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Interspecific and environment-induced variation in hypoxia tolerance in sunfish

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ABSTRACT

Hypoxia tolerance is a plastic trait, and can vary between species. We compared hypoxia tolerance (hypoxic loss of equilibrium, LOE, and critical O_2 tension, P_{crit}) and traits that dictate O_2 transport and metabolism in pumpkinseed (*Lepomis gibbosus*), bluegill (*L. macrochirus*), and the naturally occurring hybrid in different acclimation environments (wild versus lab-acclimated fish) and at different temperatures. Wild fish generally had lower P_{crit} and lower PO_2 at LOE in progressive hypoxia than lab-acclimated fish, but time to LOE in sustained hypoxia (PO_2 of 2 kPa) did not vary between environments. Wild fish also had greater gill surface area and higher haematocrit, suggesting that increased O_2 transport capacity underlies the environmental variation in P_{crit}. Metabolic (lactate dehydrogenase, LDH; pyruvate kinase, PK; citrate synthase; cytochrome c oxidase) and antioxidant (catalase and superoxide dismutase) enzyme activities varied appreciably between environments. Wild fish had higher protein contents across tissues and higher activities of LDH in heart, PK in brain, and catalase in brain, liver, and skeletal muscle. Otherwise, wild fish had lower activities for most enzymes. Warming temperature from 15 to 25 °C increased O_2 consumption rate, P_{crit}, PO_2 at LOE, and haemoglobin-O_2 affinity, and decreased time to LOE, but pumpkinseed had ≥2-fold longer time to LOE than bluegill and hybrids across this temperature range. This was associated with higher LDH activities in the heart and muscle, and lower or similar antioxidant enzyme activities in several tissues. However, the greater hypoxia tolerance of pumpkinseed collapsed at 28 °C, demonstrating that the interactive effects of hypoxia and warming temperature can differ between species. Overall, distinct mechanisms appear to underpin interspecific and environment-induced variation in hypoxia tolerance in sunfish.

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1. Introduction

Periods of low oxygen availability are a regular feature of many freshwater ecosystems, and can be elicited by both natural events (e.g., ice cover, stratification) and human activities (e.g. eutrophication, pollution) (Diaz, 2001; Ficke et al., 2007). Maintaining cellular ATP homoeostasis is critical for surviving such periods of O_2 deprivation, and can be accomplished by increasing mitochondrial O_2 supply, increasing O_2-independent ATP production, and/or reducing O_2 and ATP demands through metabolic depression (Bickler and Buck, 2007; Boutilier, 2001; Hochachka et al., 1996; Michiels, 2004; Richards, 2009; Wheaton and Chandel, 2011). More hypoxia tolerant species are generally better at preserving ATP homoeostasis in hypoxia, and maintain some aerobic scope for activity over a broader range of O_2 tensions (PO_2) (Lefrançois et al., 2005; Richards, 2009). Hypoxia tolerance is often quantified by the critical PO_2 (P_{crit}, the PO_2 below which the animal oxyconforms), or PO_2 at which loss of equilibrium (LOE) occurs during progressive hypoxia, and/or the time to LOE during a sustained level of severe hypoxia (He et al., 2015; Mandic et al., 2009; Mathers et al., 2014; Nelson and Lipkey, 2015; Petersen and Steffensen, 2003; Speers-Roesch et al., 2012).

Hypoxia tolerance is often associated with a high capacity for O_2 transport, which reduces the difference between environmental and tissue PO_2 and helps mitigate the effects of hypoxia. The surface area available for O_2 uptake at the gills often increases with hypoxia acclimation (Dabruzzi and Bennett, 2014; Hughes, 1966; Nilsson, 2007; Søllid and Nilsson, 2006), and taxa from habitats with low or variable PO_2 often have a greater gill surface area than those from open-water or well-oxygenated environments, even when compared in common-garden conditions (Chapman et al., 2002; Crampton et al., 2008; Mandic et al., 2009). Increases in gill surface area can be achieved by lengthening gill filaments (Chapman et al., 1999; Chapman and Hulen, 2001), or by reducing the inter-lamellar cell mass to increase the functional surface area of lamellae (Matey et al., 2008; Nilsson, 2007; Søllid et al., 2003).

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Alternatively, some species may decrease gill surface area with hypoxia acclimation to minimise ionoregulatory disruption due to the osmo-respiratory compromise (Borowiec et al., 2015; De Boeck et al., 2013; Matey et al., 2011; McDonald and McMahon, 1977; Wood et al., 2009; Wood et al., 2007). Haematocrit, blood haemoglobin content, and haemoglobin-O_2 affinity often increase during hypoxia acclimation as well (Borowiec et al., 2015; Lai et al., 2006; Rutjes et al., 2007; Wells, 2009), though exceptions occur (Weber et al., 1979), all of which may help increase the carrying-capacity for, and circulation of, O_2 in the blood. Only haemoglobin-O_2 affinity is typically associated with evolved (genetically based) variation in hypoxia tolerance, such that more tolerant species tend to have higher haemoglobin-O_2 affinities (Mandic et al., 2009).

The activity of metabolic and antioxidant enzymes can change during hypoxia exposure and can differ interspecifically in association with variation in hypoxia tolerance, but the responses often vary appreciably between species and tissues. For example, lactate dehydrogenase (LDH) activity increases with hypoxia exposure in the heart and liver of some (but not all) species, but LDH activity in the brain often remains unchanged (Borowiec et al., 2015; Chippari-Gomes et al., 2005; Crocker et al., 2013; Martinez et al., 2006). Contrastingly, interspecific variation in hypoxia tolerance has been associated with elevated LDH activity in the brain but not in the liver or muscle (Mandic et al., 2013). Such variation could foreseeably result from differences in the perfusion (and thus tissue PO_2) and/or the functional role of different organs and tissues in hypoxia, each of which could vary between species. It is also possible that mechanisms underlying hypoxia acclimation differ systematically from those underlying evolved variation in hypoxia tolerance, such as could occur if the acclimation response is not adaptive or even maladaptive (Ghalambor et al., 2007; Storz et al., 2010).

The objectives of this study were (i) to examine how acclimation environment and temperature affects interspecific variation in hypoxia tolerance in sunfish, and (ii) to evaluate whether interspecific and environment-induced variation in hypoxia tolerance were underpinned by similar physiological mechanisms (by measuring subordinate physiological traits that dictate capacities for O_2 transport, energy metabolism, and antioxidant defences). Bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) sunfish are abundant in temperate lakes throughout North America, and can produce unidirectional (male bluegill × female pumpkinseed), non-reproductive F_1 hybrids where they naturally co-occur (Konkle and Philipp, 1992). The parental species differ in their ecological niches (bluegill favour planktonic prey, while the pumpkinseed diet is primarily mollusks) (Mittelbach, 1984; Osenberg et al., 1992; Robinson et al., 1993), hypoxia tolerance (Farwell et al., 2007; Mathers et al., 2014), and in mitochondrial respiration and metabolic enzyme activities in their white muscle (Davies et al., 2012; Davies and Moyes, 2007; Davies et al., 2011). Here, we compare recently captured sunfish living in their native environment to sunfish acclimated to common control conditions in the laboratory. This was accomplished by comparing newly collected data on wild fish to data on lab-acclimated fish, some of which was newly collected for this study (all hybrid data, as well as bluegill and pumpkinseed data on respirometry and hypoxia tolerance, antioxidant enzyme activities, metabolic enzyme activities expressed per mg protein, and haemoglobin-O_2 binding affinities) and some of which has been previously reported (bluegill and pumpkinseed data on gill morphometrics, haematocrit, and metabolic enzyme activities expressed per g tissue) (Crans et al., 2015).

2. Materials and methods

2.1. Fish capture and holding conditions

Bluegill (*L. macrochirus*), pumpkinseed (*L. gibbosus*), and bluegill–pumpkinseed hybrids were collected from Lake Opinicon at Queen's University Biological Station in southeastern Ontario, Canada (44°35′ N, 76°20′W) by angling in August and October of 2012 (lab-acclimated group) or in June of 2013 (wild group). Fish in the wild group were held temporarily in large outdoor tanks supplied with flow-through lake water, and were used in respirometry and hypoxia tolerance experiments within 18–24 h of capture (during which time they were not fed). Fish in the lab-acclimated group were transported to McMaster University and housed for at least four weeks in 500 l tanks with a flow-through supply of dechlorinated municipal tap water at 12–15 °C (12 h:12 h light:dark photoperiod). Lab-acclimated fish were fed a mix of commercially purchased squid or beef organs (heart, liver, and kidney) four to five times weekly (~3% body mass), and were fasted for 18–24 h before any experimentation took place.

2.2. Respirometry and hypoxia tolerance experiments

Intermittent stop-flow respirometry was used to determine resting oxygen consumption rate (MO_2), P_{crit}, and PO_2 at LOE for wild and lab-acclimated fish at 25 °C, as well as at 15 °C and 28 °C for lab-acclimated fish only, using methods we have previously described (Crans et al., 2015). Repeated measurements of the same individuals were made at each temperature in these experiments with lab-acclimated fish, carried out in random order with at least 1 week of recovery between measurements. Briefly, fish were held individually in the dark for 8–12 h in 2 l respirometry chambers that were continuously flushed with normoxic water at the desired experimental temperature. MO_2 was first measured in normoxia (PO_2 of ~20 kPa), and then at each PO_2 of a step-wise exposure to progressive hypoxia in which the PO_2 was reduced by 2 kPa every 20 min. After the measurement period at 4 kPa, the chamber was sealed and the fish was then allowed to consume the remaining O_2 in the chamber until LOE. Background respiration was negligible at both sites, so MO_2 was calculated as the change in chamber oxygen concentration over time and is expressed relative to body mass. P_{crit} was calculated using a programme developed by Yeager and Ultsch (Yeager and Ultsch, 1989).

A separate set of fish was used to measure the time to LOE at a sustained level of severe hypoxia at 25 °C in groups of wild and lab-acclimated fish, as well as in lab-acclimated fish at 15 °C and 28 °C (temperatures in random order, as above). Fish were held individually for 8–12 h in 3 l plastic chambers that were continuously flushed with normoxic water. Buffer tank PO_2 was then rapidly decreased to 2 kPa (which took 13–17 min) by bubbling with N_2 gas and the PO_2 was held steady until the fish lost equilibrium.

2.3. Sampling

Wild fish were recovered and sampled within 30 min of reaching LOE during progressive hypoxia exposure in the respirometry experiments. Samples were also collected from lab-acclimated sunfish within 30 min of reaching LOE during progressive hypoxia exposure, but these samples were collected as part of a previous study that used the same holding conditions as those described in Section 2.1 (Crans et al., 2015). In both groups, fish were euthanised with an overdose of buffered MS-222 (1 g l^{-1}) and weighed. A transverse cut was made through the trunk at the anterior base of the anal fin, and blood was collected into heparinised capillary tubes and centrifuged at 14,000 rpm for 5 min to measure haematocrit. The packed red blood cells were then frozen in liquid N_2. A second transverse cut was then made ~2–3 mm anterior to the first, and the resulting block of muscle (which contained the entirety of the red and white fibres) was frozen in liquid N_2. The entire heart, brain, and liver were also frozen in liquid N_2. All frozen samples were stored at −80 °C for later assays. The entire gill basket was carefully removed, fixed in phosphate buffered saline (PBS) containing 2% paraformaldehyde and 2% glutaraldehyde for 24 h at 4 °C, and then stored in PBS until morphometric analyses were performed. Pumpkinseed and hybrid sunfish have a similar external appearance, so clips of the caudal fin were taken and preserved in 70% ethanol for later genotyping.

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