



# Critical oxygen tensions as predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in fishes



Ben Speers-Roesch<sup>\*</sup>, Milica Mandic, Derrick J.E. Groom<sup>1</sup>, Jeffrey G. Richards

Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver, British Columbia V6T 1Z4, Canada  
Bamfield Marine Sciences Centre, 100 Pachena Dr., Bamfield, British Columbia V0R 1B0, Canada

## ARTICLE INFO

### Article history:

Received 24 May 2013

Received in revised form 7 October 2013

Accepted 9 October 2013

Available online 26 October 2013

### Keywords:

Critical oxygen tension

Energy metabolism

Fishes

Hypoxia tolerance

Loss of equilibrium

$P_{crit}$

## ABSTRACT

An organism's critical oxygen tension ( $P_{crit}$ ) reflects its ability to extract environmental  $O_2$ . Consequently,  $P_{crit}$  has been used as an indicator of hypoxia tolerance in aquatic animals. The relationship between  $P_{crit}$  and hypoxia tolerance and hypoxic metabolic responses, however, remains incompletely understood. Among several species of sculpin fishes (superfamily Cottoidea), we previously demonstrated a correlation between  $P_{crit}$  and hypoxia tolerance, as measured as the time required for 50% of a group of fish to lose equilibrium ( $LOE_{50}$ ) at a water  $PO_2$  of 6.4 Torr. In the present study, we further investigated the relationship between  $P_{crit}$ , hypoxia tolerance, and hypoxic metabolic responses by examining the effects of hypoxia exposure at a fixed percentage of  $P_{crit}$  (30%; termed relative hypoxia exposure) on  $LOE_{50}$  and metabolic responses in brain, liver, and white muscle in three sculpin species that differ in  $P_{crit}$  and hypoxia tolerance at 6.4 Torr. We also assessed the tissue metabolic responses underlying hypoxic loss of equilibrium (LOE). The species, from most to least hypoxia-tolerant at 6.4 Torr and from lowest to highest  $P_{crit}$  values, were the tidepool sculpin (*Oligocottus maculosus*), staghorn sculpin (*Leptocottus armatus*), and silverspotted sculpin (*Blepsias cirrhosus*). If  $P_{crit}$  predicts hypoxia tolerance, then we expected similar  $LOE_{50}$  values and similar tissue metabolic responses across all species during relative hypoxia exposure.  $LOE_{50}$  values were similar in staghorn sculpins and tidepool sculpins, but not in silverspotted sculpins, which had a comparatively lower relative hypoxia  $LOE_{50}$  value. Thus,  $P_{crit}$ , and consequently the ability to extract environmental  $O_2$ , cannot predict hypoxia tolerance in all species, at least at a water  $PO_2$  of 30% of  $P_{crit}$ . During relative hypoxia exposure, tissue lactate accumulation and ATP levels were similar between species, suggesting that the ability to extract environmental  $O_2$  is an important determinant of cellular energy status and reliance on anaerobic glycolysis in hypoxic sculpins. However, whereas tissue glycogen content and utilization were similar between tidepool sculpins and staghorn sculpins, there were lower normoxic levels and greater hypoxic depletion in silverspotted sculpins, potentially explaining their poorer relative hypoxia tolerance. In all species, LOE was associated with depletion of brain [ATP]. Overall, caution is warranted when  $P_{crit}$  is used as an indicator of hypoxia tolerance, especially when considering temporal aspects of hypoxia tolerance and related metabolic characteristics (e.g. glycogen availability). Ideally, comparative studies of hypoxia tolerance should feature multiple measures (e.g.  $P_{crit}$  and  $LOE_{50}$ ) in order to assess the overall responses of fishes to hypoxia.

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## 1. Introduction

Environmental hypoxia is a common abiotic stressor in aquatic environments (Diaz and Breitburg, 2009). Unsurprisingly, many fishes have evolved the ability to survive periods of hypoxia, although there is large interspecific variation in the severity and duration of hypoxia that can be tolerated. An important factor thought to underlie the

variation in hypoxia tolerance is the critical  $O_2$  tension of whole-animal  $O_2$  consumption rate ( $\dot{M}_{O_2}$ ), or  $P_{crit}$ , which is the water  $PO_2$  ( $P_{wO_2}$ ) at which the  $\dot{M}_{O_2}$  of an organism transitions from being independent of, to being dependent upon, environmental  $O_2$ . Differences in  $P_{crit}$  are primarily due to variation in the physiological variables that influence the diffusive and convective movement of  $O_2$  from the water to the mitochondrion (Richards, 2011). A lower  $P_{crit}$ , in general, has been thought to be associated with greater hypoxia tolerance because it indicates improved  $O_2$  uptake and transport to tissues at low  $P_{wO_2}$ , which would decrease the requirement for  $O_2$ -independent ATP production, a relatively inefficient process that is accompanied by accumulation of deleterious by-products (e.g.,  $H^+$ ) (Nilsson and Östlund-Nilsson, 2008; Mandic et al., 2009; Speers-Roesch et al., 2012a). In fact, we recently demonstrated that  $P_{crit}$  was negatively

<sup>\*</sup> Corresponding author at: Department of Ocean Sciences, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NL A1C 5S7, Canada. Tel.: +1 709 864 4030.

E-mail address: [bensr@zoology.ubc.ca](mailto:bensr@zoology.ubc.ca) (B. Speers-Roesch).

<sup>1</sup> Present address: Department of Cell & Systems Biology, University of Toronto, 25 Harbord St., Toronto, ON M5S 3G5, Canada.

correlated with hypoxia tolerance, measured as the time required for 50% of a group of fish to show loss of equilibrium (LOE<sub>50</sub>) at a P<sub>w</sub>O<sub>2</sub> of 6.4 Torr, across 11 species of sculpins (superfamily Cottoidea) (Mandic et al., 2013). These findings corroborate the widespread use of P<sub>crit</sub> as a useful measure of hypoxia tolerance that may facilitate cross-species comparisons of the metabolic responses of aquatic organisms to environmental hypoxia (Pörtner and Grieshaber, 1993; Chapman et al., 2002; McKenzie et al., 2008; Nilsson and Östlund-Nilsson, 2008; Mandic et al., 2009; Speers-Roesch et al., 2012a). However, with the exception of Mandic et al. (2013), few studies have explored the relationship between P<sub>crit</sub> and hypoxia tolerance or responses, including determining if hypoxia exposures scaled to P<sub>crit</sub> result in similar metabolic disturbances across multiple species.

At P<sub>w</sub>O<sub>2</sub> below P<sub>crit</sub>, where O<sub>2</sub> availability is constrained, hypoxic survival is thought to be dependent upon the ability of a fish to maintain cellular energy balance (i.e., stable ATP levels) in tissues despite diminished aerobic energy supply (Richards, 2009). This may be achieved by an increased activation of O<sub>2</sub>-independent ATP production, in particular anaerobic glycolysis, as well as a reversible metabolic rate depression (Richards, 2009). Provision of exclusively aerobic fuels such as non-esterified fatty acids appears to be downregulated during hypoxia exposure in some hypoxia-tolerant fishes (Van den Thillart et al., 2002; Speers-Roesch et al., 2010). In contrast, mobilization of stored fermentable fuels (e.g., glycogen) is crucial to sustain anaerobic glycolysis and hypoxia-tolerant fishes are generally considered to have greater glycogen stores than hypoxia-sensitive fishes (Richards, 2009).

Species that can maintain cellular energy balance to lower P<sub>w</sub>O<sub>2</sub>, particularly in vital organs such as the brain, should have greater hypoxia tolerance (Richards, 2009) because they can prevent catastrophic drops in tissue [ATP] that lead to cellular dysfunction and necrosis, and, consequently, loss of equilibrium (LOE) and organismal death (Boutilier, 2001). However, direct support for the existence of a link between hypoxia-induced disruption of cellular energy balance and hypoxic survival is equivocal, with some species showing substantial decreases in [ATP] in brain and other tissues at LOE or moribundity (DiAngelo and Heath, 1987; Van Raaij et al., 1994; Van Ginneken et al., 1996; Ishibashi et al., 2002), while in other species such changes are not evident (DiAngelo and Heath, 1987; Van Ginneken et al., 1996). Even if a fish maintains cellular energy balance during hypoxia exposure, hypoxic survival time may be limited by glycogen availability as well as by metabolic acidosis associated with anaerobic glycolysis (Nilsson and Östlund-Nilsson, 2008; Richards, 2009). If glycogen stores are exhausted, loss of anaerobic glycolytic ATP production will result in the perturbed cellular energy balance described previously. Nonetheless, despite one study showing that moribund hypoxia-exposed fishes show depletion of brain [glycogen] (DiAngelo and Heath, 1987), it is unclear whether glycogen exhaustion is a major limiting factor affecting hypoxic survival. Even if glycogen stores remain available, metabolic acidosis can constrain hypoxic survival and more hypoxia-tolerant species are thought to minimize acidosis by decreasing energy demands via metabolic rate depression or by improved acid–base regulation (Jackson, 2004). Tissue lactate levels can be a simple indicator of the degree of acidosis because of the equimolar production of lactate and H<sup>+</sup> by anaerobic glycolysis (Nilsson and Östlund-Nilsson, 2008). There is some evidence that excessive lactate loads in brain and other tissues are associated with LOE during hypoxia exposure in fishes (DiAngelo and Heath, 1987; Van Raaij et al., 1994).

In the present study, we examined the relationship between P<sub>crit</sub> and hypoxia tolerance and assessed the biochemical limitations on hypoxia tolerance in three species of sculpins that vary in P<sub>crit</sub> and hypoxia tolerance as measured by LOE<sub>50</sub> at 6.4 Torr. Specifically, we addressed three questions: 1) Does exposure to hypoxia at a fixed percentage of P<sub>crit</sub> result in similar LOE<sub>50</sub> in these species?, 2) Does exposure to hypoxia at a fixed percentage of P<sub>crit</sub> result in metabolic responses in brain, liver, and muscle that are similar in all three species?, and 3)

What metabolic responses in brain, liver, and muscle are associated with hypoxic LOE in these species? Our study species were the silver-spotted sculpin (*Blepsias cirrhosus*), the staghorn sculpin (*Leptocottus armatus*), and the tidepool sculpin (*Oligocottus maculosus*), which are known to have different P<sub>crit</sub> values (44.4, 37.4, and 25.9 Torr, respectively) that are correlated negatively with the interspecific variation in LOE<sub>50</sub> values determined at 6.4 Torr (25, 281, and 538 min, respectively) (Mandic et al., 2009; Mandic et al., 2013). To address question 1, we exposed each species to a level of hypoxia equal to 30% of their P<sub>crit</sub> (henceforth termed relative hypoxia) and measured the time to LOE<sub>50</sub> (henceforth termed relative hypoxia LOE<sub>50</sub>). If variation in P<sub>crit</sub> and consequently the ability to extract environmental O<sub>2</sub>, explains interspecific variation in whole-animal hypoxia tolerance, as suggested by Mandic et al. (2013), then relative hypoxia LOE<sub>50</sub> values would be similar between species. To address question 2, we measured the metabolic profile (i.e. levels of ATP, glycogen, glucose, and lactate) in brain, liver, and white muscle of each species exposed to 6 h of relative hypoxia. If variation in P<sub>crit</sub> explains interspecific variation in hypoxia tolerance, then relative hypoxia exposure in the three species should result in similar tissue metabolic profiles. On the other hand, if factors other than P<sub>crit</sub> play a role in explaining the variation in hypoxia tolerance among these species, then tissue metabolic profiles would vary between species. To address question 3 and ascertain the proximate cause of LOE in fishes, we measured metabolic profiles in brain, liver, and white muscle of individuals of each species at the point they displayed LOE during hypoxia exposure.

## 2. Materials and methods

### 2.1. Animals

Silverspotted sculpins (7.0 ± 0.4 g, n = 58), staghorn sculpins (34.3 ± 2.0 g, n = 54), and tidepool sculpins (5.2 ± 0.4 g, n = 45) were collected using handheld nets or beach seines during the lowest tidal cycle of July 2008 at Ross Islets (48°52.4' N, 125°9.7' W) and Wizard's Rock (48°51.5' N, 125°9.4' W), near the Bamfield Marine Sciences Centre (BMSC), Bamfield, British Columbia, Canada. All three species of sculpins were transported to BMSC and held in fiberglass aquaria supplied with aerated flow-through filtered seawater (12 °C, 33 ppt). Fishes were allowed to recover from capture for at least one week before experimentation during which they were fed daily with mussels, bloodworms and frozen baitfish, except for 24 h before experimentation when food was withheld. All experiments were conducted according to guidelines set out by the Canadian Council for Animal Care and approved institutional protocols.

### 2.2. Experimental protocols

#### 2.2.1. Determination of relative hypoxia LOE<sub>50</sub> values

A total of three trials were carried out on each species using the following protocol. For each trial, eight individuals from a randomly selected species were transferred from holding tanks to a 40 L glass aquarium supplied with aerated flow-through filtered seawater and partially submerged in a wet table supplied with the same seawater (12 °C) for temperature regulation. The fishes were allowed to acclimate overnight under well-aerated conditions. Two small submersible pumps were placed in the aquarium to ensure adequate mixing of the water. Following the acclimation period, the flow-through seawater supply and aeration was stopped and hypoxia was induced by bubbling N<sub>2</sub> into the aquarium, which was covered with plastic bubble wrap to prevent O<sub>2</sub> ingress. Each species was exposed to a level of hypoxia corresponding to 30% of their P<sub>crit</sub> (relative hypoxia): P<sub>w</sub>O<sub>2</sub> = 13.2 Torr for silverspotted sculpins, 11.1 Torr for staghorn sculpins, and 7.7 Torr for tidepool sculpins. The rate of decrease of P<sub>w</sub>O<sub>2</sub> was similar across all trials and hypoxic levels were reached after approximately 30 min of N<sub>2</sub>

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