ORIGINAL PAPER

Tolerance of nonindigenous cichlid fishes (*Cichlasoma urophthalmus*, *Hemichromis letourneuxi*) to low temperature: laboratory and field experiments in south Florida

Pamela J. Schofield · William F. Loftus ·
Robert M. Kobza · Mark I. Cook · Daniel H. Slone

Abstract The cold tolerance of two non-native cichlids (*Hemichromis letourneuxi* and *Cichlasoma urophthalmus*) that are established in south Florida was tested in the field and laboratory. In the laboratory, fishes were acclimated to two temperatures (24 and 28°C), and three salinities (0, 10, and 35 ppt). Two endpoints were identified: loss of equilibrium (11.5–13.7°C for *C. urophthalmus*; 10.8–12.5°C for *H. letourneuxi*), and death (9.5–11.1°C for *C. urophthalmus*; 9.1–13.3°C for *H. letourneuxi*). In the field, fishes were caged in several aquatic habitats during two winter cold snaps. Temperatures were lowest (4.0°C) in the shallow marsh, where no fish survived, and warmest in canals and solution-holes. Canals and ditches as shallow as 50 cm provided thermal refuges for these tropical fishes. Because of the effect on survival of different habitat types, simple predictions of ultimate geographic expansion by non-native fishes using latitude and thermal isoclines are insufficient for freshwater fishes.

P. J. Schofield (✉) · D. H. Slone
U.S. Geological Survey, 7920 NW 71st Street,
Gainesville, FL 32653, USA
e-mail: pschofield@usgs.gov

W. F. Loftus
U.S. Geological Survey, 40001 State Road 9336,
Homestead, FL 33034, USA

Present Address:
W. F. Loftus
Aquatic Research & Communication, LLC,
1759 NW 20th Street, Homestead, FL 33030, USA

R. M. Kobza · M. I. Cook
South Florida Water Management District, Everglades
Division, 3301 Gun Club Road, West Palm Beach,
FL 33406, USA

Introduction

Non-native species are considered the second-greatest threat to biodiversity following habitat loss (Wilcove et al. 1998). Understanding the factors that lead to successful invasion and spread of non-native species is critical, as it should help prevent future invasions and mitigate the effects of recent invaders through early detection and the prioritisation of management measures (García-Berthou 2007). Quantifying the limits of a non-native species' tolerance to environmental variables is important in predicting its eventual geographic spread. For fishes, broad physiological tolerance to environmental variables (e.g., temperature, salinity, low oxygen) may be a key factor facilitating establishment beyond native ranges (Moyle and Light 1996; Moyle and Marchetti 2006).

Dozens of non-native freshwater fishes have established in Florida (Fuller et al. 1999; Shafland et al. 2008), and many of these successful invaders have proven exceptionally tolerant to environmental variables. Several non-native fishes can be found in southern and central Florida over a range of salinities from freshwater to marine conditions [e.g., Mayan cichlid *Cichlasoma urophthalmus* (Faunce and Lorenz 2000; Paperno et al. 2008), blackchin tilapia *Sarotherodon melanotheron* (Jennings and Williams 1992; Faunce and Paperno 1999), African jewelfish *Hemichromis letourneuxi* (Jackson and Adams, unpublished data)]. A number of successful invaders are air-breathers (e.g., walking catfish *Clarias batrachus*, brown hoplo *Hoplosternum littorale*, bullseye snakehead *Channa marulius*, Asian swamp eel *Monopterus* sp. c.f. *albus*), and at least two cichlid fishes that are not air-breathers, but are exceptionally tolerant to hypoxia (*C. urophthalmus*, *H. letourneuxi*; Schofield et al. 2007, 2009). Furthermore, non-native fishes persist in south Florida despite high levels of pesticides (Miles and Pfeuffer 1997) and mercury (Kannan et al. 1998).

While the non-native fishes of south Florida generally exhibit great physiological flexibility regarding environmental variables, one factor (i.e., cold tolerance) seems to be an exception, perhaps not surprising for a fauna almost entirely derived from the tropics (Loftus 2000). The inability to tolerate cold winter temperatures appears to be the most important factor restricting many non-native fishes to the sub-tropical southern portion of the Florida peninsula (Shafland and Pestrak 1982). The native ranges of most of the successfully established non-native fishes in south Florida include tropical and sub-tropical regions of Asia, Africa and the Americas (Fuller et al. 1999), and the similarity in climates between donor and recipient regions probably helped facilitate establishment. However, the narrow range of temperature tolerance exhibited by species introduced into south Florida may restrict their geographic expansion to the north, as well as limit their populations in south Florida habitats that do not provide refuge from cold temperatures.

The Everglades and adjacent Big Cypress Swamp comprise the largest freshwater ecosystems in south Florida. Typical natural habitats include shallow marshes, sawgrass ridges and sloughs, cypress swamps and alligator holes throughout those wetlands

(Loftus and Kushlan 1987) as well as solution holes in the limestone matrix of the southern peripheral wetlands of the Rocky Glades (Kobza et al. 2004). Water temperatures fluctuate seasonally and with differences in water depth and the influence of ground water. Hydroperiods vary both seasonally and annually; shallow marshes in the Rocky Glades and Big Cypress Swamp dry completely each year, while alligator ponds (the deepest natural habitats) hold water year-round except during the harshest droughts (Loftus and Kushlan 1987). Even during high water periods, depths in the natural habitats rarely exceed 1–1.5 m. Solution holes vary in depth and area; some continue to hold water throughout the dry season (and serve as refuges for fishes in the Rocky Glades [Loftus et al. 2006]), while most dry completely under today's water management regime. Added to those natural habitats is a complex, anastamosing matrix of canals estimated at 1,600 miles in length (Light and Dineen 1994) that provides permanent deep-water refugia (Gunderson and Loftus 1993), and harbours relatively high numbers of non-indigenous fishes (Loftus and Kushlan 1987; Trexler et al. 2000). Canals cut deep into the limestone bedrock receive warm groundwater from the aquifer and serve as permanent thermal and hydrologic refugia. Many non-native fishes are found in high densities in thermally buffered canals and deeper natural habitats; however, their distribution and invasion success in shallower, thermally less-stable natural habitats is less certain and may be limited by physiological constraints (i.e., low-temperature tolerance).

Only a few studies have tested the low-temperature tolerance of Florida's non-native fishes (e.g., Shafland and Pestrak 1982; Stauffer et al. 1984; Stauffer 1986; Zale and Gregory 1989; Jennings 1991; Stauffer and Boltz 1994). Furthermore, although there are many anecdotal observations of winter kills of non-native fishes in Florida after sudden temperature decreases, we know of no field experiments designed to test low-temperature tolerance of the fishes in situ. To accurately predict eventual spread of a species, it is important to delineate its tolerance to cold temperatures, understand how thermal regimes differ among various aquatic habitats and document the concomitant effects on survival. Herein we provide data on the low-temperature tolerance of two non-native cichlid fishes established in south Florida (African jewelfish

Hemichromis letourneuxi, Mayan cichlid *Cichlasoma urophthalmus*). The primary aims of this study were to (1) determine the low-temperature tolerance of those species using a controlled laboratory technique that has not been used previously with those species, and (2) delineate the responses of water temperatures in natural and man-made aquatic habitats to winter air temperatures, and how that in turn affects the survival of the non-native species in those habitats. The data are important for the prediction of the invasibility and spread of these species through the various aquatic habitats of south Florida (and beyond) and to development of management options for control.

Materials and methods

Study species

We chose two common cichlid fishes (*C. urophthalmus, H. letourneuxi*) that are widespread in south Florida for this study. Both species occur over a wide range of salinities in their native range (Lamboj 2004; Miller et al. 2005) as well as in south Florida (Faunce and Lorenz 2000; Rehage and Loftus 2007; Adams and Wolfe 2007; Jackson unpublished data). *Cichlasoma urophthalmus* was first found in mangrove habitats of Florida Bay in 1983 (Loftus 1987) and currently ranges throughout south Florida freshwater and coastal habitats north to Tampa Bay along the Gulf of Mexico and to Merritt Island on the Atlantic coast (Paperno et al. 2008). *Hemichromis letourneuxi* was introduced to south Florida in the 1960s (Rivas 1965, as *H. bimaculatus*). It persisted in a relatively restricted range (urban canals around Miami) until recently (2000), when it suddenly expanded its geographic range. This expansion was coincident with changes in water delivery to the Everglades National Park (Loftus et al. 2006), but might also have resulted from new introductions to the west and north (see Langston et al. 2009 for details). Currently, the geographic ranges of *C. urophthalmus* and *H. letourneuxi* in Florida are roughly equivalent.

Laboratory experiments

We used the critical thermal methodology (CTM) to quantify temperature tolerances of the two focal species, a method used extensively to determine thermal tolerances of fishes (reviewed in Beitinger et al. 2000). It consists of subjecting fish to a continuous, constant (i.e., linear) decrease in temperature until an endpoint is reached. In our experiments, we used two endpoints: loss of equilibrium (LOE) and death. Loss of equilibrium was defined as the fish lying on its side and unable to right itself. Death was defined as lack of movement by the fish after it was gently prodded while submersed; if no response was observed, the fish was removed from the water with a hand net for further examination. Studies that use the LOE endpoint sometimes assume that the experimental fish will survive if immediately returned to pre-trial acclimation temperature after LOE has occurred (e.g., Cox 1974; Ford and Beitinger 2005). We wanted to examine this assumption further, as it has important ecological relevancy. It is important to know whether fish survive after losing equilibrium; if they do not, then the LOE and death endpoints are ecologically equivalent.

Although *H. letourneuxi* and *C. urophthalmus* occur in a wide range of salinities, it is unclear how salinity affects cold tolerance (Zale and Gregory 1989; Stauffer et al. 1984). Therefore, we tested the species at three salinities (0 [freshwater], 10 [estuarine] and 35 ppt [marine]). Additionally, to examine the effect of acclimation temperature on cold tolerance, groups of fish were tested after acclimation to two temperatures (24 and 28°C). Fishes were collected from freshwater habitats in south Florida and transported to the USGS laboratory in Gainesville, Florida. At the laboratory, groups of each species were separated into six holding tanks at three acclimation salinities (0.2 [well water; hereafter termed "0"], 10 and 35 ppt) and two acclimation temperatures (24 and 28°C). To adapt fish to the salinity treatments, commercial marine-aquarium salt was added to the tanks to increase the salinity by 2–3 ppt every 2–3 days. The fishes were held at these target salinity/temperature combinations for several weeks (13 days for *H. letourneuxi*; 32 days for *C. urophthalmus*) before experiments were conducted. Fish were fed ad libitum 2 or 3 days per week during the holding period. No fish underwent more than one trial.

Low-temperature tolerance trials were conducted in November 2007 (*H. letourneuxi*) and January 2008 (*C. urophthalmus*). A large (3.1 × 6.2 m) walk-in environmental chamber (cooler) was used to house

the fishes during the low-temperature challenge. To prepare for the experiment, the environmental chamber was set at 28°C with a light cycle of 12:12 h. An additional self-contained, climate-controlled laboratory room ("warm room") was set at 25°C with the same light cycle as the environmental chamber. Racks were placed in the environmental chamber and warm room to hold individual fish containers (clear plastic boxes, 23 w × 35.5 l × 14 h cm). In the environmental chamber, plastic boxes were set up with water (at 28°C), airstones, tubing, lids (to prevent jumping escapes) and blinds (on three sides) to prevent fishes from seeing each other.

Fishes in the holding tanks at 28°C (0, 10 and 35 ppt), were weighed, measured and placed individually in prepared containers in the environmental chamber. Fishes from each salinity × holding-temperature group were divided into three treatments: control, LOE and death. Fish size was controlled so that it was similar across treatments; otherwise assignment of treatments to individual fishes was haphazard. Controls were removed from the chamber (still at 28°C) and placed in the warm room; fishes in the other treatments (LOE and death) remained in the environmental chamber at their respective salinity treatments. Sample sizes for each species × temperature × salinity × treatment (control, LOE, death) combination were generally 5–6 individuals (range 3–7). Ninety-six *H. letourneuxi* and 80 *C. urophthalmus* were used in the laboratory experiments.

We began to lower the air temperature in the environmental chamber to produce a decrease in water temperature of approximately 1.0°C h^{-1}. That rate of decrease was similar to temperature declines measured in the field during previous cold-front passages in south Florida, and to data reported by others (Provancha et al. 1986). Throughout the experiment, water temperature was monitored closely with a hand-held digital thermometer. Experimental and control fish were inspected visually every hour throughout the experiment. Four hours after the experiment began, water temperatures were 24°C in the environmental chamber. At that time, fishes held in the acclimation holding tanks at 24°C were processed in the same manner as ones from the 28°C acclimation group and added to the environmental chamber (controls were added to the warm room). Temperatures were then decreased in the environmental chamber to achieve a rate of water

temperature reduction of 1.0°C h^{-1} until reaching 8.0°C.

For the LOE treatment, we recorded the time and water temperature at which each fish attained LOE, then transferred them (in their container with cold water) to the warm room. Each succeeding hour, we recorded the increase in water temperatures in the fishes' containers and the status of the fishes. When a fish either expired or righted itself, time and water temperature were recorded. One plastic pipe elbow was placed in each LOE fish container as shelter after it was transferred to the warm room. We also placed elbows in the control-fish boxes after LOE fishes were moved to the warm room. Generally, fishes moved into the structure as soon as it was available. Control and surviving LOE fishes were maintained in the warm room for 14 days after all fishes had been removed from the environmental chamber. During the holding period, fishes were fed twice per week and their water changed twice per week.

Fishes in the death group were left in their containers in the environmental chamber until they expired. The times and temperatures at which these fishes lost equilibrium and expired were recorded. A subset of fishes used in the experiment was fixed in formalin, transferred to ethanol and deposited as identification vouchers in the Florida Museum of Natural History (*H. letourneuxi* UF 170300; *C. urophthalmus* UF 171864).

Data analysis

To ensure fishes were allocated to treatments equally, wet mass and length were compared (for each species) between holding temperatures (24 and 28°C), treatments (control, LOE and death) and salinities (0, 10 and 35 ppt) by analysis of variance (ANOVA). Levene's test was used to compare equality of variances.

We examined the factors affecting temperature at LOE and death in a general linear modeling framework (ANOVA), where the main effects were species (*H. letourneuxi* and *C. urophthalmus*), acclimation temperature (24 and 28°C) and salinity (0, 10 and 35 ppt). Model selection was based on backward-elimination following an initial assessment of potential candidate models. The analysis was begun by testing each main effect separately in univariate models. At this stage of the analysis, a liberal rejection criterion

($P = 0.2$) was used to avoid excluding effects that may have contributed within the context of interaction effects (Lee and Koval 1997; Hosmer and Lemeshow 2000). From this point, backward-elimination with a more rigid rejection criterion ($P = 0.05$) was used; however, throughout the analysis, previously excluded variables were periodically included to verify that biologically relevant models were not being artificially excluded. Post-hoc analyses were conducted on main effects that were significant in the ANOVA model but not included in interaction terms. We used Levene's test to determine whether variances were homogeneous. When variances met the assumption of homogeneity, Scheffé's post-hoc test was used; when variances were not homogenous, Dunnett's T3 test was used for post-hoc analysis.

To determine whether body size affected temperatures at which fishes lost equilibrium or died, wet mass and length were regressed against the two endpoints with both linear and growth curves to determine the best fit to the data. To determine whether salinity affected short-term survival for fishes that experienced LOE, we compared the numbers of fish that died before warming to those that righted themselves and survived the first day after LOE. For this analysis, fish survival was assessed across the three salinities tested (0, 10 and 35 ppt) separately for each species (*H. letourneuxi* or *C. urophthalmus*) with Pearson chi-square. All calculations were done with SPSS© version 12.0.

Field experiments

We conducted field experiments to examine survivorship of *H. letourneuxi* and *C. urophthalmus* in different freshwater habitats during cold snaps by placing them in cages set in major aquatic habitats used by wild individuals of the same species. The field experiments took place during two winters: In January 2008, we caged fish in mostly natural habitats (solution holes, marsh, alligator holes, plus one canal), and in January 2009, we caged fish in man-made habitats (ditches and canals). Fishes were collected from south Florida habitats proximate to the field sites and held in outdoor tanks near the field sites until a cold front was imminent. We then caged individual fish in 3-mm metal-mesh minnow traps

with openings plugged by foam to allow water flow but prevent escape.

For the study conducted in 2008, fishes (*H. letourneuxi* and *C. urophthalmus*) were placed in the field on 1 January, checked on the morning of 2 January, then retrieved from the field on 3 January. In 2009, fish (*H. letourneuxi*) were placed in the field on 20 January, checked on the morning of 21 January and retrieved on 22 January. Minimum air temperatures recorded at the University of Florida Institute of Food and Agricultural Sciences (Homestead) showed that the two cold snaps were similar. In 2008, minimum air temperatures during the field experiment were 17.3°C (1 January), 8.1°C (2 January) and 0.6°C (3 January), while in 2009 minimum air temperatures were 15.5°C (20 January), 4.6°C (21 January) and 3.1°C (22 January). We tracked water temperatures throughout the 3-day experiments with dataloggers placed in the fish cages.

For both years, *H. letourneuxi* were collected from Everglades National Park (ENP) with minnow traps and held in large outdoor containers filled with well water until they were used in the field experiment. Holding times varied, but were generally about 2 weeks. Fish were fed flake food ad libitum 2–3 days per week during the holding period. Because the fish were held in outdoor tanks, water temperatures were ambient. In 2008, *C. urophthalmus* were collected from Water Conservation Area 2A (south of Lake Okeechobee, north of Miami) and held locally in a similar manner as *H. letourneuxi*.

In 2008, individual *H. letourneuxi* were caged in three habitats in ENP: freshwater marshes (water depth: 8–15 cm), alligator ponds (water depth: 47–77 cm) and limestone solution holes (Kobza et al. 2004; water depth: 52–132 cm). Fish were placed at three replicate sites within each habitat. At each site, four cages were deployed (each with one fish), with one also housing a temperature datalogger. In deeper habitats (alligator ponds and solution holes), fish were caged at the top (surface) and bottom to test for possible thermal-stratification effects. A control group of twelve fish (three sets of four fish) were caged in outside mesocosm tanks at the Ocean Campus of Nova Southeastern University in Dania, Florida, a coastal site several degrees warmer than inland sites like ENP. Additionally, two cages, each containing a fish and a datalogger, were placed in the L-31W canal (one near the surface of the water, one

on the bottom of the canal at a depth of 214 cm) just outside of ENP. Mean fish total length (TL) was 5.5 cm ($\pm$1.1 SD, $n = 76$). *Hemichromis letourneuxi* caged at the bottom of solution holes died the first night of the experiment; we suspected this was due to hypoxia rather than low temperatures. Therefore, we placed additional cylindrical cages (1 m tall, 18 cm diameter, 3-mm plastic mesh) in the solution holes to allow fish access to the entire water column and surface.

In 2008, individual *C. urophthalmus* were caged in two habitats (Vegetated ridge [water depth: 35 cm] and slough [water depth 115 cm]) at the Loxahatchee Impoundment and Landscape Assessment (LILA) facility adjacent to the Arthur R. Marshall Loxahatchee National Wildlife Refuge (van der Valk et al. 2008). Mean fish length was 4.4 cm TL ($\pm$9.3 SD, $n = 39$). Three replicate sets of four fish (in four cages plus one datalogger) were placed on the sawgrass ridge. The deeper slough received identical sets of fishes at the surface and bottom. The control group consisted of twelve fish (three sets of four fish) caged in temperature-controlled mesocosm tanks at the South Florida Water Management District in West Palm Beach, Florida. Individual Hydrolab[©] Mini-sonde 4a dataloggers recorded temperature and dissolved oxygen at the bottom of the deep slough site and in the control mesocosm site. A single temperature logger recorded temperature at the bottom of the L-40 canal adjacent to LILA (depth: 330 cm).

In 2009, individual *H. letourneuxi* were caged in shallow ditches (water depth: 40–70 cm), deep ditches (water depth: 100–135 cm) and canals (water depth: 350 cm) near Homestead, FL. Fish were caged at four ditch sites, and at each of these, sets of four fish plus one datalogger (similar to the 2008 experiment) were placed in a shallow ditch as well as at the top and bottom of a nearby deep ditch. The four ditch sites were 10–25 km apart, and were chosen to represent inland and coastal sites. Fish were also caged (in sets of four plus one datalogger) at the top and bottom of two canal sites (one inland and one coastal). Thus, 16 fish were caged in the shallow ditches (four sites), 32 fish in the deep ditches (four sites, top and bottom) and 16 fish in the canals (two sites, top and bottom). Mean fish length was 5.8 cm TL ($\pm$1.0 SD, $n = 64$). Size of *H. letourneuxi* used in caging experiments did not differ between the 2 years (t-test, $df = 138$, $P = 0.15$).

Data analysis

We examined the field data for *H. letourneuxi* for factors that might predict their disposition (alive, Loss Of Equilibrium [LOE] or dead) after the field trial. Our preliminary models used the factors of site depth, habitat type, year and minimum temperature. First, we examined the Crosstabulation of variables to determine if there were cells that were empty or very small, thus eliminating those factors from possible consideration. Of the remaining variables, we modeled the disposition response using Ordinal Logistic Regression with Ordinary Maximum Likelihood Estimation, using disposition (alive, LOE, dead) as the response variable. The crosstabulations and logistic regressions were programmed in R 2.9.2 (R development Core team 2009), using the procedures xtabs and lrm(Design) (Harrell 2009).

Results

Laboratory experiments

For *H. letourneuxi* ($n = 96$), mean wet mass was 3.81 g ($\pm$1.59 SD; range 1.46–9.70 g) and mean total length (TL) was 6.0 cm ($\pm$0.9 SD; range 4.4–8.6 cm TL). Mass and length did not vary among the treatments (control, LOE death), salinity (0, 10, 35 ppt) or holding temperatures (28 or 24°C; one-way ANOVA, all comparisons $P > 0.10$). For *C. urophthalmus* ($n = 60$), mean wet mass was 2.44 g ($\pm$0.93 SD; range 0.81–4.63 g) and mean TL was 5.2 cm ($\pm$0.70 SD; range 3.6–6.4 cm TL). Neither mass nor length varied among treatments (control, LOE death) or salinity (0, 10, 35 ppt), and mass did not differ among holding temperatures (one-way ANOVA, all comparisons $P > 0.10$). Fish held at 28°C (mean 5.4 cm $\pm$ 0.60 SD) were slightly longer than fish held at 24°C (mean 5.0 cm $\pm$ 0.74 SD; one-way ANOVA, $F = 4.303$, $df = 1$, $P = 0.043$).

Experimentally derived endpoints (all salinities combined) were: *Hemichromis letourneuxi*: LOE: mean = 11.5°C ($\pm$0.9 standard deviation [SD]; range 9.4–13.3°C; $n = 67$); death: mean = 9.6°C ($\pm$0.9 SD; range 8.4–11.8°C; $n = 35$); and temperature at which fish became upright after LOE: mean = 15.1°C ($\pm$0.9 SD; range 13.6–16.7; $n = 22$). *Cichlasoma urophthalmus*: LOE: mean = 12.4°C ($\pm$1.1 SD;

range 9.8–17.1°C; $n = 53$); death: mean = 10.3°C (±1.2 SD; range 9.1–15.6°C; $n = 25$); and temperature at which fish became upright after LOE: mean = 16.2°C (±1.6 SD; range 13.2–19.3°C; $n = 28$).

The most parsimonious ANOVA models affecting endpoint temperatures included the three main effects (species, salinity and acclimation temperature) as well as the interaction of species and acclimation temperature. The highest salinity (35 ppt) slightly depressed low-temperature tolerance (LOE and death) for both species. Although this factor was significant in the LOE ANOVA model ($P = 0.03$), post-hoc test results comparing salinities of 0 and 10 ppt to the highest salinity (35 ppt) fell between $P = 0.05$ and $P = 0.10$ (Scheffé's test; Fig. 1). Regarding death temperatures, the effect of salinity was stronger and the post-hoc tests delineated the two lower salinities (0 and 10 ppt) from 35 ppt at $P < 0.001$ (Dunnett's T3 test; Fig. 1). Overall, differences between the two lower salinities and the highest salinity were small (mean about 1°C for both LOE and death).

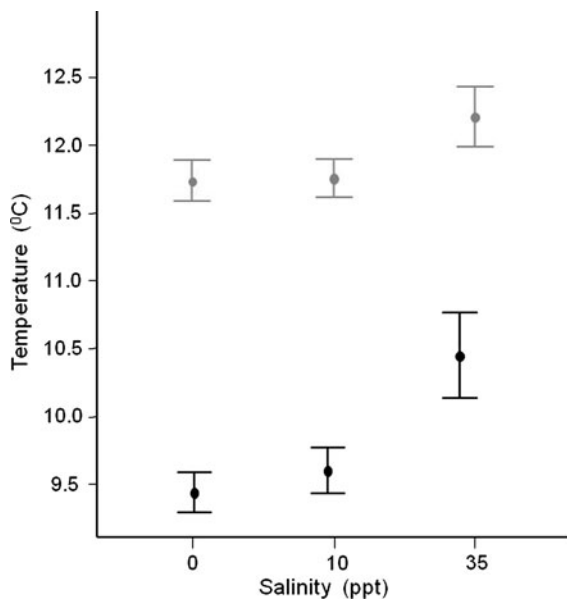Differences in acclimation temperatures affected the low-temperature tolerance of *H. letourneuxi*, but

not *C. urophthalmus*. *Hemichromis letourneuxi* held at the lower temperature (24°C) had lower LOE and death temperatures than individuals held at the higher temperature (28°C). However, no effect of acclimation temperature was seen (for either LOE or death temperatures) for *C. urophthalmus* (Fig. 2).

Salinity strongly affected survival of *H. letourneuxi* during the first 24 h after they experienced LOE ($X^2 = 21.2$, $df = 2$, $P < 0.001$). Of the 36 *H. letourneuxi* that lost equilibrium in the environmental chamber and were transferred to the warm room, 13 were in freshwater (0 ppt), 11 were at the estuarine salinity (10 ppt) and 12 were at the marine salinity (35 ppt). All 12 fish at 35 ppt died before warming. Of the 11 fish at 10 ppt, only one individual died before warming and the remaining 10 survived the 14-day holding period. For the 13 *H. letourneuxi* at 0 ppt, two individuals died before warming, three died of protistan infections several days after the challenge, and 8 survived the 14-day holding period. No *H. letourneuxi* controls died during the 14-day
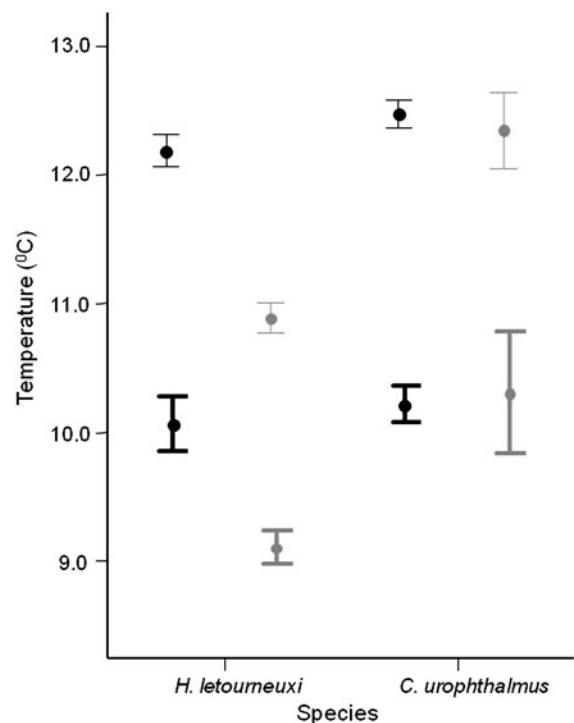

Fig. 1 Plot of mean (±1 standard error [SE]) temperatures at (*a*) loss of equilibrium (LOE; *grey values*) and (*b*) death (*black values*) for *H. letourneuxi* and *C. urophthalmus* (combined) by salinity (0, 10 and 35 ppt). Post-hoc comparisons for salinity treatments of 0 and 10 ppt were not significant for either LOE or death. For comparisons of 0 and 35 ppt as well as 10–35 ppt, $P < 0.1$ for LOE and $P < 0.001$ for death

Fig. 2 Plot of mean (±1 standard error [SE]) temperatures at loss of equilibrium (LOE; *thin bars*) and death (*thick bars*) for *H. letourneuxi* and *C. urophthalmus* by acclimation temperature. Values in *black* are for fishes acclimated at 28°C; fishes acclimated to 24°C are represented by *grey*

holding period in the warm room (eight individuals per salinity, $n = 24$ total).

Unlike *H. letourneuxi*, salinity did not significantly affect short-term survival of *C. urophthalmus* ($X^2 = 4.5$, $df = 2$, $P = 0.103$). All fish that experienced LOE and were transferred to the warm room at the freshwater ($n = 10$) and estuarine ($n = 9$) salinities survived until the end of the 14-day holding period. For fish at the marine salinity that experienced LOE and were transferred to the warm room, two died before warming and the remaining seven survived until the end of the 14-day holding period. A few of the *C. urophthalmus* controls died during the 14-day holding period (one of ten in freshwater, three of eight at the estuarine salinity, and one of eight at the marine salinity).

Neither mass nor length was related to temperatures at LOE or death for *H. letourneuxi*. For *C. urophthalmus*, mass was significantly correlated with LOE temperatures (linear regression; $F = 4.57$; $df = 51$; $P = 0.037$); however, the fraction of the variance explained by temperature was low ($R^2 = 0.082$). Regression equations for body size versus death temperatures for *C. urophthalmus* were not significant.

Field experiments

For sites with top and bottom sets of fishes, dataloggers near the water surface recorded erratic readings, probably from changes in wind, shading and the intensity of sunshine. Thus, we only present temperature data from the bottom sets of cages with loggers at deep sites (i.e., solution holes, alligator holes, ditches and canals).

Hemichromis letourneuxi at Everglades National Park (2008)

Data from the water-temperature loggers showed that the aquatic habitats responded differently to the sudden drop in air temperature (Fig. 3). Marsh temperatures fluctuated the most, followed by ponds, then solution holes (Fig. 3). The canal and control sites were the most thermally stable. Temperatures dropped more slowly in the ponds (ca. 0.5°C h^{-1}), sawgrass ridge (ca. 0.41°C h^{-1}), slough (0.33°C h^{-1}) and solution holes (ca. 0.12–0.25°C h^{-1}) than in the marshes.
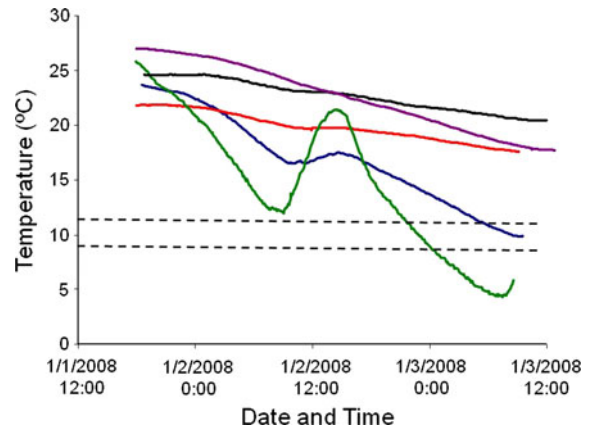

Fig. 3 Water temperatures collected from dataloggers during *H. letourneuxi* field experiment (2008). Mean values are given for marsh (*green line*, $n = 3$), alligator pond (*blue line*, $n = 3$), solution hole (*red line*; $n = 3$), control site (Nova Southeastern University, *purple line*; $n = 3$) and canal (*black line*; $n = 1$). *Horizontal dashed lines* show the mean temperatures at which *H. letourneuxi* lost equilibrium (11.5°C) and died (9.2°C) in freshwater in the laboratory experiment

All fish caged in the marsh survived the first night of the cold snap when temperatures dropped to 11.6°C. On the second night, temperatures dipped to between 3.7 and 4.8°C (the coldest temperatures recorded in this study), resulting in mortality of all caged fish.

All fish in the ponds survived the first night of the cold snap, when temperatures dropped to 14.6°C. By the second night, low temperatures in the ponds ranged from 7.8 to 11.1°C. Of the 24 pond-caged fish, seven (in the deepest, warmest pond) survived and did not exhibit LOE when checked the following morning; six fish died, and the remainder ($n = 11$) had lost equilibrium. On the morning of 3 January 2008, we observed many individual non-native fishes (but no native fishes) lying stunned or dead in the alligator ponds, including *C. urophthalmus*, pike killifish (*Belonesox belizanus*), jaguar guapote (*C. managuense*), spotted tilapia (*Tilapia mariae*) and *H. letourneuxi*.

Thermal profiles for solution holes varied both among and within sites. The WR1 solution hole had a relatively constant temperature and very little vertical stratification. It was surrounded above ground by dense shrubbery that sheltered it from winds and insulated the microenvironment. The Pa-Hay-Okee solution hole was also surrounded by some vegetation (less than WR1), while the MR2 hole was the most exposed. The first night of the caging study, when air

temperatures fell to 15.3°C, all fish caged at the top of the solution holes survived, while fish at the bottom of the solution holes died. We suspected the fish died from low dissolved-oxygen levels (mean = 0.43 mg L^{-1}) typical in some groundwater-fed solution holes, combined with the inability of the fish to reach the surface to perform aquatic surface respiration. Therefore, additional fish were placed in the solution holes inside 1-m tall cages that allowed the fish access to the entire water column, including the water surface. On the second night of the study, the lowest water temperature in WR1 was 22.2°C, and all fish (four caged at the surface, one in a tall cage) survived and did not exhibit LOE when checked the following morning. At Pa-Hay-Okee, minimum water temperature was 15.1°C. All fish (four caged at the surface, two in tall cages) survived and did not exhibit LOE the following morning. At MR2, the minimum water temperature was similar to the Pa-Hay-Okee solution hole (15.2°C); however, the four fish caged at the surface and the two fish in the tall cages had lost equilibrium when they were checked at approximately 1000 h.

The L-31W canal remained the warmest of all habitats throughout the cold snap, with a minimum water temperature of 20.4°C (Fig. 3). Neither fish caged in the canal (one at the water surface, the other near the bottom of the canal) died nor exhibited LOE. Temperatures at the control site were relatively moderate and decreased only to 17.3°C during the experiment. No control fish died or experienced LOE during the study.

Native fishes, mainly centrarchids, poeciliids, and Florida gar, showed no ill effects from the cold temperatures in any habitat, although in the marsh native fishes were not visible, probably having burrowed into the sediments (see Frederick and Loftus 1993).

Cichlasoma urophthalmus at LILA (2008)

Water temperatures in the vegetated-ridge and deep slough habitats decreased in a similar manner during the cold front, while the canal and control sites were more thermally stable (Fig. 4). The shallow vegetated-ridge site displayed moderate diel temperature variations. All fish caged on the ridge survived the first night of the study when temperatures dropped to 16.4°C. On the second night, temperatures fell to 10.1°C, and all
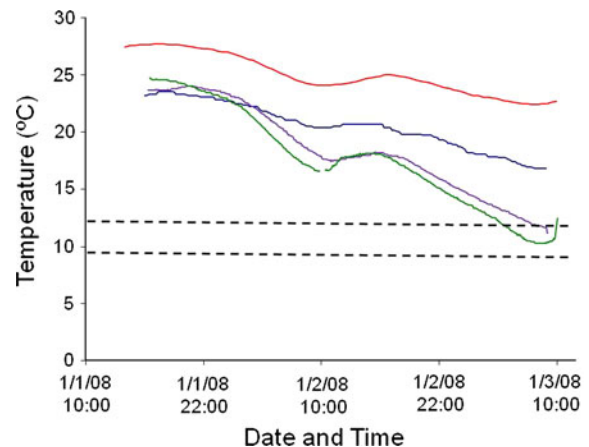

Fig. 4 Water temperatures collected from dataloggers during *C. urophthalmus* field experiment (2008). Mean values are given for ridge (*green line*; n = 3), slough (*purple line*; n = 3), control site (South Florida Water Management District, *red line*; n = 3) and canal (*blue line*; n = 1). *Horizontal dashed black lines* show the mean temperatures at which *C. urophthalmus* lost equilibrium (12.0°C) and died (9.9°C) in freshwater in the laboratory experiment

caged fish had lost equilibrium when checked the following morning. Water temperature in the slough fell to 17.4°C during the first night; the four fish caged at the top survived while three of the four at the bottom died. Those fish most likely died from low dissolved-oxygen levels (min = 0.12, mean = 1.68 mg L^{-1}), and were replaced with live fish. Dissolved-oxygen levels remained normoxic for the remainder of the study (min = 3.39, mean = 6.39 mg L^{-1}) and did not contribute to further mortality. The lowest temperature recorded during the second night in the slough was 10.8°C. One fish caged at the top died, while all other fishes caged at both top and bottom survived but experienced LOE. Canal waters responded slower to the drop in air temperature than ridge and slough habitats, declining to 16.7°C (Fig. 4). No fish were caged in the canal. Temperatures at the control site were controlled by heaters and shelter, and dropped to a low of only 20.5°C during the experiment (Fig. 4). None of the control fish died or experienced LOE during the study.

Hemichromis letourneuxi in ditches and canals (2009)

The man-made habitats (ditches and canals) responded differently to a sudden drop in air temperature, with the

shallowest habitats having the lowest temperatures (Fig. 5). As expected, shallow ditches experienced the coldest temperatures (10.2°C), followed by deep ditches (14.1°C), then canals (19.7°C; Fig. 5). All fish were healthy when checked on the first morning of the experiment (21 January). On the second (and coldest) morning, four fish (all from the same shallow ditch) exhibited LOE, while all fish in other habitats were fine. The ditch with the LOE fish was very exposed, with minimal aquatic vegetation and no marginal vegetation or structure to slow heat loss. Minimum temperatures recorded in this ditch were the lowest recorded by the study in 2009 (10.2°C). No fish died in the field during the 2009 experiment.

Hemichromis letourneuxi field experiment data analysis

Most of the factors in the field trials of *H. letourneuxi* that we screened for analysis failed the crosstabulation test because of empty cells. Of the failed factors (site, habitat type and year), site was not examined further because it was naturally correlated with the more ecologically meaningful habitat type. Year was
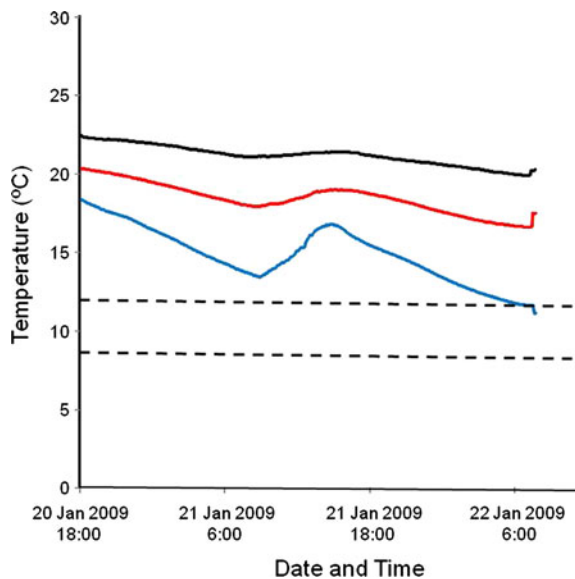

Fig. 5 Water temperatures collected from dataloggers during *H. letourneuxi* field experiment (2009). Mean values are given for shallow ditches (*blue line*, n = 4), deep ditches (*red line*; n = 4) and canals (*black line*; n = 2). *Horizontal dashed lines* show the mean temperature at which *H. letourneuxi* lost equilibrium (11.5°C) and died (9.2°C) in freshwater in the laboratory experiment

Table 1 Disposition of *H. letourneuxi* in six habitat types

Habitat type	Alive	LOE	Dead
Marsh	0	0	12
Pond	7	11	6
Shallow ditch	12	4	0
Solution hole	11	6	0
Deep ditch	32	0	0
Canal	18	0	0

Habitat types are ordered by average depth

The shallow marsh habitat type was associated with only dead fish, and the deep ditch and canal habitat types were associated with only live fish. The three intermediate habitat types showed a mix of dispositions, generally related to the depths of the individual sites

collapsed because of the crosstabulation failure, lack of habitat type replication across years and the similarity of the water temperatures across both years. Habitat type, if considered by itself, was a highly significant factor ($P < 0.0001$; Table 1), but was not used for the final model because it failed the crosstabulation test and was highly correlated with depth and minimum temperature. Habitat type should be considered an important predictor of cold-induced LOE or mortality for this species only as it relates to the depth or other temperature-buffering characteristics of the site. Here, it appears that the depth of the habitat explained almost all of the variability in disposition.

Depth passed the crosstabulation test when binned; however, as noted above, it was highly correlated with habitat type and minimum temperature in that the deepest habitats were warmer than the shallower ones (Fig. 6). When tested by itself, depth was highly significant ($P < 0.0001$), with shallower depths being associated with more mortality. Only the pond habitat type had all three dispositions (Table 1). Ponds also showed a variety of depths and associated minimum temperatures. Looking only at pond data from 2008, depth was non-significant ($P = 0.08$) as a predictor of disposition, but the data showed the expected pattern of deeper ponds having less LOE and mortality (Table 2). Given the buffering action of deeper ponds relative to shallower ponds, it is likely that greater replication of this habitat type would have shown that a greater degree of protection against cold air temperatures is afforded by deeper ponds.

Once the highly significant variable of minimum temperature was accounted for (assuming that it was
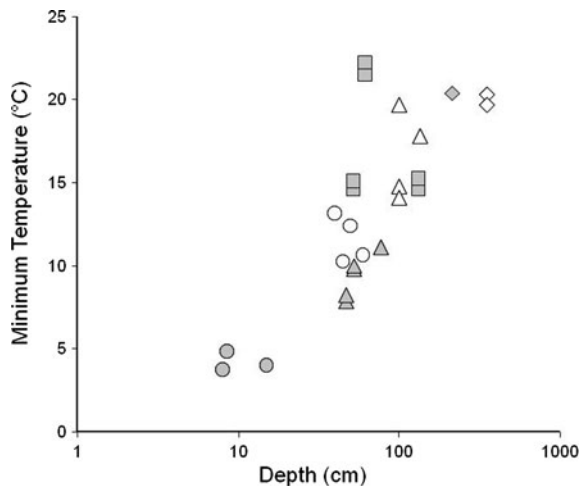
Fig. 6 Minimum temperatures measured in the field at caging sites after the passage of similar cold fronts in 2008 and 2009 (*grey circle* = marsh 2008; *grey triangle* = pond 2008; *grey square* = solution hole 2008; *grey diamond* = canal 2008; *open circle* = shallow ditch; *open triangle* = deep ditch; *open diamond* = canal 2009). Minimum temperatures were highly correlated with depth of the site and habitat type in field data of *H. letourneuxi* contained within screen enclosures. Once minimum temperature was accounted for, both habitat type and depth were non-significant, indicating no independent contributions. Exposure of the site to air temperature, versus the buffering ability of deep water bodies would explain the relationships among habitat type, depth, and minimum temperature

Table 2 Pond depths and dispositions of *H. letourneuxi*

Depth (cm)	Alive	LOE	Dead
47	0	2	6
52	0	8	0
77	7	1	0

The shallower pond was associated with fish that had LOE or had died, and the deeper pond was associated with mostly live fish
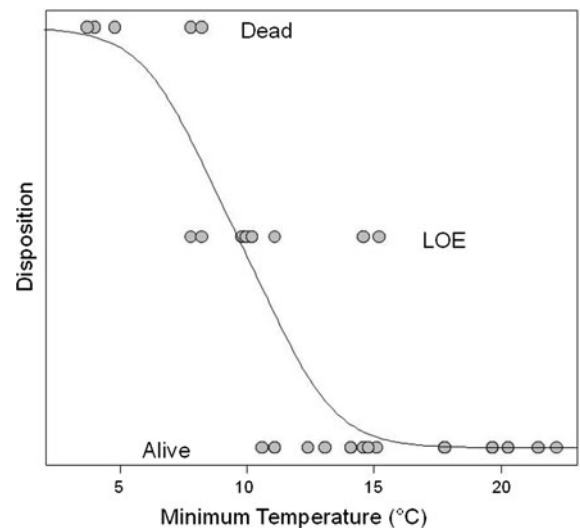

Fig. 7 Disposition of *H. letourneuxi* after exposure to different minimum temperatures in field conditions. The *heavy line* represents the fitted ordinal logistic model. Lower temperatures are associated with Loss of Equilibrium (LOE), and then mortality

the temperature and not the depth that caused the mortality), depth contributed little to the model ($P = 0.82$) and so was removed from the minimum temperature model. The minimum temperature model was the most informative, as it is assumed that the fish were responding directly to the minimum temperature in their enclosures and not the depth or habitat type (with the exception of the fish that died from anoxia and were excluded from the analyses). With disposition coded as alive = 1, LOE = 2, and dead = 3, the model coefficients using minimum temperature model were:

	Coefficient	SE	Wald Z	P
Disposition ≥ LOE	9.294	1.571	5.92	<0.0001
Disposition = dead	6.394	1.295	4.94	<0.0001
Minimum temperature	−0.819	0.135	−6.07	<0.0001

This model showed that the temperature effects in field conditions were very similar to those seen in the laboratory experiments: warmer temperatures were associated with live fish, intermediate temperatures were associated with fish that had LOE, and colder temperatures were associated with dead fish (Figs. 7, 8). The estimated break point between live and LOE was 11.2°C, which was not statistically different from the 11.5°C estimated from the laboratory experiments. The estimated break point between LOE and death was 7.9°C, which was lower than the 9.6°C estimated from the laboratory experiment, but not significantly different considering the experimental error in the laboratory and field experiments.

Discussion

Results from the laboratory study showed that both cichlid species exhibited relatively similar low-
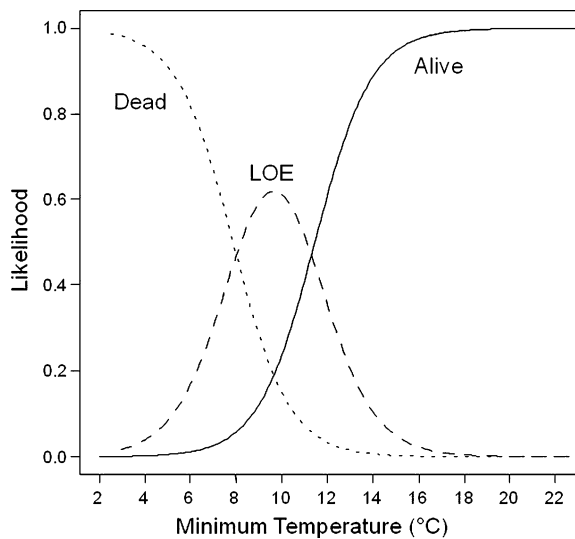
Fig. 8 Maximum Likelihood estimates for the outcome of *H. letourneuxi* after exposure to different minimum temperatures in field conditions. Below 7.9°C, exposed fish were most likely to die, while above 11.2°C, they were most likely to remain alive. In between these temperatures, they were most likely to suffer Loss of Equilibrium (LOE)

temperature tolerances, with death endpoints about two degrees lower than LOE endpoints. The results from this study agreed with the few previous studies on low-temperature tolerances of these fishes. Although both this study and Shafland and Pestrak (1982) used different acclimation temperatures and rates of temperature decrease to test the death endpoint for *H. letourneuxi*, the results were remarkably similar (9.5°C for Shafland and Pestrak 1982; 9.1–9.6°C for this study). Stauffer and Boltz (1994) tested the incipient lethal temperature for *C. urophthalmus* by immediately transferring individuals from holding conditions to test temperatures, and obtained higher thermal minima (13–15°C) than we report here (9.5–11.1°C). This was expected, as static tests (e.g., Stauffer and Boltz 1994) generally result in higher values for thermal minima than dynamic ones (e.g., this study; Bennett and Judd 1992; Beitinger et al. 2000).

The results of the field studies corroborated the laboratory thresholds for LOE and death in both species. Because of the close correspondence of those values, the effects of cold temperatures on those species can be predicted in the future from field measurements of water temperatures in habitats of interest.

Differences in acclimation temperatures had little effect on LOE and death endpoints for *C. urophthalmus*, an unusual outcome. For *H. letourneuxi*, tolerance to low temperatures was bolstered by lower acclimation temperatures. In a review by Beitinger et al. (2000), lethal thermal limits were strongly affected by thermal history prior to experimentation. Indeed, it is rare [but not unknown (e.g., Kimball et al. 2004)] that acclimation temperatures do not affect thermal tolerance. In some cases, lower acclimation temperatures confer significant advantages to fishes faced with low-temperature challenges. For example, Ford and Beitinger (2005) demonstrated a 0.41°C decrease in low-temperature tolerance of goldfish *Carassius auratus* for every 1°C decrease in acclimation temperature (over a range of 5–35°C).

The highest salinity tested (35 ppt) slightly decreased the ability of the fishes to tolerate cold temperatures (by about 1°C), while low-temperature tolerance at the brackish salinity (10 ppt) did not differ from freshwater. The physiological effects of temperature/salinity interactions in fishes are complex and not well understood. Salinity has been shown to both increase and decrease temperature tolerance of fishes (Kinne 1964). Several studies have described salinity effects on temperature tolerance by cichlid fishes. Jennings (1991) reported that cold tolerance did not differ with salinity (5, 15 or 35 ppt) for blackchin tilapia *Sarotherodon melanotheron*. However, Stauffer et al. (1984; using a different experimental design than Jennings [1991]), reported that *S. melanotheron* at 15 ppt had greater cold tolerance than individuals at 0 and 30 ppt. Zale and Gregory (1989) reported that the lethal temperature for blue tilapia *Oreochromis aureus* was lower near isosmosis (11.6 ppt) than at either lower or higher salinities. Additionally, Chervinski and Lahav (1976) showed that *O. aureus* was more tolerant of low temperatures at a 5 ppt than in freshwater. Stauffer and Boltz (1994) reported no differences in ultimate lower lethal temperatures for *C. urophthalmus* at either 0 or 15 ppt. Stauffer (1986) acclimated individuals of *O. mossambicus* to 0, 15 and 30 ppt (all at 25°C) and then transferred them directly to a series of lower temperatures. Ninety percent of the fish at 0 and 15 ppt survived direct transfer to 15°C, while only 20% of the fish at 30 ppt survived (all tests 10,000 min in duration).

Although salinity had only minimal (but significant) effects on low-temperature tolerance, it had strong effects on whether fishes ultimately survived the low-temperature challenge, especially for *H. letourneuxi*. All *H. letourneuxi* at the marine salinity (35 ppt) died while returning to ambient temperature after LOE, while most fish at 0 and 10 ppt survived after warming. A greater proportion of *C. urophthalmus* than *H. letourneuxi* survived after warming to ambient temperatures, and unlike *H. letourneuxi*, differences in survival caused by salinity were slight for *C. urophthalmus*. We have found no other published work that reported assessing fish after they had lost equilibrium to determine whether they ultimately survived the thermal challenge. However, one report (Jennings 1991) noted anecdotally that a few individuals of *S. melanotheron* at 5 and 15 ppt that were subjected to low-temperature stress and exhibited LOE recovered relatively quickly once warmed to ambient temperatures. We felt it important to determine whether fish ultimately expired after losing equilibrium because, if so, the temperature at which they lost equilibrium would be an ecological proxy for the temperature at death. For example, a small proportion of *H. letourneuxi* that experienced LOE and were subsequently returned to ambient temperature contracted protistan infections and expired during the 14-day holding period. The reduction in immune function and subsequent reduced ability of these fish to resist infectious agents is a commonly seen secondary effect of thermal stress in fishes (e.g., Loftus and Kushlan 1987; Ndong et al. 2007). Thus, the stress associated with LOE may induce death fairly quickly or may result in later secondary indirect effects, such as parasitic infection. To our knowledge, this is the first study to demonstrate that LOE may be an ecological surrogate for mortality.

Only minor body-size effects were seen in relation to the salinity tolerance of the two fishes studied. This is likely due to the relatively limited size-range of the fishes used in the study, and therefore should not be extrapolated without additional empirical data. In general, studies of effects of body size on thermal tolerance in fishes have produced mixed results. While some reports indicate body size does not affect thermal tolerance (e.g., Ospina and Mora 2004; Cortemeglia and Beitinger 2006), others show that larger fish have a greater ability to withstand

temperature changes and extremes than smaller ones (e.g., Cox 1974; Charo-Karisa et al. 2005). More information is needed to determine how body size relates to thermal tolerance for the two species studied, and for the Cichlidae introduced into the southeastern United States in general. Several other factors shown to affect the low-temperature tolerance of fishes but not examined in this study include genotype (Behrends et al. 1996), dissolved oxygen (Rutledge and Beitinger 1989), cumulative thermal stress (Bevelhimer and Bennett 2000), environmental toxins (Carrier and Beitinger 1988) and dominance or social status (Wohlfarth et al. 1983; Jennings 1991; Cnaani et al. 2000).

In the laboratory experiments, we determined low-temperature tolerance by reducing water temperatures by $1°C\ h^{-1}$, based on past data from cold-front passages in south Florida. That rate of decrease was similar to those in other studies of critical thermal tolerance (e.g., Ospina and Mora 2004). It was also the same rate of decrease that we measured in the marsh field experiment with the passing of the January 2008 cold front, when water temperature in the southern Everglades marsh dropped from about 21–4°C over 17 h. Similarly, when a cold front moved through the Indian River Lagoon in January 1985, temperature decreases were also recorded at $1°C\ h^{-1}$ (Provancha et al. 1986). In our field study, water temperatures fell fastest in the marshes, followed by the ponds (ca. $0.5°C\ h^{-1}$), sawgrass ridge (ca. $0.41°C\ h^{-1}$), slough ($0.33°C\ h^{-1}$) and solution holes (ca. $0.12–0.25°C\ h^{-1}$). The physical characteristics of Everglades marshes (shallow water [<1 m], open habitats) result in rapid cooling during cold fronts from convective and radiative heat loss from the open water surface. Therefore, cold-intolerant, non-native tropical fishes are most vulnerable to mortality in winter in that habitat. The deepest natural habitats in the freshwater Everglades are alligator holes and solution holes (Loftus and Kushlan 1987), although neither habitat typically exceeds 1–1.5 m in depth (Kobza et al. 2004). Solution holes typically are limited in surface areas, resulting in lower exposure to chilling winds that accompany cold fronts. In addition, their waters are insulated by the surrounding limestone matrix, and some holes receive warm groundwater flow through the limestone bedrock. However, the dissolved-oxygen level of groundwater is extremely low, which explains the

mortality we observed in the fish caged at the bottom of the holes. Although *H. letourneuxi* has a remarkable ability to withstand hypoxic conditions when compared to other fishes, it must be able to reach the surface to perform aquatic surface respiration or it will perish (Schofield et al. 2007). On the second night of the experiment, the test fish were caught between the warm but anoxic bottom waters in the solution hole and the cold waters at the surface, which resulted in LOE from exposure to sublethal water temperatures.

The ridge and slough habitats offer differing habitat complexity and potential temperature-buffering characteristics. Although the ridge habitats were 1–2°C cooler than slough habitats (10 vs. 12°C), the only temperature-induced mortality of this study occurred at the surface of the slough. This may have been the result of ridge vegetative complexity and deep flocculent material creating a thermal buffer around the fish cage. Ridges are typified by dense stands of sawgrass up to 3 m tall (Loftus and Kushlan 1987) that insulate and slow the loss of heat from the water column. Fish caged at the top of the open sloughs may have been subjected to more wind-generated disturbance which may have increased energetic needs and stress, thereby indirectly exacerbating the influence of low temperature. Interestingly, although air temperatures also dipped to a low of near 3°C as in ENP habitats, none of the LILA habitats experienced water temperature less than 10°C which resulted in only one mortality there. However, all fish experienced LOE and may have succumbed to hypothermia or delayed mortality had the study duration been extended until the effects of the cold front had passed. The LILA facility is supplied and surrounded by canal water and is likely more thermally stable than the proximate natural ridge and slough system. Thus, the physical structure of the facility may have buffered it against the cold and given it more protection than a natural ridge/slough system.

Alligator holes vary in area and depth but are characterized by a high surface-to-volume ratio that causes moderately-rapid cooling during cold fronts (second only to marsh habitats). During the moderate, short-lived front that coincided with our study, fish caged in alligator ponds had the second highest mortality after the marsh fish and the highest incidence of LOE.

Compared to the natural habitats, many ditches and canals are thermally buffered by their morphology and by penetration into aquifer waters that remain at about 22°C year-round. Ditches (even as shallow as 50 cm) and canals offer cold-intolerant tropical fishes a safe haven during winters.

Management implications

Our work with *H. letourneuxi* and *C. urophthalmus* demonstrates the strong effect exerted by habitat on cold-temperature survival by introduced tropical fishes. Dozens of species of non-native tropical fishes are presently established in Florida (Fuller et al. 1999; Shafland et al. 2008), and most are undesirable illegal introductions. Because management options for controlling such fishes once they become established are nearly non-existent, it is important to consider other avenues for control. The most promising possibility for achieving any level of control is to expose the fishes to cold water temperatures that accompany the periodic winter fronts. This is possible only if fishes are denied access to thermal refuges, which in south Florida consist primarily of man-made habitats such as canals, ditches, culvert pools, borrow ponds and pools at water-control structures (e.g., Stauffer et al. 1988). Those constructs are pervasive (and often inter-connected) within the south Florida landscape for moving water and providing flood control. Because the thermal properties of deep-water canals allow tropical non-native fishes to survive winter low temperatures in south Florida, wetlands connected to canals may support larger populations and higher diversity of these fishes than wetlands without canals. However, this hypothesis needs to be tested experimentally in the Everglades to determine the degree of population supplementation provided by the canals and subsequent effects on native populations of fishes and invertebrates. Our research suggests that actions such as infilling canals and pools to less than 50 cm water depth and decreasing connectivity of refugia (if done without affecting their water-management roles) would be a positive step in reducing population sizes of non-native, tropical fishes. Because canals are used for water deliveries to natural wetlands, including national parks and national wildlife refuges, the challenge is to design ways to deliver water without providing a means of ingress for undesirable aquatic invaders.

Cold-temperature effects on cichlids living in Florida waters will vary according to geographic location, salinity and habitat. Cichlids occur in aquatic habitats with a wide range of salinities. We found differences in survival of the two species resulting from salinity interacting with temperature, making generalizations for all cichlids questionable. Although all *H. letourneuxi* in our marine-salinity treatment died after being returned to ambient temperatures following LOE, little effect was seen in *C. urophthalmus*. The effects of salinity on wild fish also depend on factors of geography and habitat. The highest salinities (35 and above) occur in coastal areas where waters are buffered from the cold air temperatures experienced in interior freshwater habitats. In addition, Atlantic coastal waters in Florida remain warmer in winter than waters along the Gulf of Mexico so predictions of effects of cold interacting with salinity need to take geography into account. Thus, estuarine and marine areas may become source-population centers, serving as thermal refugia and migratory corridors to adjacent freshwater basins. Finally, we showed that habitat variables (e.g., depth, surface cover, groundwater input) are related to thermal regimes and fish survival, so that some habitats facilitate survival while others do not. Other studies have reported survival of tropical, non-native fishes in particular habitats [e.g., power plant discharge (Stauffer et al. 1988), cooling reservoirs of power plants (Peterson et al. 2005), warm springs (Nelson 1983)] north of their predicted (i.e., tropical) ranges. Fishes are able to seek and locate thermal refuges when available. Thus, while existing models often use temperature isoclines to forecast survival and eventual geographic range expansion for introduced fishes (key components of risk assessment), it is apparent that latitude alone is not a accurate predictor of potential range. Such models must consider variations in habitat type (and concomitant thermal regimes and fish survival) to produce reliable predictions.

References

Adams AJ, Wolfe RK (2007) Occurrence and persistence of non-native *Cichlasoma urophthalmus* (family Cichlidae) in estuarine habitats of south-west Florida (USA): environmental controls and movement patterns. Mar Freshwat Res 58:921–930

Behrends LL, Kingsley JB, Bulls MJ (1996) Cold tolerance in maternal mouthbrooding tilapias: heritability estimates and correlated growth responses at suboptimal temperatures. In: Pullin RSV, Lazard JM, Legendre JB, Kothias A, Pauly D (eds) The third international symposium on tilapia in aquaculture, ICLARM conference proceedings, 41:257–265

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ Biol Fish 58:237–275

Bennett WA, Judd FW (1992) Comparison of methods for determining low temperature tolerance: experiments with pinfish *Lagodon rhomboides*. Copeia 1992:1059–1065

Bevelhimer M, Bennett W (2000) Assessing cumulative thermal stress in fish during chronic intermittent exposure to high temperatures. Environ Sci Pol 3:S211–S216

Carrier R, Beitinger TL (1988) Reduction in thermal tolerance of *Notropis lutrensis* and *Pimephales promelas* exposed to cadmium. Water Resour 22:511–515

Charo-Karisa H, Rezk MA, Bovenhuis H, Komen H (2005) Heritability of cold tolerance in Nile tilapia, *Oreochromis niloticus,* juveniles. Aquaculture 249:115–123

Chervinski J, Lahav M (1976) The effect of exposure to low temperature on fingerlings of local tilapia (*Tilapia aurea*) (Steindachner) and imported tilapia (*Tilapia vulcani*) (Trewavas) and *Tilapia nilotica* (Linne) in Israel. Bamidgeh 28:25–29

Cnaani A, Gall GAE, Hulata G (2000) Cold tolerance of tilapia species and hybrids. Aquac Int 8:289–298

Cortemeglia C, Beitinger TL (2006) Projected US distributions of transgenic and wildtype zebra danios, *Danio rerio*, based on temperature tolerance data. J Therm Biol 31:422–428

Cox DK (1974) Effects of three heating rates on the critical thermal maximum of bluegill. In: Gibbons JW, Sharitz RR (eds) Thermal ecology. U.S. Atomic Energy Commission, Savannah, pp 158–163

Faunce CH, Lorenz JJ (2000) Reproductive biology of the introduced Mayan cichlid, *Cichlasoma urophthalmus*, within an estuarine mangrove habitat of southern Florida. Environ Biol Fish 58:215–225

Faunce CH, Paperno R (1999) *Tilapia*-dominated fish assemblages within an impounded mangrove ecosystem in east-central Florida. Wetlands 19:126–138

Ford T, Beitinger TL (2005) Temperature tolerance in the goldfish, *Carassius auratus*. J Therm Biol 30:147–152

Frederick PC, Loftus WF (1993) Responses of marsh fishes and breeding wading birds to low temperatures: a possible behavioral link between predator and prey. Estuaries 16:216–222

Fuller P, Nico LG, Williams JD (1999) Nonindigenous fishes introduced to inland waters of the United States. Pub 27 American Fisheries Society, Bethesda MD

García-Berthou E (2007) The characteristics of invasive fishes: what has been learned so far? J Fish Biol 71:33–55

Gunderson LH, Loftus WF (1993) The Everglades. In: Martin WH, Boyce SG, Echternacht AC (eds) Biodiversity of the Southeastern United States. John Wiley and Sons, New York, pp 199–255

Harrell Jr FE (2009) Design: design package. R package version 2.2-0. http://CRAN.R-project.org/package=Design

Hosmer DW, Lemeshow S (2000) Applied logistic regression. John Wiley and Sons, New York

Jennings DP (1991) Behavioral aspects of cold tolerance in blackchin tilapia, *Sarotherodon melanotheron*, at different salinities. Environ Biol Fish 31:185–195

Jennings DP, Williams JD (1992) Factors influencing the distribution of blackchin tilapia *Sarotherodon melanotheron* (Osteichthyes: Cichlidae) in the Indian River System, Florida. NE Gulf Sci 12:111–117

Kannan K, Smith RG Jr, Lee RF, Windom HL, Heitmuller PT, Macauley JM, Summers JK (1998) Distribution of total mercury and methyl mercury in water, sediment, and fish from south Florida estuaries. Arch Environ Contam Toxicol 34:109–118

Kimball ME, Miller JM, Whitfield PE, Hare JA (2004) Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. Mar Ecol Prog Ser 283:269–278

Kinne O (1964) The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature salinity combinations. Oceanog Mar Biol Ann Rev 2:281–339

Kobza RM, Trexler JC, Loftus WF, Perry S (2004) Community structure of fishes inhabiting aquatic refuges in a threatened karst wetland, and its implications for ecosystem management. Biol Conserv 116:153–165

Lamboj A (2004) The cichlid fishes of western Africa. Birgit Schmettkamp Verlag, Bornheim

Langston JN, Schofield PJ, Hill JE, Loftus WF (2009) Salinity tolerance of the African jewelfish *Hemichromis letourneuxi*, a non-native cichlid in south Florida, USA. Submitted: Copeia (In Prep)

Lee KI, Koval JJ (1997) Determination of the best significance level in forward stepwise logistic regression. Comm Stats: Simul Comput 26:559–575

Light SS, Dineen JW (1994) Water control in the Everglades: a historical perspective. In: Davis SM, Ogden JC (eds) Everglades. The ecosystem and its restoration. St. Lucie Press, Boca Raton, pp 47–84

Loftus WF (1987) Probable establishment of the Mayan cichlid, *Cichlasoma urophthalmus* (Günther) (Pisces: Cichlidae), in extreme southern Florida. Florida Sci 50:1–6

Loftus WF (2000) Inventory of the fishes of Everglades National Park. Fla Sci 63:27–47

Loftus WF, Kushlan JA (1987) Freshwater fishes of southern Florida. Bull Fla State Mus Biol Sci 31:147–344

Loftus WF, Trexler JC, Dunker K, Liston SE, Rehage JS (2006) Introduced fishes in short-hydroperiod wetlands: evaluation of sampling, status, and potential effects. Final report from USGS to Everglades NP for Agreement # CESI IA F5284-04-0039. Homestead, FL: U.S. Geological Survey

Miles CJ, Pfeuffer RJ (1997) Pesticides in canals of south Florida. Arch Environ Contam Toxicol 32:337–345

Miller RR, Minckley WL, Norris SM (2005) Freshwater fishes of Mexico. University of Chicago Press, Chicago

Moyle PB, Light T (1996) Biological invasions of fresh water: empirical rules and assembly theory. Biol Conserv 78:149–161

Moyle PB, Marchetti MP (2006) Predicting invasion success: freshwater fishes in California as a model. BioSci 56:515–524

Ndong D, Chen YY, Lin YH, Vaseeharan B, Chen JC (2007) The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures. Fish Shellfish Immun 22:686–694

Nelson JS (1983) The tropical fish fauna in Cave and Basin Hotsprings drainage, Banff National Park, Alberta. Can Field-Natur 97:255–261

Ospina A, Mora C (2004) Effect of body size on reef fish tolerance to extreme low and high temperatures. Environ Biol Fish 70:339–343

Paperno R, Ruiz-Carus R, Krebs JM, McIvor CC (2008) Range expansion of the Mayan cichlid, *Cichlasoma urophthalmus* (Pisces, Cichlidae), above 28°N latitude in Florida. Fla Sci 71:293–304

Peterson MS, Slack WT, Woodley CM (2005) The occurrence of non-indigenous Nile tilapia (*Oreochromis niloticus*) in coastal Mississippi, U.S.A.: ties to aquaculture and thermal effluent. Wetlands 25:112–121

Provancha MJ, Schmalzer PA, Hall CR (1986) Effects of the December 1983 and January 1985 freezing air temperatures on selected aquatic poikilotherms and plant species of Merritt Island, Florida. Fla Sci 49:199–212

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rehage JS, Loftus WF (2007) Seasonal fish community variation in headwater mangrove creeks in the southwestern Everglades: an examination of their role as dry-down refuges. Bull Mar Sci 80:625–645

Rivas LR (1965) Florida freshwater fishes and conservation. Quart J Fla Acad Sci 28:255–258

Rutledge CJ, Beitinger TL (1989) The effects of dissolved oxygen and aquatic surface respiration on the critical thermal maxima of three intermittent-stream fishes. Environ Biol Fish 24:137–143

Schofield PJ, Loftus WF, Brown ME (2007) Hypoxia tolerance of two centrarchid sunfishes and an introduced cichlid in karstic Everglades wetlands of southern Florida, USA. J Fish Biol 71(Sup D):87–99

Schofield PJ, Loftus WF, Fontaine JA (2009) Salinity effects on behavioural response to hypoxia in the non-native Mayan cichlid (Cichlasoma urophthalmus) from Florida Everglades wetlands. J Fish Biol 74:1245–1258

Shafland PL, Pestrak JM (1982) Lower lethal temperatures for fourteen non-native fishes in Florida. Environ Biol Fish 7:149–156

Shafland PL, Gestring KB, Stanford MS (2008) Florida's exotic freshwater fishes–2007. Fla Sci 71:220–245

Stauffer JR Jr (1986) Effects of salinity on preferred and lethal temperatures of the Mozambique tilapia, Oreochromis mossambicus (Peters). Water Resour Bull 22:205–208

Stauffer JR Jr, Boltz SE (1994) Effect of salinity on the temperature preference and tolerance of age-0 Mayan cichlids. Trans Amer Fish Soc 123:101–107

Stauffer JR Jr, Vann DK, Hocutt CH (1984) Effects of salinity on preferred and lethal temperatures of the blackchin tilapia, Sarotherodon melanotheron. Water Res Bull 20:771–775

Stauffer JR Jr, Boltz SE, Boltz JM (1988) Cold shock susceptibility of blue tilapia from the Susquehanna River, Pennsylvania. N Amer J Fish Manag 8:329–332

Trexler JC, Loftus WF, Jordan F, Lorenz J, Chick J, Kobza R (2000) Empirical assessment of fish introductions in southern Florida: an evaluation of contrasting views. Biol Invasions 2:265–277

van der Valk AG, Wetzel P, Cline E, Sklar FH (2008) Restoring tree islands in the Everglades:experimental studies of tree seedling survival and growth. Restor Ecol 16:281–289

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. Bioscience 48:607–615

Wohlfarth GW, Hulata G, Rothbard S, Itzkowich J, Halevy A (1983) Comparison between interspecific tilapia hybrids for some production traits. In: Fishelson L, Yaron Z (eds) International symposium on Tilapia in aquaculture. Tel Aviv University, Tel Aviv, Israel, pp 559–569

Zale AV, Gregory RW (1989) Effect of salinity on cold tolerance of juvenile blue tilapias. Trans Amer Fish Soc 118:718–720