placed inside a large water bath and acclimated to one of two temperatures (25 and 30°C). Fish were maintained in the water baths for 4 days before the experiment began. For each acclimation temperature 20 fish (10 small and 10 large) were tested and ten fish (5 small and 5 large) were maintained at the acclimation temperature (25 and 30°C) as controls. Thus, total sample size was 60—(20 experimental fish + 10 control fish) × 2 acclimation temperatures. At the start of the experiment, control fish were moved to an environmental chamber set to the acclimation temperature. Temperature of the water bath was increased 1°C per hour with a submersed heater and digital thermostat. All fish (experimental fish and controls) were checked every hour. The experiment ended when all experimental fish had died.

For the LTmin experiment, fish were acclimated to two temperatures (20 and 25°C) and maintained at these temperatures for 4 days before the experiment began. For each acclimation temperature 20 fish (10 small and 10 large) were tested and ten fish (5 small and 5 large) were maintained at the acclimation temperature (20 and 25°C) as controls. Thus, similar to LTmax, total sample size was 60—(20 experimental fish + 10 control fish) × 2 acclimation temperatures. The experiment took place in an environmental chamber where air temperature was controlled with a digital thermostat. All fish (experimental fish and controls) were placed in individual plastic bins in the environmental chamber during the 4-day pre-experiment holding period. At the start of the experiment, the control fish were moved to a climate-controlled laboratory set at the acclimation temperature and maintained there throughout the experiment. Air temperature was gradually lowered to produce a decrease in water temperature of 1°C per hour. Because we used bins that were relatively small (1.5 l of water per bin), the lag time in the water temperature changing with the air temperature was around five minutes. Control and experimental fish were checked every hour until all the experimental fish died.

Data analysis

Survival of individuals in both salinity-tolerance tests, abrupt and chronic, were evaluated using the non-parametric Kaplan–Meier survival function (Kaplan & Meier, 1958). For the chronic salinity-tolerance experiment, fish reached their target salinities at different times (i.e., fish in lower-salinity treatments reached their target salinities before higher-salinity treatments). Thus, the day the fish were transferred to their target salinity was designated as Day = 0. Fish were then maintained in their experimental salinities for at least 30 days after they had reached the target salinities. To evaluate differences between survival curves obtained by the Kaplan–Meier tests, the log-rank test was used (Kleinbaum & Klein, 1997). We tested significance of acclimation temperature, body size, and their interaction on the LTmax and LTmin with two-way ANOVA and Holm–Siddak post hoc. LTmin and LTmax were tested separately, using a 2 × 2 factorial design with two levels for each factor. The first factor was acclimation temperature and the second factor was body size. Statistical significance was considered when $P < 0.05$ (SPSS version 13.0 and SigmaPlot version 11.0).

Results

Abrupt salinity-tolerance experiment

Astronotus ocellatus tolerated rapid transfer to salinities up to 14 ppt with no mortality. At salinities above 14 ppt, larger fish survived about twice as long as smaller ones (Table 1; Fig. 1).

Chronic salinity-tolerance experiments

Nearly all fish at 28°C survived longer than fish at 18°C, regardless of salinity (Table 2; Fig. 2). For example, fish survived the 14 ppt salinity at 28°C,

Table 1 Survival of *A. ocellatus* to abrupt changes in salinity (0 [control], 14, 16, 18, and 20 ppt) over a seven-day experiment

Salinity treatment	Fish size	Percent survival over 7 day challenge	Survival time (h)		Size comparison (small vs. large; *P*)
			Mean	95% CI	
0 (control)	Small	100	No mortality	N/A	
	Large	100	No mortality	N/A	N/A
14 ppt	Small	100	No mortality	N/A	
	Large	100	No mortality	N/A	N/A
16 ppt	Small	0	87.2	77–97	
	Large	100	No mortality	N/A	0.002
18 ppt	Small	0	33.6	26–41	
	Large	0	60.4	39–81	0.012
20 ppt	Small	0	11	11–11	
	Large	0	25.4	23–28	0.003

Ten fish (5 small and 5 large) were used for each of five salinity treatments for a total of 50 fish. Kaplan–Meier survival analysis was used to compute mean survival time (in hours) and 95% confidence intervals (CI). For each salinity, fish size (small versus large) was compared with log-rank test

N/A not applicable

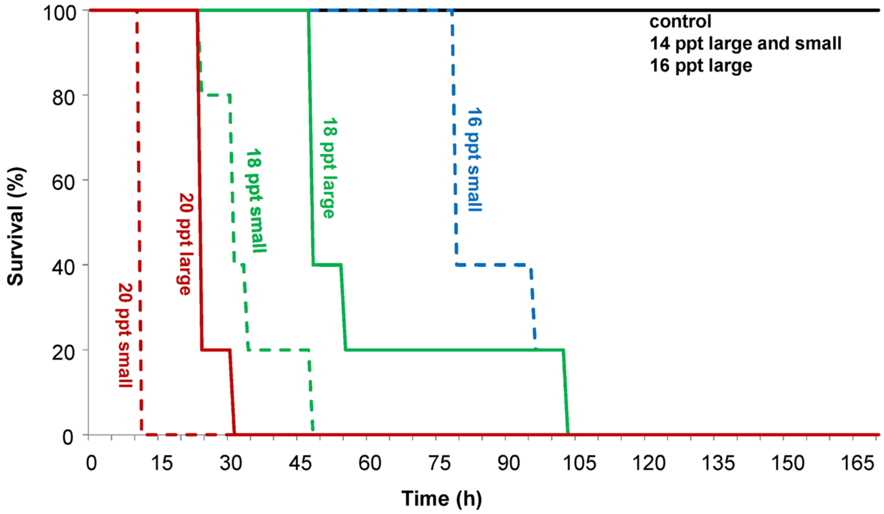

Fig. 1 Survival of *Astronotus ocellatus* after abrupt transfer from freshwater to salinity treatments (0 [control], 14, 16, 18, and 20 ppt). Sample sizes were 5 small and 5 large fish per salinity treatment × 5 salinity treatments = 50 fish. Small fish are represented by a *continuous line* and large fish by a *dashed line*

with little mortality after 30 days, but were all dead by 10 days at 18°C (Fig. 2a). Similarly, a portion of the individuals at 16 ppt survived for the entire 30-day challenge at 28°C (40% of small fish; 20% of large fish). In contrast, fish at 18°C fish in the 16 ppt salinity treatment were all dead within a week (by 6 days for large fish; 4 days for small fish) (Fig. 2b). At 28°C, larger fish survived better than the small ones at the higher salinities (18 and 20 ppt), but there was no difference in survival in salinities less than 18 ppt. Size was less important at 18°C, where both size groups exhibited similar mortality except for the lowest salinity treatment (14 ppt).

We also observed differences in feeding between the two experiments: Fish at 28°C fed normally at all salinities during the experiment. However, only control fish fed normally at 18°C. Fish in other treatment groups did not eat during the exposure to elevated salinities at 18°C.

Table 2 Tolerance of *A. ocellatus* to chronic shifts in salinity (0 [control], 14, 16, 18, and 20 ppt) at two temperatures (a) warm (28°C) and (b) cold (18°C)

Salinity treatment	Fish size	Percent survival over 30-day challenge	Survival time (h)		Size comparison (small vs. large; *P*)	Warm versus cold (*P*)
			Mean	95% CI		
(a) Warm temperature						
0 (control)	Small	100	No mortality	N/A		N/A
	Large	100	No mortality	N/A	N/A	N/A
14 ppt	Small	100	No mortality	N/A		0.002
	Large	80	638	495–781	0.317	0.002
16 ppt	Small	40	576	426–726		0.003
	Large	20	398	211–585	0.320	0.002
18 ppt	Small	0	72	39–105		0.005
	Large	0	216	155–277	0.004	0.001
20 ppt	Small	0	29	19–38		0.180
	Large	0	44	37–51	0.041	0.002

Salinity treatment	Fish size	Percent survival over 30-day challenge	Survival time (h)		Size comparison (small vs. large; *P*)
			Mean	95% CI	
(b) Cold temperature					
0 (control)	Small	100	No mortality	N/A	
	Large	100	No mortality	N/A	N/A
14 ppt	Small	0	154	142–165	
	Large	0	206	174–238	0.021
16 ppt	Small	0	53	35–70	
	Large	0	77	54–100	0.139
18 ppt	Small	0	20	15–24	
	Large	0	26	17–35	0.243
20 ppt	Small	0	3	3–3	
	Large	0	10	9–10	0.046

Ten fish (5 small and 5 large) were used for each of five salinity treatments for a total of 50 fish per temperature. Kaplan–Meier survival analysis was used to compute mean survival time (in hours) and 95% confidence intervals (CI). For each salinity, fish size (small versus large) was compared with log-rank test. For each fish size, temperature (warm versus cold) was compared with a log-rank test

N/A not applicable

Lethal thermal maximum (LTmax) and minimum (LTmin) experiments

Individuals of *A. ocellatus* survived over a temperature range of 9–41°C. The main effects of acclimation temperature and body size affected LTmax, and there was no significant interaction. In contrast, for LTmin the effects of acclimation and body size interacted (Table 3; Fig. 3). For LTmax, larger fish were able to survive higher temperatures than smaller fish. Additionally, fish acclimated to 30°C were able to tolerate temperatures greater than fish acclimated to 25°C. For LTmin, body size and acclimation interacted such that small fish died at about the same temperature regardless of acclimation. However, for large fish acclimation temperature had an effect: fish acclimated to 20°C were able to withstand cooler temperatures than fish acclimated to 25°C.

Discussion

Our results showed that *A. ocellatus* were able to survive abrupt transfer to salinities as high as 16 ppt with no mortality. When salinity change was gradual (2 ppt/day), fish in the warm-temperature experiment

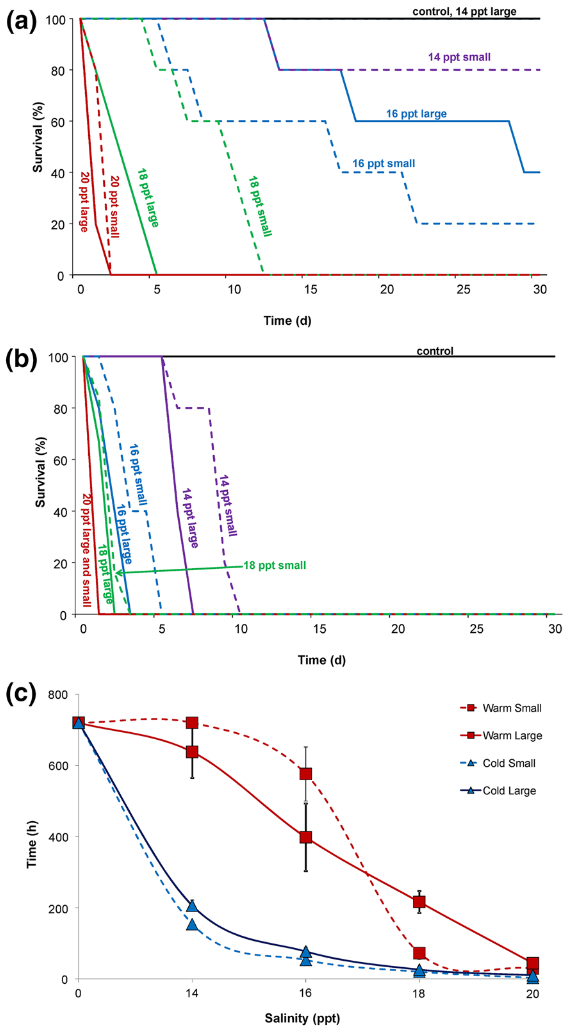

Fig. 2 Survival of *Astronotus ocellatus* over time when chronically exposed to different salinities (0 [control], 14, 16, 18, and 20 ppt). Two experimental temperatures were used: warm (28°C; *panel a*) and cold (18°C; *panel b*). Sample sizes were 5 small and 5 large fish for each salinity treatment × 5 salinity treatments × 2 temperatures = 100 fish. Small fish are represented by a *continuous line* and large fish by a *dashed line*. Mean survival times (±SE) are represent in *panel c*

(28°C) survived longer than fish in the cool-temperature experiment (18°C). Effects of fish size were variable: larger fish survived longer than smaller ones at the higher salinities when the temperature was warm, but when the temperature was cool fish size had little effect on survival. In the temperature-tolerance experiments, fish survived from 9 to 41°C for short periods of time.

Body-size effects on ecophysiological tolerance appears to be species-specific because other studies have shown that fish body size matters in thermal (Atwood et al., 2003; Charo-Karisa et al., 2005) and salinity experiments (Villegas, 1990), demonstrating that smaller fish were more susceptible to environmental changes (Sogard, 1997). However, other studies have found that body size is not a differential factor in tolerances (Ospina & Mora, 2004; Recsetar et al., 2012). Differences that we found might be attributed to the fact that smaller fish have greater surface area-to-volume ratio (Hutchison, 1976; Recsetar et al., 2012), which may lead to a more intense and earlier negative response to salinity and temperature stress. A reduced effect in larger fish also might be explained by the decrease in gill area to body mass as body mass increases (Muir, 1969), because the majority of ion exchange occurs in gills where there is direct contact with water and consequently an earlier response to environmental changes (Villegas, 1990). As showed by other studies, there are different physiological and biochemical responses between different sizes of *A. ocellatus*, with larger individuals surviving hypoxia better than smaller ones (Almeida-Val et al., 2000; Sloman et al., 2006), indicating that increased anaerobic capacity in larger individuals may lead to broader thermal and salinity tolerance. These findings suggest that there might be a positive relationship between body size and tolerance to abiotic factors for this species (Almeida-Val et al., 2000; Sloman et al., 2006). Our results showed that larger individuals were able to survive salinity stress longer in warm temperatures, but when temperatures were cool the size advantage disappeared.

In the Amazon basin, *A. ocellatus* is exposed exclusively to freshwater. Nevertheless, just like other freshwater cichlids (Briggs, 2003; Val et al., 2006; Schofield et al., 2011; Gutierre et al., 2014), *A. ocellatus* can tolerate some degree of salinity. Our specimens survived brackish-water salinities, surviving 20 ppt for 11 h, and about 16 ppt for a week after abrupt transfer. These results are similar to other cichlids such as *Oreochromis niloticus* (Linnaeus, 1758) that survived abrupt transfer to salinities up to 20 ppt (Schofield et al., 2011; Gutierre et al., 2014) and *Geophagus brasiliensis* (Quoy & Gaimard, 1824) that survived at least 6 h after abrupt transfer to 15 ppt (Gutierre et al., 2014). Fishes in the family Cichlidae (including *A. ocellatus*) are secondarily freshwater in their evolutionary history, and their closest relatives are mainly marine families such as Labridae,

Table 3 Comparison of factors affecting lethal thermal maximum (LTmax) and minimum (LTmin) for *A. ocellatus*

Source of variation	df	SS	MS	*F*	*P*
LTmax					
Body size	1	1.892	1.892	40.237	<0.001
Acclimation temperature	1	0.306	0.306	6.512	0.015
Body Size × acclimation temperature	1	0.132	0.132	2.812	0.102
Residual	36	1.693	0.047		
Total	39	4.024	0.103		
LTmin					
Body size	1	0.961	0.961	1.067	0.309
Acclimation temperature	1	8.1	8.1	8.993	0.005
Body Size × acclimation temperature	1	5.929	5.929	6.582	0.015
Residual	36	32.426	0.901		
Total	39	47.416	1.216		

Two-way analysis of variance was used to examine effects of acclimation temperature and body size (small versus large) on survival. Acclimation temperatures were 25 and 30°C for LTmax and 20 and 25°C for LTmin. For each experiment, 40 fish were tested (10 large and 10 small for each acclimation temperature)

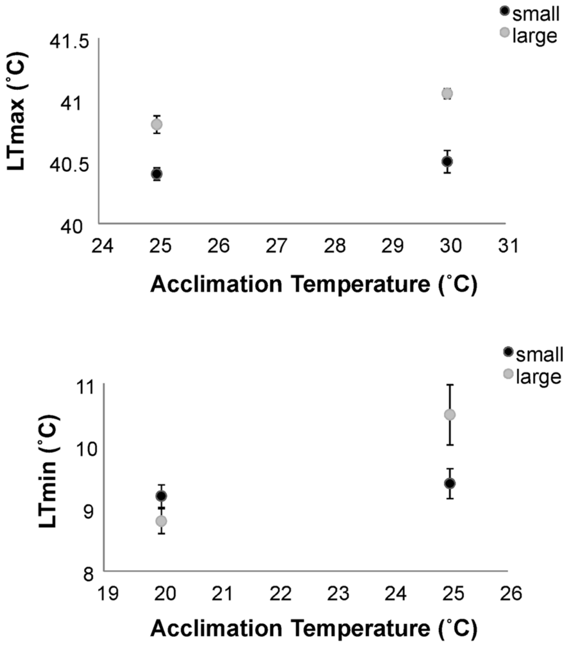

Fig. 3 Means plots (±SE) of lethal thermal maximum (LTmax) and minimum (LTmin) for *Astronotus ocellatus* by body size (large versus small) and acclimation temperature. $N = 10$ small + 10 large fish for each acclimation temperature × 2 acclimation temperatures × 2 experiments (LTmax and LTmin) = 80 fish (not including controls, see text for details). See Table 3 for results of two-way analysis of variance and post hoc comparisons

Pomacentridae, and Embiotocidae (Briggs, 2003; Nelson, 2006). Cichlids are notably euryhaline, and can adequately withstand salinity challenges while maintaining osmotic homeostasis. Previous reports have documented broad salinity tolerance in cichlids such as *Oreochromis mossambicus* (Peters, 1852), *O. niloticus*, *G. brasiliensis*, and *Hemichromis letourneuxi* (Sauvage, 1880) (Sardella et al., 2004; Langston et al., 2010; Schofield et al., 2011; Lowe et al., 2012; Gutierre et al., 2014). Those cichlids, when exposed to salinity variations (brackish or seawater), increase plasma osmotic and ionic concentrations, activate regulatory mechanisms, and return plasma concentrations to freshwater levels to maintain extracellular homeostasis (reviewed in Evans et al., 2005; Val et al., 2006).

The ability to survive an abrupt transfer to saline conditions may help explain why fish that escape from fish farms and hobbyist releases can become established in new basins or estuaries that have brackish waters (Langston et al., 2010; Gutierre et al., 2014). The ability to withstand abrupt salinity changes also can be a factor in survival when unpredicted salinity discharges result from hurricanes, storms, or human activity (Walker, 2001; Davis et al., 2004). Our results showed that *A. ocellatus* can certainly survive temporarily in brackish waters, but we need more research into salinity tolerance of its eggs and reproductive success to determine its potential to establish populations and inhabit brackish waters.

In the current study, we found that low-temperature acclimation (18°C) decreased the ability of *A. ocellatus* to tolerate higher salinities. Whereas fish acclimated to warm temperatures (28°C) survived for 30 days at 14 ppt, and about 2 days at 20 ppt, cold-acclimated fish did not survive more than 11 days at 14 ppt and less than 10 h at 20 ppt. The increased salinity tolerance in warm temperatures observed for *A. ocellatus* may be explained by its thermal history in warm freshwaters where it is native (Fontenele, 1951) and by its evolutionarily explained brackish and warm-water resistance (Briggs, 2003; Nelson, 2006; Val et al., 2006). Other studies have shown that low temperature can affect the survival of freshwater cichlids at high salinities (Stauffer, 1986; Schofield et al., 2010). Similarly, the cichlid *Oreochromis niloticus* had reduced survival to salinity changes at winter temperatures (Schofield et al., 2011). Despite the usually high salinity tolerance of tilapia species, *Oreochromis aureus* (Steindachner, 1864) and *Tilapia zillii* (Gervais, 1848) also showed decreases in salinity tolerance when exposed to low temperatures (11–16°C) versus medium temperatures (23–28°C; Lorenzi & Schlenk, 2014).

Our results showed that *A. ocellatus* can survive abrupt changes in temperature (1°C h^{-1}) within the range of 9–41°C. In our experiments, fish were only exposed to temperature extremes for a short period of time, and thus it is unclear how long they can survive in that range of temperatures. Previous results showed a low temperature tolerance of 12.9°C for Florida specimens of *A. ocellatus* previously acclimated to 28°C and with a temperature decrease rate of 2°C/day (Shafland & Pestrak, 1982). Australian specimens of *A. ocellatus* exposed to a change in temperature of 2°C every 76 h showed a tolerance range of 12.7–40.1°C (Webb, 2008). Those experiments lowered temperatures at a slower rate than the present study, with 1 or 3 days between the changes, and consequently, a longer period for fish physiologically to adjust (Beitinger et al., 2000; Beitinger & Lutterschmidt, 2011). *Astronotus ocellatus* had cold tolerance similar to other tropical invasive cichlids like *Cichlasoma urophthalmus* (Günther, 1862) (9.5°C), native to central America, and *Hemichromis letourneuxi* (9.1°C) and *Hemichromis lifalili* (Loiselle, 1979) (9.2°C), both native to tropical Africa (Shafland & Pestrak,1982; Webb, 2008; Schofield et al., 2010). The maximum temperature *A. ocellatus* could survive before perishing (about. 41°C) is similar to other tropical cichlids. For example, *Aequidens pulcher* (Gill, 1858), from tropical South and Central America, survived to 40.6°C and *Heros severus* (Heckel, 1840), native to the Amazon basin, withstood temperatures to 40.2°C (Webb, 2008). Similarly, *Etroplus suratensis* (Bloch, 1790) from tropical Sri Lanka persisted to 41°C (Rajaguru & Ramachandran, 2001). The *A. ocellatus* we used were from broodstock that had been acclimated to Florida conditions for at least a decade, yet they were still able to survive temperatures below 10°C for a short time. This result shows that the species has a good tolerance of low temperatures for short time periods, even though it is evolutionarily adapted to warm waters.

In a climate-change scenario of warmer temperate aquatic systems, invasive species such as *A. ocellatus*, with broad ecophysiological tolerances, may be better able to successfully establish and disperse into new places (Rahel & Olden, 2008). When faced with continual variations in salinity, as in estuarine regions or very dynamic habitats (such as near-shore ponds or flooded plains), stenohaline species have low chances of survival and as a consequence are less likely to establish there than euryhaline species (Ubeda et al., 2009). Species like *A. ocellatus* have a great potential for dispersal in these environments as they can withstand salinity variations for short periods of hours or days (Brown, et al. 2007; Rahel & Olden, 2008). The capacity to survive in a wide range of salinity and temperature conditions could support the species' invasiveness.

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