Responses of skilletfish *Gobiesox strumosus* to high temperature and low oxygen stress

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This study quantified physiological responses of skilletfish *Gobiesox strumosus* exposed to thermal and oxic stress. Fish acclimated at 12, 22 and 32 °C had low oxygen tolerance values (mean ± s.d.) of 0·40 ± 0·09, 0·40 ± 0·08 and 0·35 ± 0·03, and critical thermal maxima (mean ± s.d.) of 33·2 ± 0·5, 38·1 ± 0·0 and 39·5 ± 0·3°C, respectively. Furthermore, *G. strumosus* were oxygen conformers at all acclimation temperatures, i.e. the fish allowed oxygen consumption rates to decrease with ambient oxygen concentration. High temperature tolerance, low oxygen tolerance and decreasing metabolic rates during hypoxic events allow the fish to survive harsh environmental conditions encountered in their natural environment.

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INTRODUCTION

Skilletfish *Gobiesox strumosus* Cope (Gobiesocidae) inhabit clear, shallow bays and estuaries from New Jersey to Brazil and along the Gulf of Mexico (Hildebrand & Schroeder, 1928; Runyan, 1961; Saksena & Joseph, 1972). As the only North American clingfish with a geographic distribution extending into temperate waters, *G. strumosus* are exposed to a wide range of environmental extremes. Chesapeake Bay populations, for example, experience summer high temperatures of 26 °C (Runyan, 1961), whereas some Florida populations encounter mid-day highs that occasionally exceed 40 °C (Harrington & Harrington, 1961). High water temperatures reduce oxygen solubility and increase biological oxygen demand, often producing extremely hypoxic, or even anoxic, conditions. *Gobiesox strumosus* are reluctant to abandon the bivalve shells they occupy (Runyan, 1961), suggesting these fish possess behavioural and physiological adaptations to survive in water of deteriorating quality. Previous research focuses on the species' taxonomy (Briggs, 1955), distribution (Hildebrand & Schroeder, 1928; Longley & Hildebrand, 1941) or early life history and reproduction (Runyan, 1961; Saksena & Joseph, 1972) with little to no data available on physiology.

Behavioural and physiological adaptations of fishes from challenging habitats have been studied extensively (Whitmore *et al.*, 1960; Heath *et al.*, 1993; Bennett

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& Beitinger, 1997; Muusze *et al*., 1998); however, most quantify individual traits rather than functional groups of adaptations necessary for survival. It seems unlikely that a single adaptation can explain *G. strumosus* success in harsh shallow-water environments. This study quantifies high temperature tolerance, low oxygen tolerance and metabolic responses to hypoxia in *G. strumosus* acclimated to a range of water temperatures. Identifying adaptive tactics used by *G. strumosus* can lead to a broader understanding of the range of tactics used by small resident fishes to survive in challenging abiotic habitats.

**MATERIALS AND METHODS**

**COLLECTION, MAINTENANCE AND ACCLIMATION OF *G. STRUMOSUS***

Eighty-four *G. strumosus* were collected from St Joseph’s Bay (Gulf County, FL, U.S.A.: 29° 48’ N; 85° 20’ W) and transported to the Marine Ecological Physiology Laboratory at the University of West Florida. Fish were transferred into a 400 l, biologically filtered, holding aquarium and maintained at capture temperature (20°C, range ± 1.0°C) and salinity (30) for 2 weeks to ensure that they were active and feeding. Following the holding period, five or six fish were each randomly assigned to one of fifteen 110 l, biologically filtered aquaria, and groups of five aquaria assigned to a 12, 22 or 32°C temperature acclimation group (approximate winter, mid-spring and mid-autumn, and summer temperatures in St Joseph’s Bay; Fangue & Bennett, 2003). Acclimation aquaria were initially held at 20°C, range ± 0.2°C (Techne re-circulating thermoregulators, model # TE-10A; www.techne.com) within an environmental growth chamber (www.russells-tech.com) set at 11°C. Temperatures were then increased or decreased 0.17°C day⁻¹ until the desired treatment level was reached. Several studies have shown that fishes abruptly transferred between temperatures reach full acclimation between 1 day (Brett, 1956; Allen & Strawn, 1971) and about 2 weeks (Chung, 2000, Reber & Bennett, 2007). Fish in this experiment probably began acclimating within hours of temperature change, but were held for an additional 14 days even after they reached their experimental treatment temperature to ensure that acclimation was indeed complete. Fish were fed daily, but were not fed 24 h before or during experimental trials (Steffensen, 1989). Following experiments, fish were released at the site of capture.

**EXPERIMENTAL APPARATUS**

Experimental conditions during oxygen tolerance and metabolic trials were generated using a temperature-controlled oxygen depletion system described by Smale & Rabeni (1995). Briefly, counter-current gas:water flow through two gas exchange tubes (5.1 cm, inside diameter, × 360 cm) produced the desired system oxygen levels, and water temperatures were controlled with a 1500 W immersion heater (www.WATTCO.com), or a Blue M, model # PCC-24A-3 water chiller (www.coleparmer.com). Stable flow rates were established and maintained using constant head pressure from an elevated sump. The same apparatus was used during temperature trials; however, air bubbled through both gas exchange tubes assuring full oxygen saturation as heaters progressively increased water temperature.

**HIGH TEMPERATURE TOLERANCE EXPERIMENTS**

*Gobiesox strumosus* temperature tolerance was estimated as the critical thermal maximum (*T* critmax), traditionally defined as the high temperature at which 50% of fish lose the ability to escape conditions that will ultimately lead to death (Cox, 1974). The *T* critmax for fish acclimated at 12, 22 or 32°C (five replicate trials of two or three fish each), were determined by progressively increasing water temperatures at 0.30°C min⁻¹ until fish experienced loss of
equilibrium (LOE) (Becker & Genoway, 1979; Beitinger et al., 2000). The LOE was defined as failure to regain normal dorso-ventral orientation following gentle agitation of the test flask (Beitinger et al., 2000). $T_{crit\text{max}}$ were calculated for each treatment replicate as the arithmetic mean of the collective LOE endpoint temperatures (Cox, 1974; Becker & Genoway, 1979; Beitinger et al., 2000), with the population $T_{crit\text{max}}$ being the grand mean of the individual replicate values.

HYPOXIA RESPONSE EXPERIMENTS

During hypoxia trials, three fish from a given treatment replicate ($n = 14$ at $12^\circ \text{C}$, 13 at $22^\circ \text{C}$ and 15 at $32^\circ \text{C}$) were placed, one each, into 250 ml glass test flasks and held for 1 h at oxygen saturation levels. Flask size always equalled or exceeded 50 times the fish’s volume, but was small enough to discouraged excessive movement (Steffenson, 1989; Cech, 1990). A control flask was included to account for non-fish sources of oxygen consumption. Following the holding period, oxygen levels were decreased by c. 2 mg l$^{-1}$ h$^{-1}$. Flow rates were adjusted to ensure that fish would uptake between 5 and 20% of dissolved oxygen passing through the flask (Cech, 1990), and frequent checks showed that flow remained within 2% of the set rate. Oxygen depletion was paused at c. 8·0, 6·0, 4·0, 2·0 and 0·5 mg l$^{-1}$, and total oxygen consumption values (mg h$^{-1}$ at standard temperature and pressure, STP) were determined using flow-through respirometry (Cech, 1990). To avoid wash-out effect error (Steffenson, 1989), inflow and outflow oxygen levels were measured (YSI model 55 oxygen meter; www.YSIEcoSense.com, and confirmed by Winkler titration methodology; Cox, 1990) once 99% exchange of flask volume had occurred. Wash-out times varied with flow rate and metabolic uptake but typically ranged between 40 and 60 min. Oxygen consumption at each level (mg O$_2$ g$^{-1}$ h$^{-1}$, STP) was calculated as the difference between initial and final oxygen content divided by the sampling time, and multiplied by the flask volume (Cech, 1990). Oxygen consumption values were mass corrected by dividing total consumption by fish mass$^{0.8}$ (Winberg, 1960, Cech, 1990, Clarke & Johnston, 1999). Oxygen depletion was continued until fish experienced LOE, after which oxygen tension was recorded and fish masses were determined ($\pm 0.1$ g) for use in calculating individual mass-corrected resting routine metabolic rates (Cech, 1990). Population estimates of low oxygen tolerance were taken as the mean of the collective individual LOE values, and individual resting routine metabolic rates were used to determine oxygen strategy.

DATA ANALYSES

Differences in $G. \text{strumosus}$ $T_{crit\text{max}}$ and hypoxia tolerance between temperature acclimation groups were compared using one-way ANOVA followed, when appropriate, by Tukey’s multiple range test (MRT). Plots of mass-corrected metabolic rate on oxygen tension were used to determine whether fish were following an oxygen regulation or oxygen conformation strategy (Willmer et al., 2005). Oxygen conformation was concluded if (individual) metabolic rates decreased linearly across the entire range of falling ambient tensions, whereas oxygen regulation was assumed if metabolic rates conformed to low oxygen tensions but were stable at high oxygen tensions. The relationship between oxygen tension and metabolic rate across the conformational portion of each temperature plot was modelled using simple linear regression, and slopes were statistically compared. All statistical decisions were based on $\alpha = 0.05$.

RESULTS

HIGH TEMPERATURE TOLERANCE ACCRUEMENT

$Gobiesox \text{strumosus}$ exhibited a direct, but unequal pattern of high thermal tolerance accruement over the acclimation temperatures tested (Table I). Fish acclimated at 12·0, 22·0 and 32·0$^\circ \text{C}$ displayed significantly different (one-way ANOVA,
Table I. Mean ± s.d. critical thermal maxima ($T_{critmax}$), tolerance gain per °C increase (TG) and low oxygen tolerance (LOT) values for *Gobiesox strumosus*

<table>
<thead>
<tr>
<th>n</th>
<th>Acclimation temperature (°C)</th>
<th>$T_{critmax}$ (°C)</th>
<th>TG (°C)</th>
<th>LOT (mg O$_2$ l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>12</td>
<td>33.2 ± 0.5</td>
<td>—</td>
<td>0.40 ± 0.09</td>
</tr>
<tr>
<td>13</td>
<td>22</td>
<td>38.1 ± 0.1</td>
<td>0.49</td>
<td>0.40 ± 0.08</td>
</tr>
<tr>
<td>15</td>
<td>32</td>
<td>39.5 ± 0.3</td>
<td>0.14</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

$n$, number of individuals.

$F_{2,39}$, $P < 0.001$ and statistically distinct $T_{critmax}$ (Tukey’s MRT $\alpha = 0.05$). Fish accrued a total of 6.3 °C of heat tolerance over the 20 °C acclimation range. *Gobiesox strumosus* gained >70% of this increase between 12.0 and 22.0 °C.

**Oxygen Consumption Patterns**

*Gobiesox strumosus* in all temperature treatments showed a more or less continuous decrease in oxygen consumption as dissolved oxygen tensions fell, indicating a pattern of oxygen conformation (Fig. 1). The y-intercept values for all temperature treatment groups ranged from 0.006 to 0.036 mg O$_2$ l$^{-1}$ h$^{-1}$ and were not significantly different from zero ($P > 0.05$ in all cases). Regression slopes decreased with acclimation temperature (Fig. 1). Pair-wise slope comparisons using a Bonferroni correction revealed significant differences between regression slopes of *G. strumosus* acclimated at 22.0 and 32.0 °C (d.f. = 5.503; $P < 0.001$), and between fish acclimated at 12.0 and 32.0 °C (d.f. = 7.464; $P < 0.001$). Regression slopes of oxygen consumption on oxygen concentration for fish acclimated at 12 to 22 °C, however, were statistically indistinguishable (d.f. = 2.211; $P > 0.01$).

**Responses to Hypoxia**

*Gobiesox strumosus* from the three temperature treatment groups showed differing physiological and behavioural responses to hypoxia (Table I). One-way ANOVA revealed that tolerance values were statistically indistinguishable from one another ($F_{2,12}$, $P > 0.05$). In addition, fish exhibited obvious shunting of blood flow to the area just anterior to the pectoral disc as dissolved oxygen concentrations fell below 2.0 mg l$^{-1}$. Increased vasodilatation occurred more frequently in fish from higher acclimation temperatures, and conspicuous levels of anterio-pectoral perfusion were observed in 20.0, 33.3 and 53.3% of fish acclimated at 12.0, 22.0 or 32.0 °C, respectively. As dissolved oxygen levels decreased, fish did not make persistent attempts to ventilate water at the top of the flask.

**Discussion**

Fishes from thermally challenging environments such as mangrove ponds (Menasveta, 1981) or desert pools (Heath et al., 1993; Bennett & Beitinger, 1997) commonly exhibit $T_{critmax}$ near 40 °C when acclimated at temperatures of c. 30 °C. While *G. strumosus* are not probably subjected to the same thermal extremes as
Fig. 1. Mass-corrected oxygen consumption of Gobiesox strumosus acclimated at (a) 32, (b) 22 and (c) 12°C exposed to decreasing oxygen tension.

desert pools, their relatively high $T_{critmax}$ values afford necessary protection from occasional temperature peaks approaching 40°C that occur in some shallow Florida waters (Harrington & Harrington, 1961) and possibly other areas of the fishes’ geographic range. Fishes from stable thermal environments typically show proportional increases in thermal tolerance with increasing acclimation temperature (Bennett et al., 1998; Currie et al., 1998). Gobiesox strumosus, however, acquired 76% (4·8°C) of
their total heat tolerance by 22°C, well below their lethal limit. A similar thermal tolerance accrual pattern is seen in sheephead minnow Cyprinodon variegatus Lacépède, which gain 85% of their total heat tolerance by the time acclimation temperatures reached 25°C (Bennett & Beiting, 1997). Early heat tolerance gains leave fishes physiologically prepared for unpredictable hyperthermic events that might be lethal to fishes less well adapted.

Thermal tolerance attributes may be dependent on a fish’s ability to endure hypoxia, and G. strumosus tolerated mean oxygen concentrations well below 0.5 mg l⁻¹ at 32°C where activity and metabolic uptake were high. The ability of fishes to survive extreme hypoxia at low temperatures has been well documented in the literature (Doudoroff & Shumway, 1967); however, it is unusual for fishes to tolerate oxygen levels near 0 mg l⁻¹ at temperatures >25°C (Nilsson & Renshaw, 2004). Indeed, Brett (1956) noted that fishes in hypoxic conditions often succumb to high temperatures that are not lethal in well-oxygenated water. Cutaneous respiration may be one way in which G. strumosus survive extreme hypoxia. While some members of the family Gobiesocidae are known to utilize cutaneous respiration when emerged during low tide (Vargas & Concha, 1957; Ebeling et al., 1970; Gordon et al., 1970; Kramer et al., 1983; Martin et al., 1993), aerial uptake has not previously been documented in G. strumosus. Fish in this study increased blood flow to the thin, highly vascularized antero-pectoral region in response to low oxygen (pers. obs.). It is unclear whether this tactic significantly improved oxygen uptake from hypoxic water; however, increased cutaneous surface area would most certainly improve oxygen uptake in fish given access to atmospheric oxygen.

While most fishes attempt to regulate tissue oxygen levels as ambient tensions fall (Ultsch et al., 1978), G. strumosus showed a pattern of oxygen conformation over their entire thermal acclimation range. Conformation, while relatively uncommon, can be an important survival tactic in hypoxic waters (Hochachka & Lutz, 2001), in which it allows fishes to match metabolic demand to oxygen availability. In effect, conformers break the cycle of increasing metabolic demand to meet oxygen requirements, thereby allowing them to thrive in hypoxic conditions that are otherwise uninhabitable. Not surprisingly, fishes from environments that frequently become hypoxic or anoxic are most likely to exhibit oxygen conformation patterns. For example, oxygen conformation is seen in several fishes from hypoxic habitats including oyster toadfish Opsanus tau (L.) Hall, 1929), brown bullhead Amelurus nebulosus (Lesueur) (Marvin & Heath, 1968) and European plaice Pleuronectes platessa L. (Steffensen et al., 1982). Oxygen conformation is an important part of the complementary suite of adaptations used by G. strumosus to survive all but the harshest conditions.

References


Winberg, G. G. (1960). Rate of metabolism and food requirements of fishes. *Fisheries Research Board of Canada Translation Series No.* **194**.