

Salinity effects on behavioural response to hypoxia in the non-native Mayan cichlid *Cichlasoma urophthalmus* from Florida Everglades wetlands

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This study quantified the hypoxia tolerance of the Mayan cichlid *Cichlasoma urophthalmus* over a range of salinities. The species was very tolerant of hypoxia, using aquatic surface respiration (ASR) and buccal bubble holding when oxygen tensions dropped to <20 mmHg (c. 1.0 mg l⁻¹) and 6 mmHg, respectively. Salinity had little effect on the hypoxia tolerance of *C. urophthalmus*, except that bubble holding was more frequent at the higher salinities tested. Levels of aggression were greatest at the highest salinity. The ASR thresholds of *C. urophthalmus* were similar to native centrarchid sunfishes from the Everglades, however, aggression levels for *C. urophthalmus* were markedly higher.

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Key words: aquatic surface respiration; Cichlidae; dissolved oxygen; ecophysiology; non-indigenous.

INTRODUCTION

Non-native fishes have been present in U.S. fresh waters since at least the 1600s, when the goldfish *Carassius auratus* (L.) was released by early settlers (Courtenay & Stauffer, 1990). In the past few decades, however, the number of non-native fishes has grown tremendously (Fuller *et al.*, 1999), in some cases with detrimental effects on local fish populations (Lever, 1996; Pimental, 2002; Irons *et al.*, 2007). Understanding environmental factors that facilitate or hinder the spread of non-native species is crucial to predicting potential effects on native species. In this study, behavioural response to hypoxia of a non-native species of cichlid fish from the Florida Everglades at three salinities (at which it occurs in the field) was quantified to determine the degree to which tolerance to hypoxia may impede or facilitate effects of this invasion.

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Documentation of environmental tolerances of native and non-native fishes is especially important in regions undergoing re-engineering or reclamation, such as the Florida Everglades, where human manipulation of the environment may result in habitat changes of varying suitability for native and non-native species. For example, drainage of the Rocky Glades region of the Everglades National Park during recent decades has resulted in low dry season groundwater levels in this karstic wetland (Loftus *et al.*, 1992). Reduced water levels increase incidences of hypoxia (*i.e.* dissolved oxygen levels $<2.0 \text{ mg l}^{-1}$) in solution holes, where fishes are now confined for months after the marsh surface dries in autumn or early winter. Fishes very tolerant of hypoxia (*e.g.* African jewelfish *Hemichromis letourneuxi* Sauvage; Schofield *et al.*, 2007) have an ecophysiological advantage over native species such as centrarchid sunfishes and come to dominate those refuges. Chronic hypoxia can also negatively affect fishes by reducing feeding and growth (Weber & Kramer, 1983; Cech *et al.*, 1984; Bejda *et al.*, 1992; Secor & Gunderson, 1998; Pichavant *et al.*, 2000; Taylor & Miller, 2001; Stierhoff *et al.*, 2003). It is important to measure the tolerances of native and introduced fishes to hypoxia in light of habitat changes associated with drainage and, conversely, with restoration.

At least a dozen species of non-native fishes in the family Cichlidae have become established in south Florida (Fuller *et al.*, 1999; Loftus *et al.*, 2004). Of these, the Mayan cichlid *Cichlasoma urophthalmus* (Günther) is probably the most flexible with respect to habitat requirements. *Cichlasoma urophthalmus* inhabits freshwater canals, marshes, cypress wetlands-domes and mangrove forests in the Everglades (Faunce & Lorenz, 2000; Trexler *et al.*, 2000; Faunce *et al.*, 2002; Loftus *et al.*, 2004; Rehage & Loftus, 2007), in part because of its remarkable physiological tolerance of a variety of environmental variables. For example, the species is tolerant of a wide range of salinities (Stauffer & Boltz, 1994). In its home range (Central America), it is a euryhaline species, inhabiting lowland freshwater, estuarine and coastal marine environments (Martinez-Palacios & Ross, 1992; Greenfield & Thomerson, 1997; Miller *et al.*, 2005; Taylor *et al.*, 2007). When introduced into the Everglades National Park, *C. urophthalmus* quickly spread not only through freshwater wetlands (Loftus, 1987) but also became established in the coastal mangrove areas fringing the park (Faunce & Lorenz, 2000; Rehage & Loftus, 2007). Unfettered by saline waters that restrict many native freshwater fishes, it has now become the most abundant fish along some reaches of the coastal mangrove zone in north-eastern Florida Bay, where it reproduces in estuarine waters (Loftus, 1987; Faunce & Lorenz, 2000; Schofield & Powell, 2006). It has also spread northward through the Everglades into Lake Okeechobee (Nico *et al.*, 2007), the Big Cypress Swamp (Bergmann & Motta, 2005) and has moved along Florida east and west coast habitats as far north as Merritt Island (Brevard County) and Tampa Bay (Pinellas County), respectively (Paperno *et al.*, 2008).

Tolerance of hypoxia is another factor that seems to allow *C. urophthalmus* to occupy a broad range of habitats, including areas with poor water quality. The term 'hypoxia' generally refers to conditions of dissolved-oxygen levels $<2.0 \text{ mg l}^{-1}$ (Chesney *et al.*, 2000), which may be chronic or occur on a diel

or seasonal basis. Hypoxia affects fish assemblage structure by delimiting the pool of species capable of occupying the habitat (Kilgore & Hoover, 2001), however, *C. urophthalmus* is regularly found in hypoxic conditions. Taylor *et al.* (2007) reported the species from sinkholes in Belize where dissolved-oxygen levels averaged 1.7 mg l^{-1} . The fish has also been collected in Everglades habitats (*e.g.* solution holes and mangrove creeks) where dissolved-oxygen levels frequently fall to $<1.5 \text{ mg l}^{-1}$ to as low as 0.48 mg l^{-1} (W. F. Loftus, J. C. Trexler, K. Dunker, S. E. Liston & J. S. Rehage, unpubl. data). Because the species occurs in habitats across a wide salinity range, a goal of this study was to determine how salinity affects its hypoxia tolerance. Osmoregulatory theory suggests that when salinity of water deviates from isosmosis, fishes incur metabolic costs (Fry, 1971). There seems to be, however, wide variation in the strength of this effect, with some fishes exhibiting strong salinity effects while others show none (Febry & Lutz, 1987).

Behaviour responses provide flexibility to deal with variation in availability of dissolved oxygen, and behavioural responses can be used as integrative indices of relative tolerance. Aquatic surface respiration (ASR) is a widespread behavioural adaptation to hypoxia, whereby fishes use the thin layer of oxygen-rich water at the air–water interface (Kramer & McClure, 1982). When hypoxia is not severe, fishes can continue their activities away from the water surface, however, as dissolved-oxygen levels approach zero, the fishes must spend more time at the water surface performing ASR (Kramer & Mehegan, 1981; Kramer, 1983; Schofield *et al.*, 2007). The use of ASR may allow fishes to survive otherwise lethal levels of dissolved oxygen (Kramer & Mehegan, 1981; Kramer & McClure, 1982). However, it also increases a fish's susceptibility to predation (Kramer *et al.*, 1983; Poulin *et al.*, 1987) and reduces the amount of time they could be spending in other activities (*e.g.* foraging, mating and defending territory). Thus, it is advantageous for fishes to be efficient in their use of ASR. Comparisons among and within species generally equate ASR efficiency with low ASR thresholds (Kramer & Mehegan, 1981; Kramer & McClure, 1982; Chapman *et al.*, 1995; Melnychuk & Chapman, 2002). In addition to ASR, some fishes use buccal bubble holding as an additional strategy to assist them in tolerating reduced dissolved-oxygen levels. Typically, the fish takes a bubble of atmospheric air into the buccal cavity, holds the bubble during ASR and passes water over the bubble, presumably increasing the water's dissolved-oxygen content before moving to the gills (Chapman *et al.*, 1995; Olowo & Chapman, 1996). Sometimes, however, the fish may simply hold the buccal bubble without using ASR (Todd & Ebeling, 1966). Because it may be an important additional technique allowing persistence during hypoxia, *C. urophthalmus* were monitored during the study for buccal bubble holding.

In this study, behavioural responses to hypoxia in *C. urophthalmus* (aquatic surface respiration thresholds, gill-ventilation rates, bubble holding and rates of aggression) were documented at three salinities [fresh (0), brackish (10) and marine (35)]. Aquatic surface respiration thresholds and aggression by *C. urophthalmus* were also compared to an earlier study on three other species from the Everglades (two native and one non-native).

MATERIALS AND METHODS

The fish were collected in May 2007 from the Rocky Glades region of Everglades National Park (25°26'05" N; 80°46'81" W) using 3 mm metal-mesh minnow traps and a 1.3 m-diameter cast-net. After transport to the laboratory (U.S. Geological Survey, Gainesville, FL, U.S.A.), fish were divided into three groups and held in large fibreglass tanks. All three groups were initially held for several days in well water at a salinity of 0.2. Then one group was gradually shifted to a salinity of 10 and another group to 35. The third group was maintained in well water (0.2; termed 0). Salinity in the holding tanks was never increased by more than five every 2 or 3 days. Synthetic saline water for the experiment was made by adding marine aquarium salts (Forty Fathoms[®], Marine Enterprises International Inc.; www.meisalt.com) to well water. Water for the holding tanks and experimental aquarium came from an on-site well in the freshwater Floridan Aquifer, from which water emerges at 20° C year-round. Aquarium heaters submersed in the holding tanks maintained the temperature near 30° C, within the range of summer water temperatures in the Everglades. The light cycle was 12L:12D. Temperature and dissolved oxygen in each holding tank ($n = 3$) were measured once per day while the trials were run (total of 21 days). Dissolved oxygen and temperature did not differ among the holding tanks (one-way ANOVA, d.f. = 2,62, $P > 0.05$ for both comparisons; mean \pm s.d. temperature = $30.4 \pm 0.9^\circ$ C; mean \pm s.d. dissolved oxygen = 6.0 ± 1.0 mg l⁻¹).

The experimental aquarium in which the fish were subjected to progressive hypoxia was a modified 37.85 l glass aquarium. Two mesh panels inset with silicone cement on each side of the aquarium divided it into three sections. The centre portion of the tank measured 380 \times 250 mm. Each side compartment was 60 \times 250 mm. An airstone was placed into each of the side compartments, and the tops of these compartments were sealed using Plexiglas panels and silicone. During the trials, nitrogen gas was bubbled into the aquarium through the airstones, which diffused through the mesh panels into the centre compartment. Dissolved-oxygen levels could not be lowered beyond c. 0.8 mg l⁻¹ by purging with nitrogen gas. Once that threshold was reached, a small amount (c. 3 g) of sodium sulphite was added after the 15 min readings to reduce the oxygen content of the water further. Generally, one addition of sodium sulphite was adequate to lower oxygen levels sufficiently to complete the trial, however, in rare instances, sodium sulphite was added twice during a trial (a total of c. 6 g). Previous studies have shown no differences in fish behaviour when water was deoxygenated with sodium sulphite v. nitrogen gas (Lewis, 1970), and use of sodium sulphite is common in hypoxia-tolerance experiments (Kramer & Mehegan, 1981; Melnychuk & Chapman, 2002). To increase water circulation, a small stirring magnet was placed at the bottom of the centre portion of the aquarium. A Yellow Springs Instruments (YSI model 556 MPS Yellow Springs Instruments Inc.; www.ysi.com) probe was placed in the centre compartment of the aquarium to record dissolved oxygen and temperature. Water was removed from the aquarium at the end of each trial and replaced for each trial. Water temperature in the experimental aquarium was slightly cooler for trials at a salinity of 35 (mean \pm s.d. = $30.1 \pm 0.3^\circ$ C) than salinities of 0 (mean = $30.4 \pm 0.6^\circ$ C) and 10 (mean = $30.5 \pm 0.3^\circ$ C.; one-way ANOVA, d.f., = 2,465, $P < 0.001$; *post hoc* with Dunnett's T3, $P > 0.10$ for comparison of salinities of 0 and 10; $P < 0.001$ for all other comparisons).

At the beginning of each trial, the experimental aquarium was filled with water from the holding tank for the salinity to be tested that day (0, 10 or 35). One hour before the beginning of each trial, three fish were placed in the experimental aquarium. The acclimation period was limited to 1 h because longer times allowed the fish, which were quite aggressive, to fight and injure each other. No individual was used in more than one trial. Body size, mass (M) and total length (L_T) of the fish did not differ among treatments (mean \pm s.d. $M = 8.18 \pm 4.18$ g, range 2.06–21.44 g; one-way ANOVA, d.f. = 2,60, $P > 0.05$; mean \pm s.d. $L_T = 80.1$ mm \pm 12.8 mm, range 53.0–107.0 mm; one-way ANOVA, d.f. = 2,60, $P > 0.05$). For each trial, body sizes of the three fish were matched as closely as possible. The species generally reaches sexual maturity

between *c.* 75 and 80 mm L_T (Miller *et al.*, 2005), although gonadal development can occur at as small as 60 mm L_T (Chávez-López *et al.*, 2005). Thus, specimens used in this study were most likely a mixture of juveniles and young adults.

An opaque paper blind with a small viewing port was attached around the aquarium once fish were added. Atmospheric air was pumped into the aquarium through an airstone during the 1 h acclimation period (before dissolved-oxygen levels were lowered). At the beginning of the hypoxia trial, the air source was turned off and nitrogen gas was pumped in. Every 15 min, the following data were recorded: dissolved oxygen, temperature, number of fish using ASR (recorded at 10 s intervals, 10 times), number of fish exhibiting aggressive behaviours (*e.g.* chasing and biting, recorded at 10 s intervals, 10 times), number of fish holding a buccal bubble and number of gill ventilations (recorded twice for each fish at 15 s intervals). When a fish began bubble holding, the duration of each bout of bubble holding and ASR was recorded for the remainder of the experiment. For each of the three salinities (0, 10 and 35), seven sets of three fish each were tested for a total of 63 fish. Aquatic oxygen levels were measured in mg l^{-1} . For analyses and presentation, dissolved oxygen concentrations were converted to oxygen tensions (mmHg) using the tables in Colt (1984).

At the beginning of the trials, dissolved-oxygen levels were generally between 7 and 9 mg l^{-1} , and were reduced to $<0.10 \text{ mg l}^{-1}$ over a period of *c.* 4.5–5.0 h. Dissolved-oxygen levels were then held at $<0.10 \text{ mg l}^{-1}$ for 1 h, after which the trial was ended. If a fish lost equilibrium (*i.e.* inability to maintain upright position) during the trial, it was removed and immediately transferred to a well-oxygenated aquarium to recover. Time, temperature and dissolved oxygen were recorded when each individual lost equilibrium. Following recovery, fish were weighed (to the nearest 0.1 g after being blotted dry) and measured (to 10 mm L_T). A series of *C. urophthalmus* used in this study was deposited as voucher specimens at the Florida Museum of Natural History (UF 170301).

DATA ANALYSIS

The level of oxygen at which 10 (A_{SR10}), 50 (A_{SR50}) and 90% (A_{SR90}) of the fish performed ASR was estimated by fitting curves to plots of dissolved-oxygen tension and % of A_{SR} . The % of A_{SR} was calculated as the number of fish in a group using ASR divided by the total number of fish, averaged over the 10 observations in a given sample. Linear regression analyses were run separately for each salinity treatment (using both linear and growth curves) and indicated no relationship between M and L_T and ASR thresholds.

Aquatic surface respiration thresholds were compared using one-way ANOVA with salinity (0, 10 and 35) as the main effect and ASR thresholds (A_{SR10} , A_{SR50} and A_{SR90}) as the response variables. Levene's test was used to examine homogeneity of variances, and because the results were not significant ($P > 0.05$ for all response variables), Scheffé's *post hoc* tests were used to compare variables.

To compare hypoxia tolerance of *C. urophthalmus* to other Everglades fishes, data from this study were compared to data adapted from Schofield *et al.* (2007) for two native species: warmouth *Lepomis gulosus* (Cuvier) and dollar sunfish *Lepomis marginatus* (Holbrook), and the non-native African jewelfish *H. letourneuxi*. One-way ANOVA was used within each ASR threshold to compare across species, and Dunnett's T3 test was used for *post hoc* comparisons as variances were not homogeneous. For *C. urophthalmus*, only data from the freshwater trials were used because all other species in the earlier study were tested in fresh water.

Bubble-holding frequency was calculated for each 15 min observation (number of fish in a group holding a buccal bubble, divided by total number of fish, averaged over the 10 observations in a sample) for each species. Because bubble holding never occurred at oxygen tensions $>6 \text{ mmHg}$ (*c.* 0.3 mg l^{-1}), the dataset only included observations at $\leq 6 \text{ mmHg}$. Frequency of bubble holding was compared among salinities with one-way ANOVA and Dunnett's T3 *post hoc*. To compare the percentage of time each fish spent

bubble holding v. ASR, a different sub-set of the data was used, including only readings taken during the last hour of the experiment [when dissolved-oxygen values were $<0.1 \text{ mg l}^{-1}$ (c. 2 mmHg)]. This sub-set of data only included fish that used both ASR and bubble holding ($n = 35$); fish that only used ASR were excluded from this dataset.

To assess whether gill-ventilation rates varied with ASR, a sub-set of the data (readings $< 30 \text{ mmHg}$) was used. These data were further divided into readings that were taken before and after the onset of ASR. Because fish began to use ASR at c. 15 mm Hg, delimiting the dataset to $\leq 30 \text{ mmHg}$ provided approximately equal datasets for comparison. Pre-ASR and post-ASR readings were compared with Mann–Whitney *U*-tests for each salinity. Gill-ventilation rates (divided into pre-ASR and post-ASR) were then compared across salinities with the non-parametric Kruskal–Wallis test.

Per cent aggression was calculated for each 15 min observation (number of fish in a group showing aggression, divided by total number of fish and averaged over the 10 observations in a sample) for each species. To examine overall frequency of aggressive behaviour, data from this experiment (freshwater only) were compared to data collected in the same manner from three other Everglades species: two native centrarchid sunfishes (*L. gulosus* and *L. marginatus*) and the non-native *H. letourneuxi* (Schofield et al., 2007). Aggression rates were compared among the species with one-way ANOVA and Dunnett's T3 *post hoc*. The data were then divided into pre-ASR and post-ASR and compared (within species) with Mann–Whitney *U*-test. SPSS version 12.0 (SPSS Inc., Chicago; www.spss.com) was used for all statistical tests.

RESULTS

Sixty-three *C. urophthalmus* were used in the experiment (three fish per trial \times three salinities \times seven replicates per salinity). Of these, 11 fish lost equilibrium (one at 0, two at 10 and eight at 35).

Aquatic surface respiration thresholds for *C. urophthalmus* were (mean \pm s.e.): $A_{\text{SR}10} 15.7 \pm 1.2 \text{ mmHg}$, $A_{\text{SR}50} 10.9 \pm 0.9 \text{ mmHg}$, $A_{\text{SR}90} 6.5 \pm 0.5 \text{ mmHg}$. There was little variation with salinity (Fig. 1). Thresholds for $A_{\text{SR}50}$ and $A_{\text{SR}90}$ did not differ with salinity. For $A_{\text{SR}10}$, fish at 35 salinity had higher A_{SR} thresholds than at 10 (one-way ANOVA comparing three salinities, $P < 0.01$; Scheffé's test comparing 10 and 35, $P < 0.05$; all other multiple comparisons $P > 0.05$; Fig. 1). Compared with Everglades centrarchid sunfishes, *C. urophthalmus* ASR thresholds did not differ from those of *L. marginatus* at $A_{\text{SR}10}$ and $A_{\text{SR}50}$, or from *L. gulosus* at any ASR threshold (Fig. 2). ASR thresholds for the non-native *H. letourneuxi* were significantly lower than all other species.

Cichlasoma urophthalmus were observed holding buccal bubbles when oxygen tensions dropped to $<6 \text{ mmHg}$. This behaviour was not consistent as only about half of the fish used bubble holding (35 of 63). Bubble-holding frequency was related to salinity (one-way ANOVA, d.f. = 2,113, $P < 0.01$). Bubble holding was least frequent at salinities of 0 and 10, and those two treatments did not differ (Dunnett's T3, $P > 0.05$). At 35 salinities, *C. urophthalmus* used bubble holding more frequently than at 0 (Dunnett's T3, $P < 0.05$). There was no significant difference between 10 and 35 salinities (Dunnett's T3, $P > 0.05$). When holding buccal bubbles, fish did not perform ASR, or otherwise ventilate their gills. Instead, fish took in a bubble of atmospheric air, gently dropped to the floor of the tank and remained there motionless with opercles clamped. Generally, fish alternated between bubble holding and ASR. The majority of time was spent in ASR (mean \pm s.d. = $85.4 \pm 19.3\%$; range 28.4–99.6%); less time was spent in bubble holding (mean \pm s.d. = $8.8 \pm 10.4\%$ range

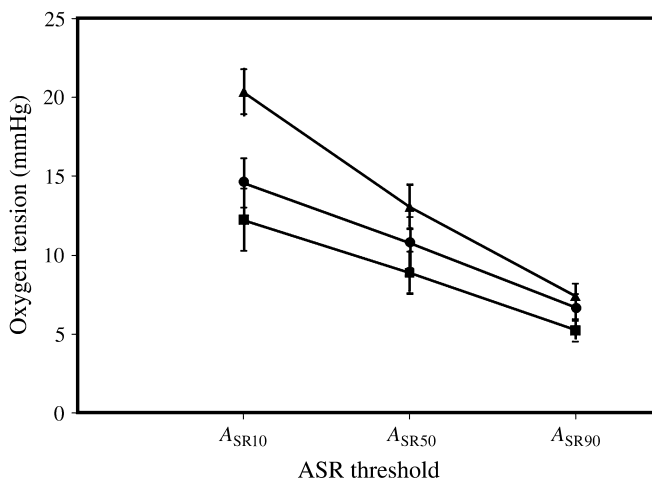


FIG. 1. Mean \pm s.e. aquatic surface respiration (ASR) thresholds [the levels of oxygen at which 10 (A_{SR10}), 50 (A_{SR50}) and 90% (A_{SR90}) of the fish performed ASR] for *Cichlasoma urophthalmus* at three salinities [0 (●), 10 (■) and 35 (▲)]. For A_{SR50} and A_{SR90} , there was no difference among salinities (one-way ANOVA, $P > 0.10$). For A_{SR10} , the threshold for salinity at 35 was significantly higher than at 10 (one-way ANOVA comparing three salinities, $P < 0.01$; Scheffé's test comparing 10 and 35, $P < 0.05$; all other multiple comparisons $P > 0.05$).

0.4–36.2%). Bubble-holding bouts lasted a mean \pm s.d. of 29.1 (± 70.5 s), although the range was large (4–600 s).

Gill-ventilation rates did not vary across salinities (analysed separately for pre-ASR and post-ASR onset). Fish, however, lowered their gill-ventilation

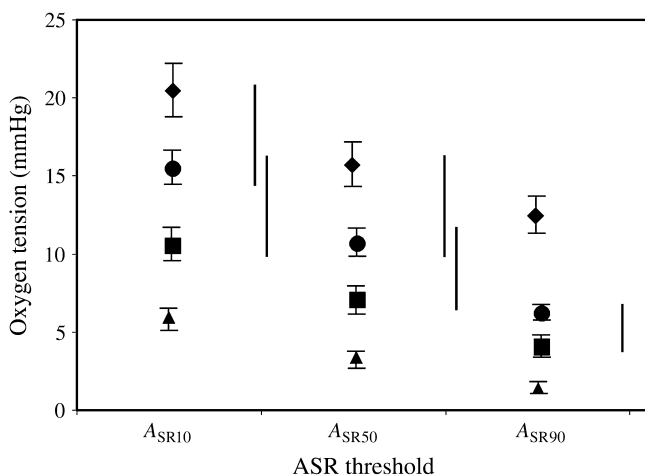


FIG. 2. Comparison of ASR thresholds (see Fig. 1) for four species from the Everglades: *Lepomis marginatus* (◆, native), *Cichlasoma urophthalmus*; freshwater only (●, non-native), *Lepomis gulosus* (■, native) and *Hemichromis letourneuxi* (▲, non-native). Data for *C. urophthalmus* are from this study; all other data are from Schofield *et al.* (2007). One-way ANOVA (with Dunnett's T3 *post hoc*) was used to compare across species within each ASR threshold. Species joined with solid vertical line to the right are not significantly different at $P < 0.05$ for that ASR threshold.

rates after the initiation of ASR at all three salinities ($P < 0.01$ for all three comparisons).

Aggression varied significantly with salinity (one-way ANOVA, d.f. = 2,467, $P < 0.05$). Aggression levels were lowest at a salinity of 10, highest at 35 (Fig. 3) and differed significantly (Dunnett's T3 test, $P < 0.05$); no other comparison differed ($P > 0.05$). *Cichlasoma urophthalmus* was more aggressive than native centrarchid sunfishes. Pre-ASR aggression levels for *C. urophthalmus* (fresh water only) were higher than for *L. gulosus* and *L. marginatus* (Fig. 4). As with the other species, *C. urophthalmus* reduced its aggression levels after the onset of ASR (Mann–Whitney *U*-test, $P < 0.01$). Although its aggression level was attenuated during ASR, *C. urophthalmus* remained more aggressive than the two native centrarchids and the non-native *H. letourneuxi* when they performed ASR (Fig. 4).

DISCUSSION

Behavioural response of the non-native *C. urophthalmus* from Everglades wetlands suggests high tolerance to hypoxia in this fish as well as the ability to withstand a high level of variation in salinity under hypoxic conditions. When comparing salinities of 0 and 10, there were no differences in ASR thresholds, gill-ventilation rates, bubble-holding frequency or aggression. At 35, however, A_{SR10} threshold and bubble-holding frequency were higher than for 0 and 10, suggesting the highest salinity was more stressful for the fish during hypoxia. Overall, the fish compensated well for osmotic differences and demonstrated only small behavioural modifications related to increased stress when it encountered hypoxia at the highest salinity tested.

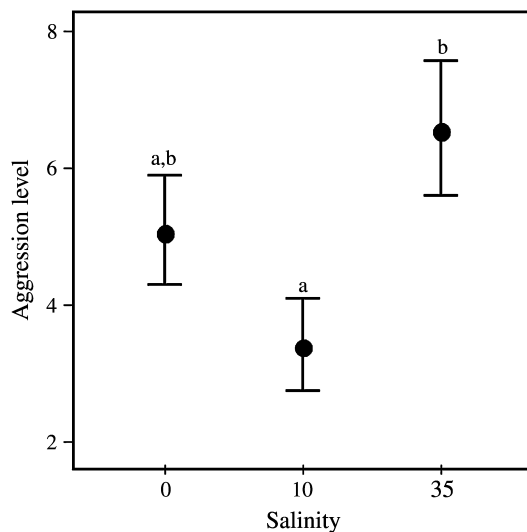


FIG. 3. Mean \pm s.e. aggression levels (aggression frequency calculated for each 15 min observation, $n = 10$) of *Cichlasoma urophthalmus* at three salinities (0, 10 and 35). Aggression levels at 10 and 35 were significantly different (Dunnett's T3, $P = 0.026$), while levels at 0 did not differ from those at 10 or 35 ($P < 0.05$, as indicated by similar lower case letters above bars).

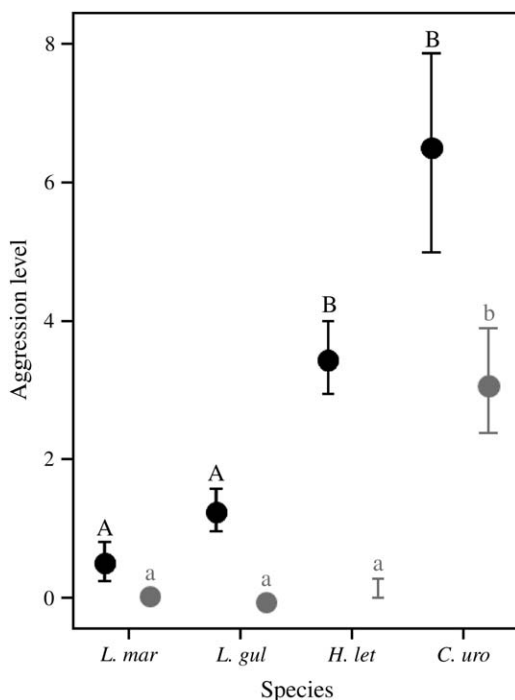


FIG. 4. Mean \pm S.E. aggression levels (aggression frequency calculated for each 15 min observation, $n = 10$) for four species of Everglades fishes: *Lepomis marginatus* (*L. mar*; native), *Lepomis gulosus* (*L. gul*; native), *Hemichromis letourneuxi* (*H. let*; non-native) and *Cichlasoma urophthalmus*; fresh water only (*C. uro*; non-native). ●, before aquatic-surface-respiration (ASR) onset; ●, after ASR onset (*i.e.* during ASR). Different upper and lower case letters denote significantly different aggression levels among species ($P < 0.05$).

The fish began ASR when oxygen tensions dropped to to <20 mmHg (*c.* 1.0 mg l^{-1}). After initiation of ASR, gill-ventilation rates decreased, indicating that the species is relatively efficient in its use of ASR. The ASR thresholds of the fish were slightly lower than the average reported by Kramer & McClure (1982) for small tropical fishes but were approximately equivalent to native centrarchid sunfishes from the Everglades that occupy seasonally hypoxic habitats (Schofield *et al.*, 2007). Compared with other cichlids, it had higher ASR thresholds than the Jack Dempsey *Cichlasoma octofasciatum* (Regan) (Obordo & Chapman, 1997) and *H. letourneuxi* (Schofield *et al.*, 2007). These results agreed with a previous study that indicated *C. urophthalmus* tolerated low-oxygen levels well and could survive virtual anoxia for up to 2 h (Martinez Palacios & Ross, 1986). Additionally, the results corroborated field observations of *C. urophthalmus* from waters with very little dissolved oxygen (Taylor *et al.*, 2007; W. F. Loftus, J. C. Trexler, K. Dunker, S. E. Liston & J. S. Rehage, unpubl.data).

Osmoregulatory theory suggests that additional metabolic costs are brought on by an increase in gradient away from isosmosis, and that oxygen uptake is metabolically more expensive at increased salinities (Rao, 1968; Bœuf & Payan,

2001). Additionally, the solubility of oxygen, and consequently the absolute amount held in solution, decreases with increasing salinity (Kinne, 1964). This reduced solubility results in less oxygen available to the fish at the air–water interface that is the target of ASR, while, in turn, dissolved oxygen is more rapidly depleted at higher salinities (Gee & Gee, 1991). Thus, stress from hypoxia should be lowest at 10 (nearest isosmosis), higher at 0 and highest at 35. Demands from osmoregulation, however, may not always present metabolic challenges to fishes (Nordlie, 1978; Febrý & Lutz, 1987; Bœuf & Payan, 2001; Chatelier *et al.*, 2005). Indeed, the relationship between osmoregulation and metabolism is unclear and has not been accurately addressed in whole fishes (Febrý & Lutz, 1987). The literature describes studies of metabolic rates measured in acclimated fishes that have variously increased, remained unchanged or decreased as salinity increased (Febrý & Lutz, 1987). *Cichlasoma urophthalmus* seems to compensate for the increased osmotic stress well. A previous study of growth rates of fish at salinities from 0 to 35 documented no significant differences (Martinez-Palacios *et al.*, 1990). Physiological changes made to compensate for osmoregulation (*e.g.* hormone secretion), however, may have unknown secondary effects in other arenas of a fish's physiology (Bœuf & Payan, 2001).

Cichlasoma urophthalmus exhibited an unusual bubble-holding technique by gulping atmospheric air, descending to the substratum while holding the bubble and remaining motionless, sometimes for long periods of time. This type of bubble holding is not unique and has been recorded in other studies (Todd & Ebeling, 1966). It is very different from typical bubble-holding behaviour, however, in which the fish holds the bubble in the buccal cavity during ASR and passes water over the bubble, presumably increasing the water's dissolved-oxygen content before moving to the gills (Chapman *et al.*, 1995; Olowo & Chapman, 1996). In this study, bubble-holding behaviour was most frequent at a salinity of 35. It is suggested that the reduced solubility of oxygen in saline waters, rapid depletion of dissolved oxygen in saline surface waters and increased cost of osmoregulation at higher salinity reduced the utility of ASR so that fish increased their use of bubble holding.

Hypoxia attenuated aggression somewhat in *C. urophthalmus*. Despite the reduction in aggression levels after the onset of ASR, they remained remarkably high relative to other species (Fig. 4), in keeping with the pugnacious, territorial reputation of this species (Hervey & Hems, 1953; Axelrod & Vorderwinkler, 1962; Nico *et al.*, 2007). Aggression was most frequent for *C. urophthalmus* at the highest salinity (35). Jennings (1991) found similar behaviour in blackchin tilapia *Sarotherodon melanotheron* Rüppell that were more aggressive at salinities of 15 and 35 than at 5. Aggression in the native (Iberian) killifish *Aphanius fasciatus* (Valenciennes) did not change with increased salinity, although the non-native *Gambusia holbrooki* Girard was more aggressive at higher salinities (Alcaraz *et al.*, 2008). Although aggression was not specifically studied, some studies have demonstrated reductions in overall activities of euryhaline fishes at higher salinities (Swanson, 1998; Plaut, 2000). At this time, it is unclear why *C. urophthalmus* expressed more aggression at presumably more metabolically costly (*i.e.* higher) salinities, although there may be a relation to increased blood levels of certain hormones

(e.g. cortisol) in saline waters. Future research is needed to clarify the interrelations between salinity and aggression in this (and other) euryhaline species.

Overall, because *C. urophthalmus* tolerated hypoxia well, its continued geographic range expansion and survival in Florida habitats are unlikely to be hindered by hypoxia. Indeed, the species has already colonized much of the southern half of the Florida peninsula, especially lowland coastal areas. The species also exhibits additional characteristics thought to be conducive to invasion: biparental care of young, broad diet, aggression and wide physiological tolerance of environmental variables. Based on laboratory work and field observations, the most likely restriction to its geographic expansion through Florida will be its intolerance of cold water temperatures during the winter (P. J. Schofield, unpubl. data).

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