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REVIEW

Can variation among hypoxic environments explain why different fish species use different hypoxic survival strategies?

Milica Mandic[1,*,‡] and Matthew D. Regan[2,*]

ABSTRACT

In aquatic environments, hypoxia is a multi-dimensional stressor that can vary in O_2 level (partial pressure of O_2 in water, Pw_{O_2}), rate of induction and duration. Natural hypoxic environments can therefore be very different from one another. For the many fish species that have evolved to cope with these different hypoxic environments, survival requires adjusting energy supply and demand pathways to maintain energy balance. The literature describes innumerable ways that fishes combine aerobic metabolism, anaerobic metabolism and metabolic rate depression (MRD) to accomplish this, but it is unknown whether the evolutionary paths leading to these different strategies are determined primarily by species' phylogenetic histories, genetic constraint or their native hypoxic environments. We explored this idea by devising a four-quadrant matrix that bins different aquatic hypoxic environments according to their duration and Pw_{O_2} characteristics. We then systematically mined the literature for well-studied species native to environments within each quadrant, and, for each of 10 case studies, described the species' total hypoxic response (THR), defined as its hypoxia-induced combination of sustained aerobic metabolism, enhanced anaerobic metabolism and MRD, encompassing also the mechanisms underlying these metabolic modes. Our analysis revealed that fishes use a wide range of THRs, but that distantly related species from environments within the same matrix quadrant have converged on similar THRs. For example, environments of moderately hypoxic Pw_{O_2} favoured predominantly aerobic THRs, whereas environments of severely hypoxic Pw_{O_2} favoured MRD. Capacity for aerial emergence as well as predation pressure (aquatic and aerial) also contributed to these responses, in addition to other biotic and abiotic factors. Generally, it appears that the particular type of hypoxia experienced by a fish plays a major role in shaping its particular THR.

KEY WORDS: Aquatic hypoxia, Total hypoxic response, Aerobic metabolism, Anaerobic metabolism, Metabolic rate depression, Fish

Introduction

Environmental hypoxia is a multi-dimensional stressor of many aquatic ecosystems, typically involving variations in O_2 level (partial pressure of O_2 in water, Pw_{O_2}), rate of hypoxia induction and duration of exposure. Aquatic hypoxic environments can therefore differ from one another considerably. For example, intertidal pools oscillate between ~80 and 0 kPa Pw_{O_2} each day (Richards, 2011), whereas oceanic oxygen minimum zones (OMZs), which typically occur at depths of 200 to 1500 m, remain at stable hypoxic Pw_{O_2} (≤4.2 kPa; Seibel, 2011). Orders of fishes have independently evolved abilities to tolerate and exploit different hypoxic environments, and the literature is rife with studies that reveal the different strategies that allow them to do this. What remains unknown, however, is whether similar hypoxic environments beget similar hypoxic survival strategies, independent of phylogenetic relationships.

Our Review will explore variation in the hypoxic survival strategies of different fish species in the context of these species' native hypoxic environments. We will represent each species' hypoxic survival strategy using the total hypoxic response (THR; see Glossary), which we define as the combination of these three metabolic modes an animal uses in hypoxia: (i) sustained aerobic metabolism via a wide array of mechanisms that enhance capacities for O_2 uptake, transport and delivery; (ii) increased use of anaerobic metabolism; and (iii) metabolic rate depression (MRD; see Glossary). The THR is useful in this respect because it is a complex phenotype that is contributed to by numerous traits operating at lower levels of organization. Although these traits can vary among species [e.g. some species increase haemoglobin (Hb)–O_2 binding affinity, whereas others increase haematocrit (see Glossary)] or be similar but achieved through different mechanisms (e.g. high gill surface area that is constitutively expressed or increased via remodeling/plasticity), they may result in consistent responses at higher levels of organization (e.g. sustained aerobic metabolism). Interpreting hypoxic responses at these higher levels of biological organization may reveal patterns of convergent evolution (see Glossary) that are less visible at lower levels.

There are costs associated with hypoxic survival strategies

Although the metabolic strategies that contribute to the THR – aerobic metabolism, anaerobic metabolism and MRD – benefit hypoxic survival (Fig. 1; and see Box 1 for descriptions of these metabolic modes and the data that may be used to interpret them), each strategy has limits and potential costs that may influence how it is (or is not) used in certain hypoxic environments. The main limit of aerobic metabolism is a critical Pw_{O_2} (P_{crit}; see Glossary) below which aerobic reliance becomes severely compromised (see Box 1, Fig. 1). Capacity for aerobic metabolism may be improved through different mechanisms of increasing O_2 supply, but these too can come at a cost. For example, increasing respiratory surface area aids in oxygen extraction from the water, but with potential negative consequences of compromising ion and water fluxes, increasing detrimental uptake of ammonia and other toxic substances, and increasing the likelihood of bleeding (reviewed in Nilsson and Randall, 2010). Similarly, an increase in ventilation and haematocrit can improve O_2 extraction and delivery, but an increase in ventilation incurs a significant energetic cost (Hughes and Sunders, 1970; Steffensen, 1985), and high haematocrit increases blood viscosity, thus increasing blood flow resistance (reviewed in Gallaugher and Farrell, 1998).

[1]Department of Biology, University of Ottawa, Ottawa, ON, Canada, K1N 6N5. [2]Comparative Biosciences Department, University of Wisconsin-Madison, Madison, WI 35706, USA.
*These authors contributed equally to this work

‡Author for correspondence (mmandic@uottawa.ca)

M.M., 0000-0002-9377-4173; M.D.R., 0000-0001-9341-5747

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List of symbols and abbreviations

ASR	aquatic surface respiration
CrP	creatine phosphate
Hb	haemoglobin
Hb P_{50}	partial pressure of O_2 at which Hb is 50% saturated with O_2
$\dot{M}_{O_2}$	oxygen consumption rate
MRD	metabolic rate depression
OMZ	oxygen minimum zone
Pw_{O_2}	partial pressure of oxygen in water
THR	total hypoxic response

For anaerobic metabolism (i.e. anaerobic glycolysis, hereafter referred to as glycolysis), the main limit is a finite substrate pool (glucose, glycogen) that restricts the time over which an animal may rely on glycolysis (Wang et al., 2009). The main cost of prolonged anaerobic reliance is a metabolic acidosis (see Glossary) that jeopardizes the fish's health and hypoxia tolerance (Driedzic and Gesser, 1994; Hochachka and Somero, 2002; Nilsson and Östlund-Nilsson, 2008). And more generally, glycolysis is relatively inefficient at converting energy stored in food to forms that are usable by the cell (ATP).

For MRD, the costs can be physiological or ecological. Physiologically, these include oxidative damage (Carey et al., 2000), reduced growth, repair and immunocompetence (Burton and Reichman, 1999), and cognitive impairments stemming from neuronal damage (Popov et al., 1992; Lefevre et al., 2017). Ecologically, costs include ceased reproduction (Humphries et al., 2003) and increased susceptibility to predation stemming from significantly reduced awareness and motor activity (Draud et al., 2004; Kokurewicz, 2004; Lanszki et al., 2006; Wiklund et al., 2008; Estok et al., 2009; Sommer et al., 2009; Olofsson et al., 2011).

There is great variation in how different fish species use each metabolic strategy in response to hypoxia. Some species prioritize aerobic and anaerobic metabolism (e.g. intertidal sculpins), whereas others rely on anaerobic metabolism coupled with MRD (e.g. crucian carp). It is possible that the diversity of THRs is partly attributable to the aforementioned costs, which may be more or less relevant to a given species depending on its ability to mitigate them. This ability may be influenced by factors such as genetic constraint or the ecological environment. However, no study has explicitly examined the causes underlying why different hypoxia-tolerant species have (or have not) evolved particular THR profiles.

Glossary

Aquatic surface respiration
A breathing technique that involves skimming the relatively well-oxygenated surface layer of the water column.

Bohr effect
A reduced haemoglobin–oxygen binding affinity resulting from a reduced pH.

Convergent evolution
Independent evolution of similar traits in different evolutionary lineages.

Eutrophic
A body of water enriched with nutrients and minerals, resulting in excessive growth of plants and algae.

Haematocrit
Proportion of whole blood that consists of red blood cells.

Macrophyte
An aquatic plant that grows in or near water, and is emergent, submergent or floating.

Metabolic acidosis
Reduced pH resulting from metabolic activity.

Metabolic rate depression
A coordinated and reversible depression of metabolic rate below standard metabolic rate.

Oligotrophic
A body of water with low accumulation of nutrients and minerals, resulting in sparse growth of plants an algae.

Critical oxygen tension (P_{crit})
The lowest P_{O_2} at which an animal can sustain routine $\dot{M}_{O_2}$, below which $\dot{M}_{O_2}$ progressively decreases.

Pleiotropy
Phenotypic effect of a gene on more than one trait.

Total hypoxic response
The hypoxia-induced combination of sustained aerobic metabolism, enhanced anaerobic metabolism and metabolic rate depression (encompassing also the mechanisms underlying these metabolic modes) that an animal uses to survive hypoxia.

Ventilation amplitude
Volume of water pumped over the gills during a single breath.

Ventilation rate
Number of breaths per minute.

Water column stratification
Vertical distribution of water layers that form as a result of low mixing between water masses of different properties (e.g. hypoxia, salinity, density, temperature).

White muscle
A rapid or fast-twitch muscle fibre type.

THRs may arise through different mechanisms

Despite multiple THRs arising among the hypoxia-tolerant fishes, there is a finite number of evolutionary solutions and most species can be broadly grouped into clusters of similar metabolic strategies. Three mechanisms may influence this probability of convergence: genetic constraints, phylogenetic history and/or natural selection (Christin et al., 2010; Losos, 2011; Rosenblum et al., 2014).

Genetic constraints, such as pleiotropy (see Glossary) or limited genetic variation, increase the likelihood of repeated evolution of a given phenotype because the constraints decrease the number of evolutionary paths available in response to an environmental stressor such as hypoxia (Chevin et al., 2010; Conte et al., 2012). Phylogenetic history may also lead to similar THRs, whereby the shared genetic backgrounds of closely related species increase their probabilities of evolving parallel solutions (Rosenblum et al., 2014). Natural selection may also be a possible driver of convergence, where the ecological environment is the primary determinant of evolution of a similar THR across species. If the environment is a driving factor in the evolution of a particular THR, then mining the extensive hypoxia literature should reveal a pattern whereby distantly related species native to similar hypoxic environments display similar THRs. Conversely, closely related species native to different hypoxic environments may display different THRs.

Exploring the role of the environment in THR evolution

To begin to explore how variation in the hypoxic environment influences the THR of fishes, we first devised a four-quadrant matrix (Q1–Q4; Fig. 2) that bins different aquatic hypoxic environments according to their time and Pw_{O_2} dimensions: Q1, hypoxia that is short in duration and moderate in Pw_{O_2}; Q2, short in duration and severe in Pw_{O_2}; Q3, long in duration and moderate in Pw_{O_2}; and Q4, long in duration and severe in Pw_{O_2}. Although this broad binning has its shortcomings (e.g. it is insensitive to finer-scale environment variations), it is to our knowledge the first meta-analysis to incorporate this level of hypoxic complexity, a necessary step towards understanding the fundamental aspects of hypoxia tolerance.

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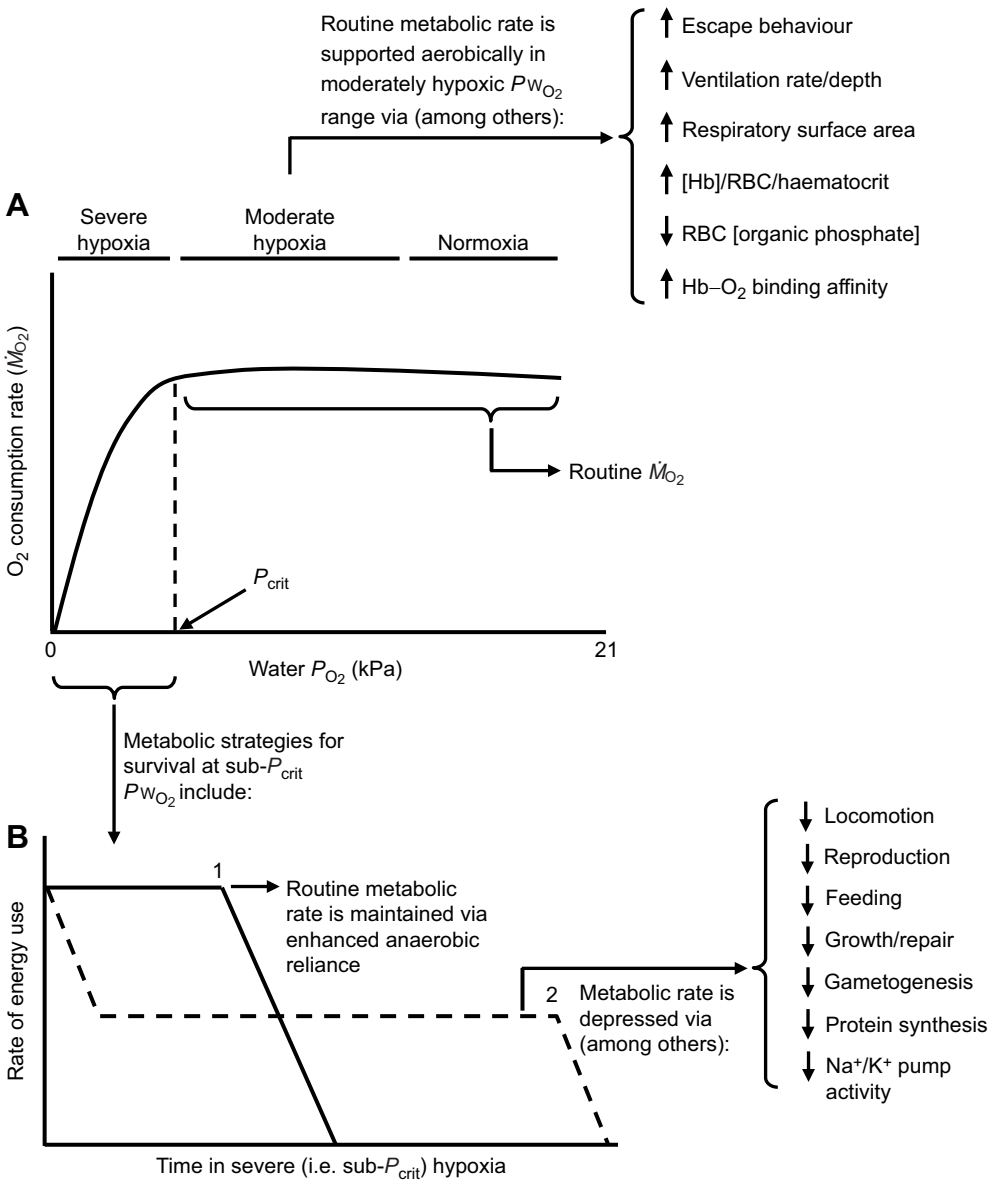

Fig. 1. The aerobic, anaerobic and metabolic rate depression (MRD) responses of a fish to hypoxia. (A) The aerobic response of a typical fish to decreasing partial pressure of oxygen in water ($P_{W_{O_2}}$). The solid curve represents the oxygen consumption rate ($\dot{M}_{O_2}$) that is required to support routine metabolic rate, and a typical fish can maintain this $\dot{M}_{O_2}$ over a broad $P_{W_{O_2}}$ range (including moderately hypoxic $P_{W_{O_2}}$) using the mechanisms listed on the right. The ability to use these mechanisms varies among species. P_{crit} (vertical dashed line) is the critical $P_{W_{O_2}}$ at which the animal is unable to extract sufficient environmental O_2 to support routine $\dot{M}_{O_2}$. $P_{W_{O_2}}$ values below P_{crit} are severely hypoxic, and, because aerobic ATP supply is compromised at these $P_{W_{O_2}}$ values, the fish's survival depends on enhanced reliance on anaerobic ATP supply pathways (glycolysis) and/or MRD. (B) Theoretical metabolic responses of an animal at sub-P_{crit} (i.e. severely hypoxic) $P_{W_{O_2}}$ as a function of time, with the *y*-axis representing the rate of energy use instead of $\dot{M}_{O_2}$. Line 1 (solid line) represents a fish that maintains routine metabolic rate in severe hypoxia by supplementing its reduced aerobic ATP supply capacity with an enhanced reliance on glycolysis. Line 2 (dashed line) represents a fish that induces MRD to better match its ATP demand to its reduced ATP supply capacity, and this is accomplished by reducing processes such as those listed on the right. Both metabolic responses to severe hypoxia are ultimately unsustainable, but the MRD response (line 2), here illustrated with its approximately 50% depression of metabolic rate, allows this hypothetical fish to survive for twice as long in severe hypoxia.

We then turned to the literature for examples of particularly well-studied species native to environments within these four quadrants. We found 10 hypoxic environment types with well-studied resident species (or multi-species systems) with respect to THR-related characteristics. Other relevant species–environment combinations exist in both the literature and the natural world, but we chose ours based on well-resolved understandings of the O_2 dynamics of the environment and an understanding of at least two of the three metabolic modes of the THR. We mined the literature systematically (see Table S1), but were limited in some cases by information availability (especially regarding MRD, the least studied metabolic mode) and the possibility of ascertainment bias regarding some traits and species (i.e. certain phenomena are better suited to study in some species than others). This may have influenced some of our interpretations. However, for the most part, compiling results from across multiple studies allowed us to piece together most species' THRs, something that had not been done before. Interpreting these THRs in the context of each species' natural hypoxic environment revealed patterns whereby distantly related species under similar hypoxic pressures rely on similar THRs, and suggested that other factors such as predation also likely play roles in shaping the THR.

The following sections are organized by quadrant, and within each section are descriptions of that quadrant's representative case studies. Although we have elected to describe Q1 below, species belonging to these environments are not particularly hypoxia-tolerant. As such, they are not the focus of the Review but have been included as a comparison case of how mildly tolerant species may respond to hypoxia. We have detailed our literature-mining process in Table S1, and have also included an extensive outline of these studies and their reported THR-related values. In the final section of the paper, we summarize our findings and reflect on what they might mean for the evolution of hypoxic survival strategies.

Q1: Moderate $P_{W_{O_2}}$ of short duration

Many aquatic species encounter low $P_{W_{O_2}}$ in the environment, but most avoid or escape these hypoxic zones and are therefore only transiently exposed to moderate hypoxia. One such species is the well-studied rainbow trout (*Oncorhynchus mykiss*), an inhabitant of streams and lakes, where pockets of O_2-depleted water

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Box 1. The metabolic responses of fishes to hypoxia

Energy metabolism

Energy metabolism is the rate of ATP turnover of a cell, tissue or whole animal (or organism). ATP is supplied by oxidative phosphorylation and/or anaerobic glycolysis, and is consumed by energy-demanding biological processes ranging from whole animal behaviour to protein translation. For a hypoxia-exposed animal, energy metabolism is the combined sum of aerobic metabolism, anaerobic metabolism and metabolic rate depression.

Aerobic metabolism

Aerobic metabolism centers on oxidative phosphorylation, the O_2-dependent process by which ATP is produced in the mitochondria. Optimizing steps along the O_2-transport cascade to more efficiently move O_2 from the environment to the mitochondria can aid hypoxic survival (Weibel, 1984), and hypoxia-adapted fishes have evolved traits at each step to do so (Sollid et al., 2003; Gracey et al., 2001; Affonso et al., 2002; Lai et al., 2006; Turko et al., 2014; Holeton and Randall, 1967a,b; Itazawa and Takeda, 1978; Tzaneva et al., 2011; Vulesevic and Perry, 2006; Sundin et al., 1995). The collective effectiveness of these traits to extract O_2 from hypoxic water is quantified by the critical $P\mathrm{w}_{O_2}$ (P_{crit}; Fig. 1) of O_2 uptake rate ($\dot{M}_{O_2}$). The lower the P_{crit}, the greater the $P\mathrm{w}_{O_2}$ range over which the fish can maintain routine metabolic rate aerobically. As $P\mathrm{w}_{O_2}$ drops below P_{crit} and aerobic metabolism becomes compromised, a fish's survival requires either supplementing aerobic metabolism with anaerobic metabolism to maintain routine metabolic rate, or depressing ATP-consuming processes to better match reduced ATP supply rates.

Data that are relevant to aerobic metabolism

$\dot{M}_{O_2}$; P_{crit} ; behaviours that maximize O_2 uptake (from water or air); ventilatory responses; respiratory surface anatomy; haematology and Hb function; circulatory anatomy and physiology; aerobic enzyme function; mitochondrial function.

Anaerobic metabolism

Anaerobic metabolism includes anaerobic glycolysis and creatine phosphate (CrP) hydrolysis. CrP is important for activity, but because CrP reserves are small and quickly depleted, they play a minimal role in hypoxic survival. Glycolysis is beneficial in O_2-limited environments because it allows for an O_2-independent supply of ATP. Most fishes, tolerant and intolerant, upregulate glycolysis during hypoxia, but tolerant fishes display a suite of biochemical adaptations that collectively enhance their anaerobic potentials (Hochachka and Somero, 2002; Farwell et al., 2007; Mandic et al., 2013; Lushchak et al., 1998; Abbaraju and Rees, 2011; Johnston, 1977; Shoubridge and Hochachka, 1980). However, even in hypoxia-tolerant species, should the hypoxic exposure last too long, glycolysis cannot sustain sufficient ATP supply and survival becomes dependent on the reduction of metabolic demand.

Data that are relevant to anaerobic metabolism

A species' reliance on anaerobic glycolysis may be represented by tissue/plasma lactate accumulation, tissue/whole-body glycogen depletion, tissue/plasma ethanol accumulation, or ethanol excretion to the water; tissue/whole-body [glycogen] quantifies a species' anaerobic fuel stores; glycolytic enzyme function represents potential glycolytic flux rates.

Metabolic rate depression

Metabolic rate depression (MRD) is a reduction in metabolic rate below standard metabolic rate (SMR; Hochachka and Somero, 2002; Richards, 2009). Metabolic rate is reduced to SMR through adjustments at behavioural and physiological levels, and below SMR (i.e. MRD) through adjustments at physiological and cellular/biochemical levels (Brett and Groves, 1979; Chiba, 1983; Pedersen, 1987; Nilsson et al., 1993; Schurmann and Steffensen, 1994; McKenzie et al., 1995; Hochachka et al., 1996; Hochachka and Somero, 2002; Thomas et al., 2006; Fitzgibbon et al., 2007; Perry et al., 2009; Richards, 2009; Wang et al., 2009; Wu, 2009). MRD is an effective mechanism for maintaining energy balance when ATP supply is limited at sub-P_{crit} $P\mathrm{w}_{O_2}$, but although some fishes use it, others do not. Those that do use MRD tend to be highly tolerant.

Data that are relevant to MRD

Calorimetric measurements of metabolic heat production, though rare in the fish literature, are the best indicator of MRD because they inherently account for aerobic and anaerobic contributions to metabolic rate (Nelson, 2016); simultaneous measurements of $\dot{M}_{O_2}$ and anaerobic reliance (see above); reduced rates of ATP-consuming processes such as ATPase activity and protein translation may (but not necessarily) indicate MRD; whole-body quiescence reduces ATP demand, though is not MRD because it does not reduce metabolic rate below standard levels.

periodically form. Like most salmonids, rainbow trout are not particularly hypoxia tolerant (Doudoroff and Shumway, 1970) and avoid hypoxic areas by migrating vertically away from the reduced $P\mathrm{w}_{O_2}$ of deeper waters (Rowe and Chisnall, 1995). Studies performed under controlled laboratory conditions have found that, similar to hypoxia-tolerant species (discussed in subsequent sections), rainbow trout rapidly (minutes to hours) attempt to maximize O_2 uptake during hypoxia by increasing ventilation rate (Holeton and Randall, 1967a,b) and amplitude (Hughes and Sunders, 1970) (see Glossary), and blood Hb–O_2 affinity via reduced intraerythrocytic ATP concentrations (Tetens and Lykkeboe, 1981). In contrast to tolerant species that typically maintain O_2 consumption rate ($\dot{M}_{O_2}$) as $P\mathrm{w}_{O_2}$ drops towards P_{crit}, rainbow trout increase $\dot{M}_{O_2}$, likely the result of enhanced ventilatory efforts (Hughes and Saunders, 1970). Further $P\mathrm{w}_{O_2}$ reductions see the trout enhance anaerobic metabolism (Dunn and Hochachka, 1986), but, unlike most hypoxia-tolerant fishes, the trout's anaerobic metabolism does not appear to be fuelled by hepatic glycogen stores (Dunn and Hochachka, 1987; Van Raaij et al., 1996). And perhaps most indicative of the trout's relative

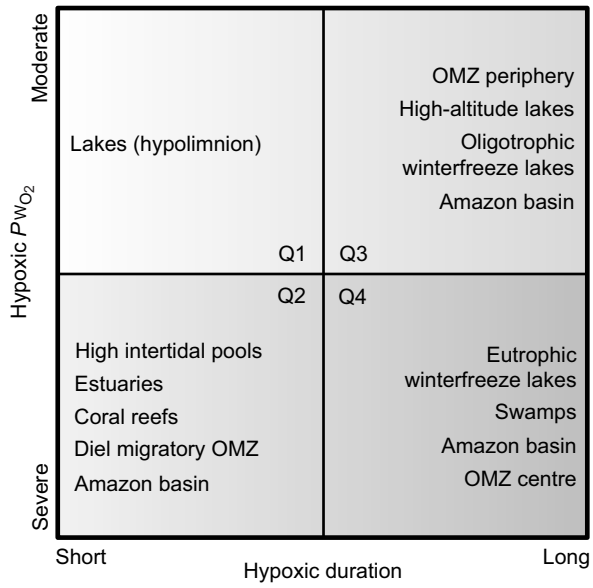

Fig. 2. An environmental hypoxia matrix. Natural hypoxic environments are grouped according to their O_2 levels ($P\mathrm{w}_{O_2}$) and time durations over which they remain hypoxic. Quadrant 1 (Q1) portrays moderately hypoxic $P\mathrm{w}_{O_2}$+short-duration environments. Q2 portrays severely hypoxic $P\mathrm{w}_{O_2}$ + short-duration environments. Q3 portrays moderately hypoxic $P\mathrm{w}_{O_2}$ + long-duration environments. Q4 portrays severely hypoxic $P\mathrm{w}_{O_2}$ + long-duration environments. Note that as the Amazon basin encompasses a wide diversity of hypoxic habitats, it is listed under Q2–Q4 (for greater description, see 'Heterogeneous hypoxic environments' section).

intolerance, hypoxia exposure rapidly and significantly reduces cellular [ATP], [creatine phosphate] ([CrP]) and energy charge in crucial tissues such as the heart (Dunn and Hochachka, 1986).

The rainbow trout therefore employs a primarily aerobic THR with some contribution of anaerobic metabolism in severe hypoxia. This is similar to many species (see below), but the trout's relatively limited capacity for these strategies results in cellular [ATP] imbalance and, consequently, a low hypoxia tolerance. Nevertheless, given the moderate $P_{W_{O_2}}$ and escapable nature of the trout's hypoxic environment, this THR is sufficient.

Q2: Severe $P_{W_{O_2}}$ of short duration

Intertidal zones

Fishes routinely found in high intertidal pools experience dramatic and acute changes in $P_{W_{O_2}}$, unlike their subtidal counterparts that inhabit much more O_2-stable environments (see environment description in Box 2). Intertidal sculpins, such as the tidepool sculpin (*Oligocottus maculosus*) and the intertidal triplefin twister (*Bellapiscis medius*), show lower P_{crit} values than do subtidal sculpins and triplefins, respectively (Hilton et al., 2008; Mandic et al., 2009a). The intertidal fishes' lower P_{crit}, which indicates their ability to maintain routine $\dot{M}_{O_2}$ to lower $P_{W_{O_2}}$, is achieved through behavioural and physiological modifications to the O_2 cascade that maximize O_2 extraction and delivery to the tissues, reducing the impact of environmental O_2 limitation on aerobic respiration.

Behaviourally, intertidal fishes respond to hypoxia with aquatic surface respiration (ASR; skimming of the relatively well-oxygenated surface layer of the water column; see Glossary) and aerial emergence, where the fish leave the tidepool to respire in air (reviewed in Bridges, 1988; Martin, 1995). Initiation of ASR and/or emergence occurs as $P_{W_{O_2}}$ falls below P_{crit} (Congleton, 1980; Innes and Wells, 1985; Hill et al., 1996; Mandic et al., 2009b). If intertidal fishes are restricted from the surface water or air, $\dot{M}_{O_2}$ continually declines with a decrease in $P_{W_{O_2}}$ below P_{crit}; however, given the opportunity to access surface water or air, intertidal fish will use ASR and/or emerge to breathe air, thus maintaining routine $\dot{M}_{O_2}$ (Yoshiyama and Cech, 1994; Martin, 1996). Indeed, a number of studies report similar or only slightly reduced respiratory rates in air compared with in water (Wright and Raymond, 1978; Daxboeck and Heming, 1982; Martin, 1991; Yoshiyama and Cech, 1994; Sloman et al., 2008). These behavioural responses uncouple the intertidal fishes from their aquatic habitat and allow them to maintain $\dot{M}_{O_2}$ by accessing the well-oxygenated upper layer of water or air when the bulk water of a tidepool becomes severely hypoxic (i.e. sub-P_{crit}) (Yoshiyama and Cech, 1994; Martin, 1996).

Although these behaviours enhance hypoxic survival, they also significantly elevate the fishes' risk of aerial predation (Kramer, 1983). A perceived predatory threat from above will send the intertidal fishes back into the tidepool's severely hypoxic water (or delay their emergence from it; Hugie et al., 1991; Shingles et al., 2005; Sloman et al., 2008), and in these scenarios, survival ultimately depends on a suite of physiological and biochemical adaptations (Brix et al., 1999; Mandic et al., 2009a; Craig et al., 2014; Lau et al., 2017). Compared with their subtidal counterparts, intertidal fishes exhibit: higher mass-specific gill surface area (Mandic et al., 2009a); higher haematocrits and blood–O_2 carrying capacities (Craig et al., 2014); higher Hb–O_2 affinities (Mandic et al., 2009a; Brix et al., 1999, respectively); and higher cytochrome *c* oxidase O_2 affinities (Lau et al., 2017). These adaptations allow intertidal fishes to rely primarily on aerobic metabolism even if predatory threats deny them surface access for periods of time. However, if $P_{W_{O_2}}$ in the tidepool falls below P_{crit}, then the fishes' survival may depend on anaerobic metabolism and/or MRD.

Intertidal sculpins have high capacities for anaerobic glycolysis. Specifically, their glycogen reserves are large and their glycolytic enzyme activity levels in brain are significantly higher than those of closely related subtidal sculpins (Mandic et al., 2013). Similarly, plainfin midshipman males, which are exposed to repeated hypoxic bouts while tending their nests, have higher glycogen reserves (liver) and glycolytic enzyme capacities (gill, skeletal muscle) than the less-tolerant females, which do not tend the nests (LeMoine et al., 2014). And both tidepool sculpins and the plainfin midshipman exhibit significant accumulation of plasma lactate when exposed to ecologically relevant hypoxic bouts lasting 4 to 6 h, indicating the activation of anaerobic glycolysis (Speers-Roesch et al., 2013; Craig et al., 2014).

Scant information exists on MRD in intertidal fishes. It is known that tidepool and rosylip sculpins (*Ascelichthys rhodorus*), both intertidal species, reduce locomotor activity and enter quiescent states when denied access to air, a measure that helps conserve energy (Yoshiyama et al., 1995). However, it is not known whether cellular MRD occurs during this quiescent state. Parental male plainfin midshipman have been suggested to induce cellular MRD during hypoxia (Craig et al., 2014), but the evidence is based solely on reduced $Na^{+/}K^{+}$-ATPase activity levels in the gill (and no decrease in liver). It may be that the intertidal environment, which is rich in predators, may not favour MRD owing to the reduced predator avoidance abilities that accompany a metabolically depressed state. In any case, careful work on these intertidal species' MRD use would compliment the extensive work that has been done on their aerobic and anaerobic hypoxic defenses.

Taken together, the available evidence thus far suggests that intertidal fishes prioritize aerobic metabolism under all possible hypoxic conditions, and likely rely on anaerobic glycolysis (and perhaps not MRD) when forced to spend time in severely hypoxic water.

Estuaries

Estuarine fishes experience O_2 regimes (severe $P_{W_{O_2}}$ that is short in duration; see Box 2) and aerial predation pressures similar to those of intertidal fishes (Kneib, 1982; Burnett et al., 2007). Two well-studied estuarine species are the Atlantic killifish (*Fundulus heteroclitus*) and the gulf killifish (*Fundulus grandis*), whose P_{crit} values are similar to those of the intertidal fishes (see Table S1; Cochran and Burnett, 1996; Virani and Rees, 2000; McBryan et al., 2016). As estuarine $P_{W_{O_2}}$ falls, both *Fundulus* species typically skim the water's surface and perform ASR (Wannamaker and Rice, 2000; Love and Rees, 2002), an important behavioural mechanism that contributes to alleviating the negative effect of hypoxia on growth rate in gulf killifish (Stierhoff et al., 2003). Akin to the intertidal fishes, the killifishes' THR is predominantly aerobic and anaerobic metabolism rather than MRD, although how these metabolic strategies are combined depends on the hypoxic time frame.

During initial hypoxia exposures, there is an increase in transcription of genes associated with oxidative phosphorylation, suggesting that enhanced aerobic enzyme activity is among the first lines of metabolic defense in the gulf killifish (Everett et al., 2012). In both killifish species, sub-P_{crit} $P_{W_{O_2}}$ levels activate anaerobic metabolism in the liver and the white muscle (see Glossary) during short-term hypoxia (Virani and Rees, 2000; Richards et al., 2008), but prolonged exposure (days to weeks) causes a shift to reliance on

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Box 2. Aquatic hypoxic environments

Each aquatic hypoxic environment is unique in the way physical and biological factors create its hypoxic events. However, we have binned 12 different hypoxic environments according to their Pw_{O_2} and durations over which they remain hypoxic using a four-quadrant matrix: Q1, hypoxia that is short in duration and moderate in Pw_{O_2}; Q2, short duration and severe; Q3, long duration and moderate; Q4, long duration and severe. We describe these environments below.

Q1: Thermally stratified lakes

The hypolimnion is the lower layer of a thermally stratified lake, and is frequently hypoxic, especially during the summer months (Rowe and Chisnall, 1995; Roberts et al., 2009).

Q2: Intertidal zones

Tidepools high in the intertidal zone become isolated from the ocean for hours to days with the falling tide. Their small water volumes and often-dense biota result in enormous fluctuations in Pw_{O_2} reaching anoxia at night and up to 80 kPa in the day (Truchot and Duhamel-Jouve, 1980; Burggren and Roberts, 1991; Richards, 2011).

Q2: Estuaries

Estuarine waters are subject to tides and strong winds that upwell O_2-poor bottom waters and cause severe diel variation in Pw_{O_2} levels, with hypoxic events often occurring at night (Breitburg, 1990; D'Avanzo and Kremer, 1994). Dense biota and water column stratification (see Glossary) further reduce Pw_{O_2} (Breitburg, 1990).

Q2: Coral reefs

Pools on coral reef flats become hypoxic when isolated during tidal cycles, and nocturnal O_2 consumption among the coral colonies themselves can severely reduce Pw_{O_2} to as low as 0.4 kPa (Goldshmid et al., 2004; Nilsson and Ostlund-Nilsson, 2006; Nilsson et al., 2007).

Q2, Q3: Oceanic oxygen minimum zones (OMZs)

OMZs occur at depths between 200 and 1500 m, where certain biological and physical processes combine to reduce Pw_{O_2} to <6.4 kPa around the OMZ's periphery and 0.5 kPa in its centre. Biologically, a high density of aerobic bacteria reduce the OMZ's O_2 levels as they feed upon the organic matter falling from the mixed layer above, while physically, a lack of atmospheric contact and low levels of convective mixing keep these waters low in O_2.

Q3: High-altitude lakes

Atmospheric pressure decreases by ∼0.91 kPa with every 100 m of altitudinal ascent. Water bodies at higher altitudes consequently have lower partial pressures for all gases, including O_2. These hypoxic exposures are typically chronic and moderate in Pw_{O_2}, reaching ∼14 kPa as a result of altitude in lakes at 3750 m above sea level (about the highest at which fish species have been studied).

Q3, Q4: Winterfreeze lakes

Winterfreeze lakes generally occur at high elevations or at far northern or southern latitudes, where low wintertime atmospheric temperatures freeze the lake's surface layer. Biological activity reduces Pw_{O_2}, and the ice/snow layer prevents photosynthesis and water–atmosphere mixing until the spring thaw (Ultsch, 1989; Barica and Mathias, 1979; Mathias and Barica, 1980). The relatively low biological activity levels of oligotrophic lakes (Fig. 2, Q3) can cause moderately hypoxic Pw_{O_2}, whereas the high activity levels of eutrophic lakes can cause anoxia for months (Fig. 2, Q4) (Vornanen, 2004).

Q4: Swamps

Swamps, such as those surrounding Lake Victoria, typically experience hypoxia that is chronically low in Pw_{O_2}, varying from 0.6 kPa in the bottom layers at night to a maximum of 3.2 kPa in the upper layer during the day (Chapman et al., 2002) as a result of high biological activity.

Q2, Q3, Q4: The Amazon

The Amazon basin floods each year when the Amazon River spills over its riverbanks into the surrounding forests (igapo) and floodplains (varzea), bringing many of the Amazon's 5600+ fish species with it (Albert and Reis, 2011). The basin is a network of complex heterogeneous environments where interacting biological and physical factors create long- and short-term fluctuations in O_2 levels (Val et al., 1998). At the peak of the wet season, all of the flooded areas are interconnected, allowing fish to move among them. The hypoxia that occurs during this season arises from extensive floating macrophytes (see Glossary) and occurs mainly in the varzea lakes (Val and Almeida-Val, 1995). As the season wears on, the water levels recede and leave behind smaller, isolated water bodies that become hypoxic (even anoxic) as a result of high biological activity and a lack of light penetration (Val and Almeida-Val, 1995; Val et al., 1998). These habitats can remain deeply hypoxic for months at a time, even chronically, or undergo large diurnal changes in O_2, all depending on the shape, size, depth, winds and vegetation of the varzea lakes (Val and Almeida-Val, 1995).

MRD in the white muscle but not in the liver (Kraemer and Schulte, 2004; Martinez et al., 2006; Richards et al., 2008; Abbaraju and Rees, 2011). This indicates that the type of hypoxia strongly influences the metabolic response of estuarine fishes. Borowiec et al. (2015) explored this idea by acclimating Atlantic killifish to either chronic or intermittent (diel cycles) hypoxia exposures. Both exposure types lowered P_{crit} and routine $\dot{M}_{O_2}$, but only killifish acclimated to intermittent hypoxia upregulated mechanisms that enhance glycolytic capacity and the processing of glycolytic end-products. Killifish acclimated to chronic hypoxia exhibited modified gill morphology (reduced filament length; reduced mitochondrion-rich cells that potentially decrease ion loss and cost of osmoregulation) in a way that may decrease metabolic demand (Borowiec et al., 2015).

Overall, it appears that killifish exposed to short-term intermittent hypoxia rely on an aerobic-anaerobic THR, while those exposed to long-term hypoxia rely less on anaerobic metabolism and perhaps more on MRD. Given that the killifishes' natural habitat typically experiences tidally influenced intermittent hypoxia, the former is likely the predominant THR, similar to the intertidal fishes. This THR may be well suited – and perhaps selected for – in predator-rich environments that experience rapid and severe fluctuations in Pw_{O_2}.

Coral reefs

Hypoxic events in coral reefs tend to be severe, cyclical and short in duration (see Box 2), and so we would predict THRs among coral reef inhabitants that are similar to those of intertidal and estuarine fishes. Generally, coral reef fishes have relatively low P_{crit} (3.1–6.1 kPa; Nilsson and Ostlund-Nilsson, 2004; Nilsson et al., 2004; Wong et al., 2018), but in-depth THR information is scant. The best-studied species in this regard is the epaulette shark (*Hemischyllium ocellatum*), a reef flat inhabitant. The shark's P_{crit} is approximately 5.1 kPa (Speers-Roesch et al., 2012a), the lowest of any elasmobranch tested (Routley et al., 2002; Speers-Roesch et al., 2012a) and similar to those of teleost reef inhabitants. A comparison with a much less-tolerant elasmobranch, the shovelnose ray (*Aptychotrema rostrata*), revealed the epaulette shark to have a higher Hb–O_2 affinity, higher arterial blood O_2 content, higher $\dot{M}_{O_2}$ at sub-P_{crit} Pw_{O_2} and better maintained routine cardiovascular

function in hypoxia (Speers-Roesch et al., 2012a,b; Hickey et al., 2012), suggesting an aerobic-focused THR. Furthermore, elevated ventilatory frequencies (Routley et al., 2002) and altered blood flow patterns that enhance blood supply to the gills and return it directly to the heart (Stenslokken et al., 2004) support the epaulette shark's aerobic-focused THR.

Though epaulette sharks are known to clamber over land in search of water when the receding tide exposes tidal flats (Goto et al., 1999), they are not known to exploit the air's (or surface waters') higher O_2 levels like intertidal and estuarine fishes. When Pw_{O_2} falls to ~3.7 kPa (i.e. sub-P_{crit}) as it typically does each night (Routley et al., 2002), anaerobic metabolism contributes to ATP production, as indicated by significant lactate accumulation with progressive, cyclical hypoxia exposures (Wise et al., 1998; Routley et al., 2002). There is conflicting information about whether the shark also uses MRD in these situations. Evidence for MRD use includes the shark's loss of righting reflex with anoxia exposure despite stable brain ATP levels (Renshaw et al., 2002), and reduced neuronal oxidative demand with cyclical severe hypoxia exposure (Mulvey and Renshaw, 2000). Relatively low cardiac lactate levels of hypoxia-exposed epaulette sharks are speculated to be due to MRD in extra-cardiac tissues, which would leave more O_2 available for the heart (Speers-Roesch et al., 2012b). However, other studies have found no evidence of MRD in epaulette sharks (Dowd et al., 2010), nor a loss of body posture or voluntary movement with cyclical severe hypoxia (Wise et al., 1998). The conflicting results are possibly a function of the studies' different experimental hypoxia exposure protocols, but nevertheless, the presence of MRD in even some of these studies suggests that the epaulette shark has evolved an ability to use MRD, and perhaps does so in the wild. This is unlike the previously discussed intertidal and estuarine species, which are not known to employ MRD when exposed to tidally relevant (i.e. short-term intermittent) hypoxia despite their similar natural hypoxic habitats (though killifish may do so when acclimated to chronic hypoxia; see above). We speculate that the reason may involve predation pressure. Whereas the previous species are small and subject to predation during their hypoxia exposures, the shark is relatively large and less likely to encounter predators during its hypoxia exposures. Thus, the reduced predation risk may allow the shark to depress metabolism during hypoxia.

Oceanic OMZs – migratory residents

There are two types of OMZ resident: permanent and migratory. Permanent residents, which we discuss later, spend their entire lives in the OMZ and therefore experience chronic moderate-to-severe Pw_{O_2} levels (Q3 and Q4 in Fig. 2). Migratory residents, by contrast, spend their days in the OMZ's severely hypoxic centre and migrate vertically into well-oxygenated surface waters each night to feed in the cover of darkness (Seibel, 2011). This migratory pattern exposes these animals to progressively changing Pw_{O_2}, becoming normoxic with upwards migration and hypoxic with downwards migration. The hypoxic exposures experienced by these animals are therefore severe (Pw_{O_2}) and short in duration (see Box 2), similar to those described above. Despite this similarity, migratory OMZ residents use a different THR. Although they tend to possess traits that enhance O_2 extraction (e.g. Seibel, 2013; Trueblood and Seibel, 2013) and glycolytic capacity (e.g. Gonzalez and Quiñones, 2002; Torres et al., 2012), migratory OMZ residents rely primarily on MRD while in the deeply hypoxic OMZ during the day (Seibel, 2011; Seibel et al., 2016). For example, the jumbo (or Humboldt) squid (*Dosidicus gigas*) depresses metabolic rate by 87% when held at 0.6 kPa, the Pw_{O_2} at which it typically spends the daytime in the OMZ (Rosa and Seibel, 2010; Trueblood and Seibel, 2013). Migratory krill (*Euphausia eximia* and *Nematoscelis gracilis*) from this same OMZ region also employ MRD at this Pw_{O_2} (Seibel, 2011; Seibel et al., 2016). These are different THRs than those employed by tidepool sculpins and killifish despite similar environmental O_2 characteristics, and the reason may involve predation risk. As discussed, predation risk in tidepools and estuaries is high, particularly for small animals in MRD. But predation risk in the OMZ is relatively low owing to low light and activity levels, a diffuse distribution of animals (see Childress, 1995; Drazen and Seibel, 2007; Seibel and Drazen, 2007; Seibel et al., 2000), and low O_2 levels that tend to keep top ocean predators such as sharks, tunas and billfishes out (Brill, 1994; Nasby-Lucas et al., 2009; Vetter et al., 2008). Consequently, animals living in the OMZ – particularly those that migrate into oxygenated surface waters to complete necessary behaviours such as feeding and mating – can employ MRD with a relatively low risk of being eaten.

Q3: Moderate Pw_{O_2} of long duration

Oceanic OMZs – permanent residents

Permanent OMZ residents experience hypoxia that is moderate (Pw_{O_2}) and chronic (see Box 2). These animals, which include many fish and invertebrate species, tend to live towards the OMZ's periphery, where Pw_{O_2} levels are not as severe as in its centre (Childress and Seibel, 1998). Probably owing to the detrimental effects of chronic reliance on anaerobic glycolysis and/or MRD, these animals rely primarily on aerobic metabolism through a suite of highly effective O_2 extraction adaptations.

From a THR perspective, by far the best-studied permanent OMZ resident is the giant red mysid (*Gnathophausia ingens*). The red mysid has a high ventilatory capacity (Childress, 1971), a large mass-specific gill surface area (Childress, 1971), a small blood–water diffusion distance across the gills (Seibel, 2011), a high circulatory capacity (Belman and Childress, 1976), and a haemocyanin with an extremely high affinity for O_2 and a large Bohr effect (see Glossary) to facilitate tissue O_2 delivery (Sanders and Childress, 1990a,b). Combined with an extremely low routine metabolic rate (a common trait of permanent OMZ residents; see Childress, 1995), this results in a P_{crit} value of 0.8 kPa (Seibel, 2011), coincident with the minimum Pw_{O_2} that the mysid typically encounters in the OMZ (Childress and Seibel, 1998). In fact, across a wide range of OMZ residents, P_{crit} has been shown to correlate at near-unity (or below) with the minimum Pw_{O_2} each of these animals experience in the wild (Childress, 1975; Cowles et al., 1991; Donnelly and Torres, 1988; Torres et al., 1994).

Highly developed mechanisms of O_2 extraction and delivery are present in a diverse array of permanent OMZ resident species beyond the giant red mysid (Childress and Seibel, 1998; Lamont and Gage, 2000; Levin, 2003), and these mechanisms may preclude a significant reliance on anaerobic glycolysis. It is believed that anaerobic glycolysis is used by permanent OMZ residents in the same way it is used by species from normoxic habitats, not to support routine metabolic rate, but to supplement supra-routine metabolic rates (Childress and Seibel, 1998; Levin, 2003). Consistent with this, the capacities for anaerobic metabolism (as indicated by maximal rates of anaerobic enzymes) of the permanent OMZ residents that have been investigated are no higher than those of closely related species (or conspecifics) from outside the OMZ (Childress and Somero, 1979; Yang and Somero, 1993; Vetter et al., 2008; Childress and Seibel, 1998; Friedman et al., 2012). Exceptions exist, however. Some permanent OMZ residents such as the copepod *Gaussia princeps* (Childress, 1976) and the

isopod *Anuropus bathypelagicus* (Childress, 1975) have been caught at Pw_{O_2} levels that are lower than their measured P_{crit} values (0.8 kPa for *G. princeps*, 0.6 kPa for *A. bathypelagicus*) and they may therefore use anaerobic glycolysis to support routine metabolic rates. But such examples are rare (see Childress and Seibel, 1998).

In summary, permanent OMZ residents use a predominantly aerobic THR to survive in their chronically hypoxic habitat. Anaerobic glycolysis is typically reserved for supplementing supra-routine metabolic rates, while MRD has not been measured in these species. This THR enables these species to carry out routine behaviours and life-history events without accumulating a significant O_2 debt, despite living permanently in hypoxia. Predominantly aerobic THRs may be selected for in other chronic hypoxic environments of moderate Pw_{O_2}, such as high-altitude (<3000 m) lakes. The single physiology study on species from these environments that we know of revealed a highly plastic gill surface area in Lake Qinghai (3205 m) naked carp (Matey et al., 2008). However, more work is needed to say anything general about hypoxic adaptations in high-altitude fishes.

Oligotrophic winterfreeze lakes

Oligotrophic (see Glossary) winterfreeze lakes experience hypoxia that is moderately severe (Pw_{O_2}) and long in duration (see Box 2). A recent study examined the THRs of two threespine stickleback (*Gasterosteus aculeatus*) populations from two isolated lakes in British Columbia: Alta Lake, which experiences long-term hypoxia owing to winterfreeze, and Trout Lake, which does not (Regan et al., 2017b). The Alta Lake fish were found to be significantly more hypoxia-tolerant than the Trout Lake fish, and although P_{crit} and lactate accumulation did not differ, the Alta Lake fish used MRD at sub-P_{crit} Pw_{O_2} levels and the Trout Lake fish did not. Interestingly, aspects of the Alta Lake fish's MRD were different than those of another MRD-inducing species, the goldfish (discussed in detail below). Alta Lake sticklebacks depress metabolic rate by 33% and do so at 2.8 kPa Pw_{O_2}, whereas goldfish depress metabolic rate by 80% and wait until near-anoxia to initiate it. These MRD differences may relate to variation in each species' hypoxic environment. Although the native lakes of goldfish likely become anoxic during wintertime (like the native lakes of crucian carp; Vornanen, 2004), apart from at the water–sediment interface (Dunnington et al., 2016), Alta Lake waters do not reach anoxia (Jacques Whitford AXYS Ltd, 2007). Selection may therefore be acting on hypoxic survival strategies at higher Pw_{O_2} values in the Alta Lake environment than in the more severe goldfish environment.

Q4: Severe Pw_{O_2} of long duration

Eutrophic winterfreeze lakes

Eutrophic (see Glossary) winterfreeze lakes are typified by hypoxia that is severe (Pw_{O_2}) and long in duration (see Box 2). Consequently, they tend to be colonized by highly tolerant fishes, such as the well-studied crucian carp (*Carassius carassius*; Vornanen et al., 2009) and its congener, the goldfish (*Carassius auratus*; Ultsch, 1989). These fishes employ a complex THR that involves highly effective mechanisms of aerobic metabolism, anaerobic metabolism and MRD. Aspects of this THR are altered not only in response to Pw_{O_2}, rate of hypoxia induction and duration of hypoxia exposure, but also in anticipation of the naturally occurring hypoxic season. Despite this THR's complexity, the evidence suggests it is altered so as to maintain routine metabolic rate in as many hypoxic environments as possible. This is accomplished in different ways depending on time: for rapid induction rates and acute exposure durations (−21 kPa Pw_{O_2} h^{-1}, 1 h exposure), goldfish upregulate glycolysis to buffer ATP supply so as to maintain routine metabolic rate (Regan et al., 2017a); whereas for gradual induction rates and long exposure durations (−2.6 kPa Pw_{O_2} h^{-1}, 8 h exposure), goldfish and carp increase environmental O_2 extraction by increasing gill surface area and Hb–O_2 affinity so as to support routine metabolic rate aerobically (Sollid et al., 2003; Tzaneva et al., 2011; Dhillon et al., 2013; Regan and Richards, 2017). The combination of a highly plastic gill surface area, the highest Hb–O_2 binding affinities reported for vertebrates (Burggren, 1982; Sollid et al., 2003; Regan et al., 2017a), and a generally low routine metabolic demand allows goldfish to maintain routine metabolic rates down to ∼1 kPa Pw_{O_2}.

At Pw_{O_2} below ∼1 kPa, goldfish rapidly depress metabolic rate to ∼20% of routine values in less than 20 min (van Ginneken and van den Thillart, 2009; Regan et al., 2013). O_2 content in the water is negligible or altogether absent at these Pw_{O_2} levels, and the goldfish and carp become solely reliant on glycolysis to supply the ATP required to fuel their reduced metabolic demands. The use of MRD and glycolysis initially leads to an accumulation of lactate and protons (Regan et al., 2017a), but as the hypoxic/anoxic exposure duration lengthens, the fish begin to convert these end-products into ethanol, which they excrete across their gills and thus mitigate a metabolic acidosis (Shoubridge and Hochachka, 1980; Holopainen et al., 1986; Regan et al., 2017a). The glycogen stores of goldfish and crucian carp are larger than those of any other fish species (Richards, 2009), and, similar to fat stores in hibernating mammals, these stores significantly increase in size over the late summer and early autumn to levels that are sufficient to fuel the fish's depressed metabolic rates in anoxia during the winter months (Vornanen et al., 2009).

Another species system native to eutrophic winterfreeze lakes is the centrarchid sunfishes. The ranges of two closely related sunfish, the bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*), overlap, but the most northern lakes – the ones that experience the most severe winterfreeze hypoxia – contain only pumpkinseed (Mittelbach, 1984; Farwell et al., 2007). Unsurprisingly, pumpkinseed have repeatedly been shown to be more hypoxia-tolerant than bluegill (Farwell et al., 2007; Mathers et al., 2014; Borowiec et al., 2016). The pumpkinseed's greater tolerance does not appear to result from greater aerobic abilities, as the two species do not differ in P_{crit} (Mathers et al., 2014; Borowiec et al., 2016) or a wide array of underlying mechanisms (Crans et al., 2015; Borowiec et al., 2016). Where the species do differ is in their capacities for anaerobic metabolism, with the more tolerant pumpkinseed displaying higher lactate dehydrogenase activities in axial muscle (Farwell et al., 2007; Borowiec et al., 2016) and heart (Borowiec et al., 2016) than the less tolerant bluegill. The pumpkinseed's greater anaerobic capacity may contribute to its greater hypoxia tolerance, but because anaerobic glycolysis on its own is a limited long-term strategy owing to glycogen depletion and end-product accumulation, it is unlikely to solely explain how pumpkinseed can tolerate the more hypoxic northern lakes that bluegill cannot. It may be that pumpkinseed, like goldfish, rely on MRD and/or plastic mechanisms that enhance O_2 extraction, and that their capacities for these traits are greater than the bluegill's. This warrants further investigation.

Swamps

The swamps surrounding Lake Victoria experience hypoxia that is chronic and severe in Pw_{O_2} (see Box 2). The haplochromine cichlids that live here rely on a primarily aerobic THR. Compared with

closely related lake-dwelling (i.e. normoxic) species or conspecifics, the swamp-dwelling fish have larger gill surface areas, higher haematocrits and Hb concentrations, and lower routine O_2 demands. These modifications result in swamp-dwelling fishes exhibiting extremely low P_{crit} values that are approximately half those of lake-dwelling fishes and just slightly higher than the lowest Pw_{O_2} measured in these habitats (Chapman et al., 2002). Their hypoxia tolerance is further enhanced by effective ASR abilities, which are a central component of their overall THR (Chapman et al., 1995). Moreover, swamp dwellers engage ASR at significantly lower Pw_{O_2} than do lake dwellers (which are also capable of ASR; Chapman et al., 2002), a possible advantage given the high daytime predation pressures exerted by pied kingfishers (*Ceryle rudis*) (Randle and Chapman, 2004). The swamp dwellers' low P_{crit} values enable them to remain in deeper, safer waters throughout the day when the kingfisher is active and when photosynthetic activity elevates Pw_{O_2} above P_{crit}.

It is not known whether these fishes use MRD, but it is known that the swamp dwellers' capacities for anaerobic metabolism are no higher than those of closely related lake-dwelling fishes (as indicated by anaerobic enzyme activities in various tissues of *Pseudocrenilabrus multicolor* populations from swamp and lake habitats; Crocker et al., 2013). It therefore appears that swamp dwellers employ a predominantly aerobic THR, though additional MRD-focused work is needed to provide a complete picture of their THR.

Heterogeneous hypoxic environments in the Amazon

Amazonian fishes can experience hypoxia that is mild or severe in Pw_{O_2}, and short or long in duration (see Box 2). Perhaps because of the diversity and widespread persistence of Amazonian hypoxic habitats, many Amazonian fish species have independently evolved high tolerances to hypoxia (Almeida-Val and Val, 1993). Most of these species achieve this through behavioural and/or morphological features that maximize their abilities to acquire O_2 in their O_2-depleted habitats, the prime examples being ASR and air breathing. Many Amazonian fish families have independently evolved morphological features to optimize ASR and air breathing, including extensible lower lips to syphon O_2-rich water directly across the gills, and a wide variety of air-breathing organs ranging from modified buccal cavities to lungs (Val et al., 1998). These morphological features, and the behaviours they optimize, are believed to have evolved in response to aquatic hypoxia (Graham, 1997; Kramer and McClure, 1982). It is no surprise then that they are widely used among the Amazon's hypoxia-dwelling fish species.

One study collected the resident species from an isolated Amazonian lake (Camaleao Lake) after it had become severely hypoxic, and then determined the primary hypoxia tolerance strategy used by each species. Of the 11 families caught (numerous species for most), seven used air-breathing as their primary means of tolerating hypoxia, two used ASR, one used Hb–O_2 affinity modulation and one used MRD (Junk et al., 1983). In a similar study in which 20 species were caught in a hypoxic Amazonian lake, 10 species used ASR as a primary means of tolerating hypoxia, four used air-breathing, four positioned themselves directly adjacent to O_2-secreting plant roots, one combined a large gill surface area with a high Hb–O_2 affinity and one used MRD (the Amazonian oscar, *Astronotus ocellatus*, the most tolerant of the group; Soares et al., 2006). Furthermore, although air breathing and/or ASR behaviours increase the susceptibility to aerial predation (Kramer and Mehegan, 1981), some of these fishes have evolved complex group behaviours to mitigate this risk (Sloman et al., 2009).

In addition to ASR and air breathing, hypoxia-adapted Amazonian fishes tend to possess characteristics across the multiple steps of the O_2 cascade that enhance O_2 extraction and delivery. These include high ventilation rates, large gill surface areas (Saint-Paul, 1984), and high blood–O_2 carrying capacities through increased red blood cell count, [Hb] and Hb–O_2 binding affinities (Saint-Paul, 1984; Val and Almeida-Val, 1995; Muusze et al., 1998; Val et al., 1998; Affonso et al., 2002). Beyond sustaining aerobic metabolism, hypoxia-exposed Amazonian fishes also strongly activate anaerobic metabolism (e.g. glycolysis, CrP hydrolysis) (Chippari-Gomes et al., 2005; MacCormack et al., 2006; Richards et al., 2007; Scott et al., 2008). And at least one species – the Amazonian oscar – uses MRD, evidenced by suppressed $\dot{M}_{O_2}$ and ATP-consuming processes and enzymes, such as protein synthesis and Na^+/K^+-ATPase, respectively (Muusze et al., 1998; Lewis et al., 2007; Richards et al., 2007; Scott et al., 2008). Given the Amazon's species diversity and hypoxic heterogeneity, it is perhaps unsurprising that a wide range of THRs would be seen among Amazonian fishes. However, as not all aspects of the THR are available for all species discussed (e.g. scarcity of MRD information), it is also possible that THRs of Amazonian fishes are more similar than the data currently suggest.

Summary and perspectives

We can combine the 10 case studies summarized above with the environmental hypoxia matrix in Fig. 2 to draw some general conclusions on how the THR may relate to the hypoxic environment (Fig. 3). Importantly, these are generalizations based on the limited number of available studies, and more specifically, the relatively

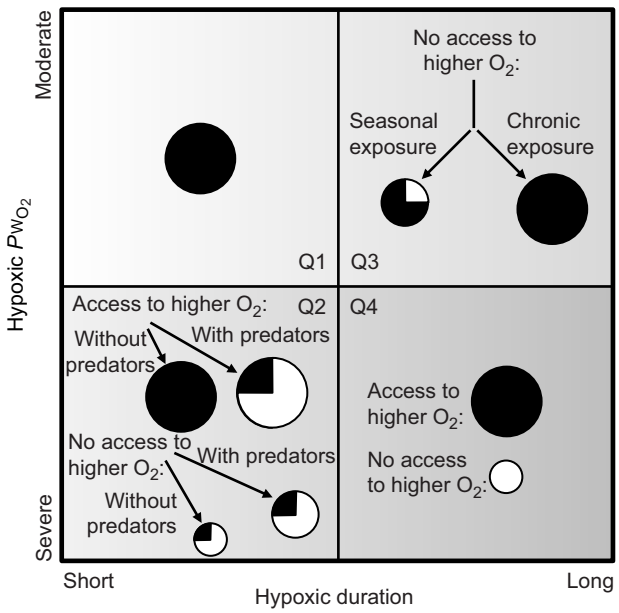

Fig. 3. The total hypoxic responses (THRs) of species inhabiting the different hypoxic quadrants. The circular pie charts represent THR. Each pie chart's total area qualitatively represents its total metabolic rate relative to the size of the pie chart in Q1, which represents normoxic routine metabolic rate. A smaller-diameter pie chart therefore represents a depressed metabolic rate. The black-filled portion of a pie chart qualitatively represents the aerobic contribution to that THR's metabolic rate, and the white-filled portion represents the anaerobic contribution (ratios are approximate). All-black and all-white pie charts therefore represent fully aerobic and anaerobic THRs, respectively. 'Access to higher O_2' labels indicate environments in which the inhabitants may access higher Pw_{O_2} regions within their hypoxic environment (e.g. via aerial emergence).

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small number explored here. As additional studies are completed on species native to environments within each hypoxia matrix quadrant, these generalizations will become more refined and likely modified in various ways. For now, however, we will use currently available information from across studies to build an initial environment-focused THR framework. The individual studies we used to do so (which are highlighted in Table S1) were chosen based on two main criteria: (i) they used sound methods to measure mechanisms that are directly related to one or more THR metabolic modes; and (ii) they were carried out on species native to hypoxic environments that are well characterized.

For hypoxia exposures that are short (time) and moderate (Pw_{O_2}) (Q1), we believe the THR may vary with the ability to escape to more oxygenated waters. If escape to more oxygenated waters is not possible, the fishes tend to employ a primarily aerobic THR with some contribution of anaerobic metabolism. Generally, Q1 fishes are hypoxia intolerant relative to Q2, Q3 and Q4 species.

For hypoxia exposures that are short and severe (Q2), we believe the THR may be heavily influenced by aerial/surface access and predators (Fig. 3); if the air–water interface is accessible (e.g. tidepools), fishes living in these environments tend to prioritize aerobic metabolism by using ASR and/or air breathing. If an aerial predator presents itself, fishes tend to dive into the hypoxic water and buffer routine metabolic rate using anaerobic glycolysis, which in this environment may be more practical than inducing MRD because it allows the fishes to maintain cellular energy balance (though not indefinitely) without impairing predator avoidance behaviour as a result of a metabolic shut-down. The costs of anaerobic metabolism (fuel depletion, end-product accumulation) are positively correlated with time spent in the hypoxic environment, and, for these fishes, this would typically be short: either the predation threat will subside and allow the fish to re-emerge and/or perform ASR, or the O_2 will be replenished by photosynthesis and/or the rising tide. The costs accrued with anaerobic reliance would therefore be low. For Q2 environments that lack an air–water interface, the response tends to depend on Pw_{O_2}. If Pw_{O_2} is above residents' P_{crit} (e.g. less hypoxic OMZ regions), then aerobic metabolism will be prioritized. However, if Pw_{O_2} is below residents' P_{crit} (e.g. more hypoxic OMZ regions), then aerobic metabolism cannot be sustained. Animals living in these environments tend to use MRD over anaerobic metabolism, perhaps enabled by relatively low predation pressures. Deep MRD is used if predation risk is low to absent (e.g. jumbo squid), and moderate MRD is used if predation risk is moderate to low (e.g. krill).

For exposures that are long and moderate (Q3), we believe the THR may vary as a function of exposure duration (Fig. 3). Species that live in chronically moderate hypoxic environments (e.g. OMZ periphery, high-altitude lakes) tend to rely on enhanced O_2 extraction abilities to support metabolic rate aerobically. Because

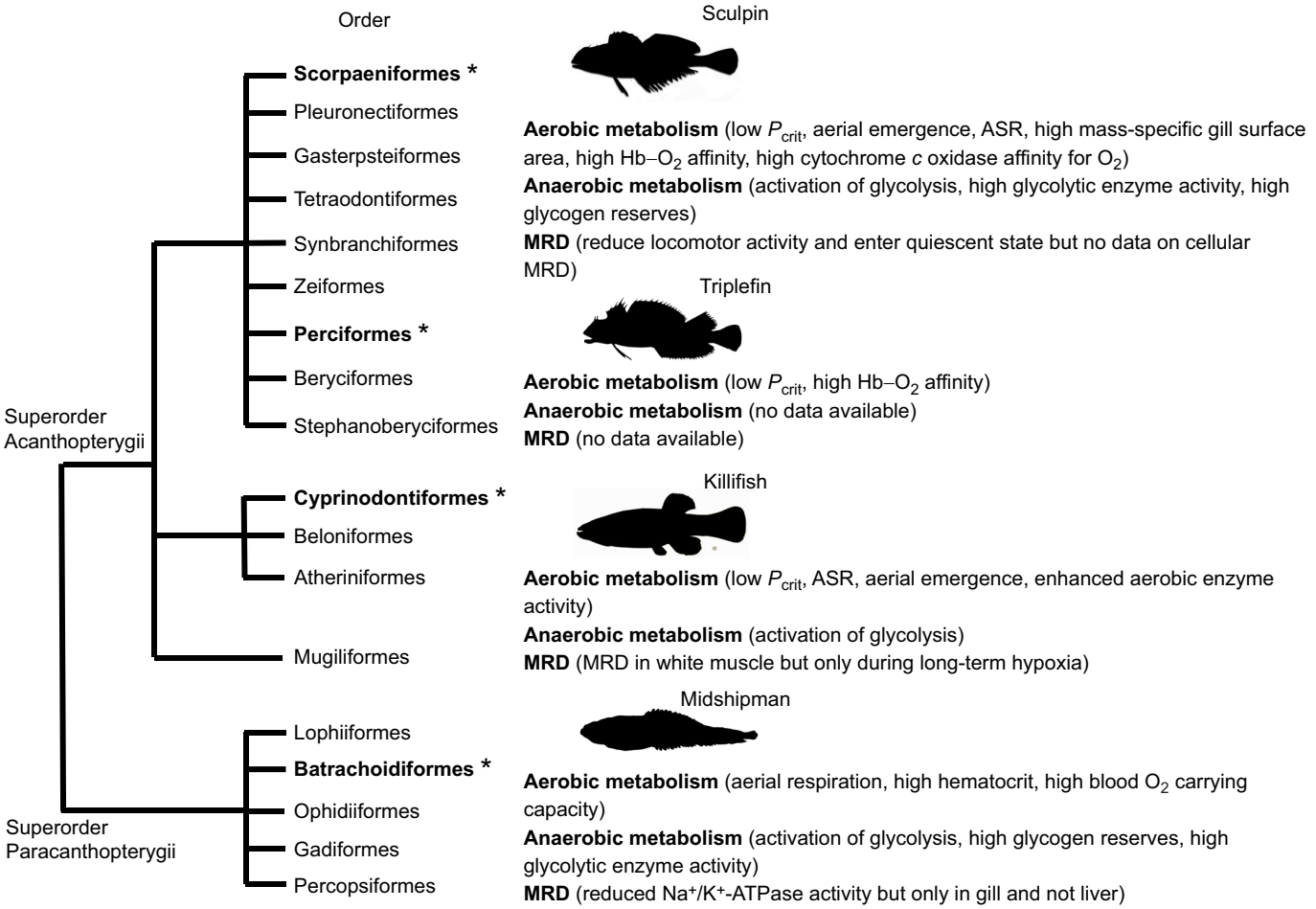

Fig. 4. Phylogeny and the traits underlying the THR of representative fishes from Q2. A truncated phylogeny of well-studied examples of Q2 fishes – sculpins (order Scorpaeniformes), triplefins (order Perciformes), killifish (Cyprinodontiformes) and plainfin midshipman (order Batrachoidiformes) – is represented (modified from Nelson, 2006). Listed are the known traits underlying the THR of each group of species.

these species infrequently enter fully oxygenated waters, they need to support routine activities such as feeding and mating in chronic hypoxia, and this makes an aerobically based THR ideal. Species that live in seasonally moderate hypoxic environments (e.g. oligotrophic winterfreeze lakes) tend to use modest MRD, perhaps because energetically costly routine activities such as mating and reproduction are typically accomplished during the oxygenated months of the year. More work is needed on species from seasonally fluctuating moderately hypoxic environments.

Finally, for exposures that are long and severe (Q4), we believe the THR may vary as a function of aerial/surface access (Fig. 3). If it is accessible (e.g. Amazon basin), then the fishes living in these environments tend to exploit its high O_2 content using ASR and/or air breathing, effectively uncoupling themselves from their severely hypoxic aquatic environment. If the air–water interface is not available (e.g. eutrophic winterfreeze lakes), then aerobic metabolism is not an option and the fishes living here tend to employ deep MRD so as to conserve limited anaerobic fuel reserves. A general lack of predators in these environments allows these fishes to surrender locomotor performance with minimal predation-related consequences, while the return of O_2 with spring thaw allows them to complete routine activities such as feeding and mating in oxygenated waters.

Looking across these studies as a function of hypoxic environmental variation, it is apparent that the hypoxic environment is not the sole influence on THR. It is possible that other factors such as genetic constraint, rate of environmental change (e.g. climate change-related) and complex interactions with other abiotic factors (e.g. temperature, $P_{W CO_2}$, pH) may also contribute to a species' THR, and divorcing these effects from those of the hypoxic environment is difficult. However, there appear to be common patterns in the THRs across species that depend on their native hypoxic environments. Using the extensively studied Q2, we illustrate that distantly related species share similar THRs, suggesting that convergent evolution has played a role in shaping the hypoxic metabolic phenotype. Spanning different phylogenetic orders, sculpins (Scorpaeniformes), triplefins (Perciformes), killifish (Cyprinodontiformes) and the plainfin midshipman (Betrachoidiformes) are commonly found to have an enhancement of aerobic metabolism along with contributions from anaerobic metabolism in defense of hypoxic stress (although the scarcity of direct MRD measurements in ecologically relevant time frames should be noted; Fig. 4). The THR is more similar in the distantly related species than with the species' respective taxonomic counterparts. For example, hypoxia-tolerant sculpin and triplefin species inhabiting the intertidal zone share more similar traits with each other, e.g. low P_{crit} and high Hb–O_2 affinity, than with the less tolerant subtidal sculpin and triplefin species, respectively, e.g. high P_{crit} and low Hb–O_2 affinity (Mandic et al., 2009a; Brix et al., 1999). This would suggest a convergence of similar metabolic strategies among distantly related species. However, disentangling the roles of phylogenetic history and the hypoxic environment per se on shaping species' THRs will require a more widespread use of rigorous, phylogenetically appropriate comparisons.

As current human practices increase the prevalence and severity of hypoxia among the world's aquatic environments (IPCC, 2014; Schmidtko et al., 2017; Smith et al., 2006), understanding how metabolic strategies associate with different hypoxic environments is probably more important than ever. Knowing which THRs are most conducive to survival in which types of hypoxic environments may help us to better identify potentially vulnerable species, and better predict their redistribution patterns as their environments become increasingly hypoxic.

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M.M. and M.D.R. co-developed the ideas and co-wrote the manuscript.

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Table S1. Aerobic metabolism, anaerobic metabolism and metabolic rate depression traits of species in different aquatic hypoxic environments.

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