Research article

Themed Issue Article: Conservation Physiology of Marine Fishes

A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level ($P_{\text{crit}}$)

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Hypoxia is a common occurrence in aquatic habitats, and it is becoming an increasingly frequent and widespread environmental perturbation, primarily as the result of anthropogenic nutrient enrichment and climate change. An in-depth understanding of the hypoxia tolerance of fishes, and how this varies among individuals and species, is required to make accurate predictions of future ecological impacts and to provide better information for conservation and fisheries management. The critical oxygen level ($P_{\text{crit}}$) has been widely used as a quantifiable trait of hypoxia tolerance. It is defined as the oxygen level below which the animal can no longer maintain a stable rate of oxygen uptake (oxyregulate) and uptake becomes dependent on ambient oxygen availability (the animal transitions to oxyconforming). A comprehensive database of $P_{\text{crit}}$ values, comprising 331 measurements from 96 published studies, covering 151 fish species from 58 families, provides the most extensive and up-to-date analysis of hypoxia tolerance in teleosts. Methodologies for determining $P_{\text{crit}}$ are critically examined to evaluate its usefulness as an indicator of hypoxia tolerance in fishes. Various abiotic and biotic factors that interact with hypoxia are analysed for their effect on $P_{\text{crit}}$, including temperature, CO₂, acidification, toxic metals and feeding. Salinity, temperature, body mass and routine metabolic rate were strongly correlated with $P_{\text{crit}}$; 20% of variation in the $P_{\text{crit}}$ data set was explained by these four variables. An important methodological issue not previously considered is the inconsistent increase in partial pressure of CO₂ within a closed respirometer during the measurement of $P_{\text{crit}}$. Modelling suggests that the final partial pressure of CO₂ reached can vary from 650 to 3500 µatm depending on the ambient pH and salinity, with potentially major effects on blood acid–base balance and $P_{\text{crit}}$ itself. This database will form part of a widely accessible repository of physiological trait data that will serve as a resource to facilitate future studies of fish ecology, conservation and management.

Key words: Carbon dioxide, critical oxygen tension, metabolic rate, oxygen and capacity limitation of thermal tolerance, physiological trait

Editor: Steven Cooke
Received 17 December 2015; Revised 17 March 2016; accepted 19 March 2016

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Introduction

In recent decades, there has been growing concern regarding the increasingly widespread and frequent occurrence of hypoxia in aquatic environments, associated with the increased discovery of hypoxic zones globally (Diaz, 2001; Diaz and Breitburg, 2009; Zhang et al., 2010). Although periods of hypoxia can develop naturally in many aquatic systems, anthropogenic influences have been shown to be a major driver of hypoxic events in both freshwater and marine habitats (Friedrich et al., 2014). In particular, eutrophication associated with increased anthropogenic nutrient loading of lakes, rivers and coastal waters leads to blooms of algae and phytoplankton, the death of which subsequently fuels microbial respiration and the depletion of dissolved oxygen (Smith, 2003). Hypoxia has been shown to result in losses of biodiversity and to trigger widespread mortality events (Vaquer-Sunyer and Duarte, 2008). In the marine environment, more than 400 coastal systems have been reported as eutrophication-associated ‘dead zones’ (Diaz and Rosenberg, 2008). Global warming is likely to exacerbate hypoxia in aquatic systems owing to increased microbial respiration rates and reduced oxygen solubility with increasing water temperatures (McBryan et al., 2013). In addition, potential modifications to oceanic circulation linked to future climate change are predicted to result in greater stratification and ‘deoxygenation’ of the oceans (Keeling and Garcia, 2002; Keeling et al., 2009). In summary, in the future, reduced oxygen concentrations are predicted to occur more extensively, more frequently and for longer periods of time (IPCC, 2014). Fish are among the more hypoxia sensitive of aquatic taxa and, as such, the sequential loss of fauna from aquatic ecosystems during hypoxic events is commonly initiated by the loss or relocation of fish populations (Vaquer-Sunyer and Duarte, 2008). Understanding the physiological responses of individual organisms to environmental stressors, such as hypoxia, provides a mechanistic link between environmental change and population-level effects, which may be key to predicting future ecological impacts (Chown, 2012; Seebacher and Franklin, 2012; Cooke et al., 2013).

Fish can show various behavioural responses to hypoxia, such as rising to the surface to breathe the uppermost layer of water in contact with air, increasing activity to escape the hypoxic area or decreasing activity to reduce oxygen demand (Chapman and McKenzie, 2009; Urbina et al., 2011; Domenici et al., 2012). Beyond these behavioural responses, fishes can engage numerous profound physiological responses, such as changes in ventilation, cardiac activity and haemoglobin-O2 binding (Richards, 2009). These physiological responses work primarily to sustain oxygen extraction from the environment in order to maintain aerobic ATP production. This allows the majority of fishes to maintain stable oxygen uptake rates across a wide range of ambient partial pressures of oxygen (P02), a response known as ‘oxyregulation’ (reviewed by Perry et al., 2009). When, however, oxygen reduces to a threshold below which oxygen uptake rate cannot be maintained, oxygen uptake declines linearly with a decrease in ambient P02, a response known as ‘oxyconform- ing’ (Pörtner and Grieshaber, 1993; Claireaux and Chabot, 2016). This threshold, when oxygen uptake transitions from regulation to conforming, is referred to as the critical P02 (PCrit; Beamish, 1964; Ultsch et al., 1978). As a measure of whole-animal oxygen extraction capacity, which varies extensively across species and among populations, PCrit is widely used to describe the degree of hypoxia tolerance in fishes (Ulteh et al., 1978; Chapman et al., 2002; Nilsson et al., 2007a,b; Mandic et al., 2009; reviewed by Chapman and McKenzie, 2009; Speers-Roesch et al., 2012).

Oxygen, the key variable in PCrit measurements, is used by aerobic organisms as an electron acceptor in order to drive the production of ATP. As such, the rate of oxygen uptake is widely considered as a proxy for the rate of aerobic metabolism, at least when in a steady state (Brown et al., 2004; Nelson, 2016). Standard metabolic rate (SMR) is the oxygen uptake rate of an entirely inactive, post-absorptive fish and reflects its minimal cost of living at a given temperature (Beamish and Mookherjii, 1964; Chabot et al., 2016). Routine metabolic rate (RMR) provides a similar estimate of the cost of living but takes into account energy expended on maintaining posture and making the small movements that are typical of most fishes even when in a quiescent state (McBryan et al., 2013). In contrast, maximal metabolic rate (MMR) is the highest rate of oxygen uptake that can be attained in defined environmental conditions (Clark et al., 2013; Norin and Clark, 2016). The difference between SMR and MMR is referred to as aerobic scope and provides for the oxygen demands of higher functions, such as locomotion, growth, behaviour and reproduction (Farrell and Richards, 2009; Claireaux and Chabot, 2016). In the context of this aerobic hierarchy, several levels of critical P02 are represented in Figure 1. As this conceptual diagram illustrates, MMR is the
first rate to become limited as ambient oxygen decreases (\(P_{\text{crit}}\)), from which point a decline in MMR leads to a reduction in aerobic scope. Secondly, the \(P_{\text{crit}}\) for RMR is reached, whereby oxygen supply cannot sustain even minimal levels of aerobic activity. Finally, the \(P_{\text{crit}}\) for SMR indicates that oxygen supply cannot meet even basic oxygen demands (Portner and Lannig, 2009; Claireaux and Chabot, 2016). Below this threshold, anaerobiosis or suppression of metabolic rate are required to sustain life (Richards, 2009). Each of the three levels of \(P_{\text{crit}}\) may indicate the difference between mortality and survival. If so, \(P_{\text{crit}}\) may have major implications for the fitness of fishes living in environments prone to hypoxia and, as such, each of these levels can be considered as functional traits (McGill et al., 2006; Claireaux and Chabot, 2016).

The examination of trait variation across populations and communities, and its ecological implications, are increasingly becoming the basis for predicting and potentially mitigating the effects on biodiversity of environmental change (Chown, 2012). Such trait-based approaches are facilitated by the collection and dissemination of trait data. Large-scale multi-trait databases have been compiled for various taxa, including plants (Kattge et al., 2011), mammals (Jones et al., 2009), marine polychaetes (Faulwetter et al., 2014) and North American freshwater fishes (Frimpong and Angermeier, 2009). As a quantifiable measure of hypoxia tolerance that is measured on individuals and is applicable at population level, \(P_{\text{crit}}\) is useful for incorporation into trait-based approaches to the conservation physiology of fishes (Frimpong and Angermeier, 2009).

The field of fish physiology has generated a large body of literature on \(P_{\text{crit}}\) across a wide range of species and in highly variable abiotic and biotic conditions (Perry et al., 2009). Owing to the discrete and nuanced nature of each study, it is challenging to make broad generalizations. The aims of the present work were as follows: (i) to assemble a database of the \(P_{\text{crit}}\) values reported for fishes, from published literature, in a format suitable for future incorporation into multi-trait-based analyses; (ii) to analyse the data to identify how biotic and abiotic factors (particularly temperature) interact with hypoxia and affect \(P_{\text{crit}}\); and (iii) to appraise methodologies for measuring \(P_{\text{crit}}\) critically, and thereby evaluate its usefulness for quantifying hypoxia tolerance in fishes. This new analysis not only provides an opportunity for further quantitative considerations but also serves as a tangible link between the physiology and the conservation of fishes.

**Methods**

**Literature search**

The citation and abstract indexes, Scopus® and Web of Science®, were used to collect relevant peer-reviewed literature. The literature search was conducted in December 2014 using the following terms: ‘critical oxygen’, ‘critical \(PO_2\)’, ‘oxygen threshold’, ‘\(P_{\text{crit}}\)’, ‘oxyregulate’, ‘oxyconform’ or ‘hypoxia tolerance’. Approximately 400 papers from relevant subject areas were identified. Each of these articles was individually assessed for relevance based on their title and abstract. Finally, 144 papers were downloaded for a full read of the manuscript. Of these, only 96 papers reported \(P_{\text{crit}}\) measurements in at least one fish species.

**Database construction**

In order to maximize the future usefulness of the database and to ensure that it fully reflects the variation in abiotic/biotic conditions in which \(P_{\text{crit}}\) has previously been measured in fishes, it was necessary to extract multiple parameters from each study. For each \(P_{\text{crit}}\) entry, 66 columns summarize information on the species and origin, acclimation parameters, animal characteristics, experimental method, results, statistical analyses, general comments and bibliographic information (Table 1). The database was constructed as a single Microsoft Excel file, with individual columns for each parameter and rows for each \(P_{\text{crit}}\) determination in a particular species or treatment group. As such, a single study may occupy several rows depending on the number of treatment groups and/or species for which \(P_{\text{crit}}\) is reported. Values for \(P_{\text{crit}}\) were reported in a variety of different oxygen units across the literature (millimetres of mercury, torr, percentage air saturation, milligrams of oxygen per litre and micromolar), but were converted here to a partial pressure of oxygen (in kilopascals) based on oxygen solubility values reported by Green and Carritt (1967) and assuming standard atmospheric pressure at sea level (760 mmHg), if not otherwise reported. Likewise, all values of oxygen uptake rate were converted to milligrams of oxygen per kilogram per hour. To enable unbiased inter-species comparison, a subset of the full database was produced, which included only those \(P_{\text{crit}}\) measurements made in fishes meeting the following conditions: (i) in an unfed or post-absorptive state; (ii) undergoing no additional (to hypoxia) abiotic stressor; and (iii) where temperature acclimation lasted for >2 days.

**Database analysis**

The frequency of \(P_{\text{crit}}\) measurements across families and climate zones was calculated based on the full database. However, comparisons of \(P_{\text{crit}}\) values were made using the subset ‘control’ database described above. Based on the latitude of where the studies were conducted, each entry was labelled as tropical, sub-tropical, temperature or polar. Analysis of variance was used to test for an effect of climate zone on \(P_{\text{crit}}\) using the Sidak post hoc test.

Potential influences of varying respirometry methodologies and hypoxia exposure methods on \(P_{\text{crit}}\) were explored using the subset ‘control’ database, in which there are 297 data points. Similar to the full database, the majority of studies measured \(P_{\text{crit}}\) using closed static respirometry on individual fish, where oxygen is reduced via the oxygen consumption of the fish \((n = 202)\). Where there were sufficient data to compare methods between respirometry methods within a species, a Student’s unpaired \(t\)-test was used to compare between groups. It was not possible to test for differences in hypoxia exposure methods within species because there were insufficient data from at least two methods.
Stepwise multiple linear regression analysis was used to develop a model for predicting $P_{\text{crit}}$ based on biotic (body mass, RMR) and abiotic (temperature, salinity) variables. Earlier analysis detected no significant within-species effect of respirometry method (closed or flow through) on $P_{\text{crit}}$ and it was therefore not included in the linear regression model. Acclimation variables such as temperature, $P_{O_2}$, and salinity were not included in this analysis because they were very highly correlated with the equivalent variables reported during the trials. Minimal $P_{O_2}$ was not included in the model because it is driven by $P_{\text{crit}}$.

As the multivariate model identified salinity as a relevant factor, the potential effect of salinity on $P_{\text{crit}}$, was explored further by comparing $P_{\text{crit}}$ values measured in seawater (150 entries from 82 species) with $P_{\text{crit}}$ values measured in freshwater (116 entries from 50 species). This approach was taken because most of the studies were conducted either in freshwater [~0.1 practical salinity units (PSU)] or seawater (~30–38 PSU). Values of $P_{\text{crit}}$ were calculated as the partial pressure of oxygen (in milligrams per litre), using the solubility coefficient based on experimental temperature and salinity (Green and Carrit, 1967). Potential differences between groups were then tested by a Mann–Whitney U-test, because normality assumptions were violated.

## Results and discussion

### Database coverage

Of the 96 studies reviewed, 331 measurements of $P_{\text{crit}}$ across 151 species were incorporated into the database. Across the global database, 58 families are represented, with Cyprinidae ($n = 44$), Pomacentridae ($n = 41$), Gobiidae ($n = 24$), Cichlidae ($n = 23$), Salmonidae ($n = 19$), Cottidae ($n = 18$), Apogonidae ($n = 17$), Percidae ($n = 13$) and Sparidae ($n = 12$) the most frequently represented. freshwater and marine (including euryhaline) species account for 40 and 60% of $P_{\text{crit}}$ entries, respectively. Water temperatures at which $P_{\text{crit}}$ values were determined ranged between ~1.5 and 36°C, with a mean (±SD) of 21.7 ± 7.6°C. Values for $P_{\text{crit}}$ over the entire data set ranged between 1.02 kPa (Pseudocrenilabrus multicolor victoriae; Reardon and Chapman, 2010) and 16.2 kPa (Solea

### Table 1: List of the parameters incorporated into the database alongside each reported critical oxygen level value

<table>
<thead>
<tr>
<th>Species and origin</th>
<th>Stock acclimation</th>
<th>Sample characteristics</th>
<th>Experimental method</th>
<th>Results</th>
<th>Statistical analysis</th>
<th>Comments and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude and longitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMR, basal metabolic rate; DOI, digital object identifier; MMR, maximal metabolic rate; $M_{O_2}$, oxygen uptake rate; $P_{O_2}$, partial pressure of carbon dioxide; $P_{\text{crit}}$, critical oxygen level; $P_{O_2}$, partial pressure of oxygen; RMR, routine metabolic rate; SMR, standard metabolic rate.
solea larvae; McKenzie et al., 2008) with a mean (±SD) $P_{\text{crit}}$ in the ‘control’ data set of 5.15 ± 2.21 kPa. Plots of species and their reported $P_{\text{crit}}$ values from the subset data set are provided in the Supplementary Data (Supplementary Fig. 1).

The geographical coverage of the database includes at least one entry from every continent, although North America, Europe and Australasia are by far the most heavily represented and, when combined, account for 87% of $P_{\text{crit}}$ entries. Perhaps unsurprisingly, most studies of $P_{\text{crit}}$ in fishes have been concentrated around the major fish physiology research groups in Europe, North America and Australia. Arguably, this introduces an element of bias into the database, given the incomplete representation of all habitats and species at a global scale. Based on the full database, tropical studies are the most frequently represented ($n = 125$ $P_{\text{crit}}$, measurements, dominated by Lizard Island Research Station, Australia, $n = 98$), followed by subtropical ($n = 104$) and temperate regions ($n = 100$), dominated by Canada and Europe. The polar regions are the most under-represented ($n = 2$). Within the subset ‘control’ database, there was a significant difference in mean $P_{\text{crit}}$ across climatic regions (ANOVA, $F_{2,297} = 4.054$, $P = 0.018$), where tropical fishes had the lowest $P_{\text{crit}}$ (mean ± SEM: 4.92 ± 0.190 kPa) < sub-tropical fishes (5.0 ± 0.24 kPa) < temperate fishes (5.74 ± 0.24 kPa). However, the Sidak post hoc test suggested that $P_{\text{crit}}$ values for tropical fishes were significantly lower only than temperate fishes ($P = 0.021$). There was no difference in mean $P_{\text{crit}}$ between subtropical and either tropical ($P = 0.991$) or temperate $P_{\text{crit}}$ ($P = 0.085$). Owing to low sample size, the polar $P_{\text{crit}}$ values were not included in the ANOVA across temperatures but, interestingly, had a higher mean $P_{\text{crit}}$ than the other three climatic zones (7.9 ± 1.6 kPa).

Additionally, the species studied tend to be those conducive to respirometry trials. In particular, large, active or highly sensitive species, such as those of the Scombridae family (tuna, mackerels and bonitos) are generally under-represented in the literature (Blank et al., 2007). For example, the majority of $P_{\text{crit}}$ values reported in the database were measured on fish <1 kg body mass.

### Methodology used to determine critical oxygen level

The relationship between ambient $P_{\text{O}_2}$ and oxygen uptake in fishes has been investigated since the study of Keys (1930). Even at that early stage, there was considerable discussion among physiologists regarding the validity of different methodological developments, particularly methods for measuring dissolved oxygen content such as galvanic oxygen electrodes and, more recently, fibre-optic sensors, have made the performance of high-resolution measurements of oxygen uptake in fishes increasingly common (Clark et al., 2013; Nelson, 2016). Nevertheless, the literature examined for the purpose of building this database is characterized by considerable variation in terms of methods used to determine $P_{\text{crit}}$. For example, the majority of studies (56%) used closed respirometry for $P_{\text{crit}}$ estimates, 21% used flow-through respirometry, 20% used intermittent respirometry, and 3% used other approaches, such as indirect estimation of gill oxygen uptake (Table 2). Most studies (70%) depleted ambient oxygen through the fish’s own respiration, whereas 30% of studies bubbled nitrogen gas into the water to reduce ambient oxygen levels. The majority of studies (80%) measured RMR for $P_{\text{crit}}$ estimates; the remaining 20% measured SMR. These methodological differences and their implications are important to consider when interpreting collated $P_{\text{crit}}$ data.

Closed respirometry, whereby the fish is placed within a sealed chamber from which water is intermittently sampled for measurement of dissolved oxygen content, provides the simplest method of measuring oxygen uptake rate (Steffensen, 1989), as follows:

$$M_{\text{O}_2} = [(V_i - V_t) \times \Delta O_2] \times (\Delta t \times bw),$$

where $M_{\text{O}_2}$ represents oxygen uptake rate, $V_i$ is respirometer volume, $V_t$ is fish volume, $\Delta O_2$ is change in ambient oxygen content, $\Delta t$ is time, and bw is fish mass (‘body weight’). Importantly, water needs to be recirculated within the chamber to ensure adequate mixing, thus preventing the stratification of dissolved

### Table 2: The breakdown of the number of data points representing each respirometry type and oxygen removal method in the subset database

<table>
<thead>
<tr>
<th>Respirometry type</th>
<th>Fish respiration</th>
<th>$N_2$ equilibration</th>
<th>$N_2$ and $O_2$ equilibration</th>
<th>$N_2$ and $CO_2$ equilibration</th>
<th>$N_2$, $O_2$ and air equilibration</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed static (individual)</td>
<td>202</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>203</td>
</tr>
<tr>
<td>Closed static (grouped)</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Closed flow-through</td>
<td>13</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Intermittent flow</td>
<td>13</td>
<td>26</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Mesocosm (grouped, large tuna)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Open flow-through</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Opercular mask</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
oxygen within the chamber (Keys, 1930). Whether spontaneous movements and ventilation are sufficient to provide mixing depends on the species and achieving the correct fish-to-respirometer volume ratio. For closed determinations of \( P_{\text{crit}} \), hypoxia is generated by allowing the fish to deplete available oxygen through its own respiration, therefore negating the need to strip dissolved oxygen from the water artificially through equilibration with nitrogen. For this reason, closed respirimetry is particularly useful for conducting measurements of \( P_{\text{crit}} \) in the field or at remote locations where facilities such as a supply of \( N_2 \) may not be readily available (Rosenberger and Chapman, 2000; Nilsson et al., 2007b).

However, there are several important considerations regarding the use of closed respirimetry for determination of \( P_{\text{crit}} \). For instance, the rate of oxygen depletion during closed respirimetry is determined by the ratio of fish size (or oxygen uptake rate) to respirometer volume. A lack of control over the development of hypoxia can be problematic in comparative studies that use the same respirometer to measure \( P_{\text{crit}} \) in fish of different size and/or metabolic rate. As an illustrative example, the depletion of oxygen levels from 20 to 1 kPa by Australian barramundi \((Lates calcarifer)\) took between 1.5 and 4 h depending on the temperature (26 or 36°C; Collins et al., 2013). From our database, it is evident that there is very little, if any, standardization in terms of the rate of oxygen depletion between \( P_{\text{crit}} \) studies, irrespective of which respirimetry method is employed. This is in contrast to measurements of other physiological threshold traits, such as the determination of critical temperature, which tends to be made at consistent warming or cooling rates among studies (0.2–0.3°C min\(^{-1}\); Beitinger et al., 2000; Mora and Maya, 2006; Murchie et al., 2011). It is unclear whether the rate of decline in ambient oxygen will significantly affect \( P_{\text{crit}} \), but it is likely that a longer time scale would allow for greater respiratory adjustments, and hence, reveal lower \( P_{\text{crit}} \) values than more acute hypoxic exposures. Indeed, our own anecdotal observations in European flounder \((Platichthys flesus)\) suggest that these fish tend to oxyconform across the entire range of ambient \( P_O \) when exposed to a very rapid reduction of oxygen (from 21 to 2 kPa in <2 h).

A further issue associated with closed respirimetry is the build-up of the waste products of metabolism, in particular \( CO_2 \) (Keys, 1930; Steffensen 1989; Urbina et al., 2012). It has been argued that the amount of \( CO_2 \) accumulation within a closed respirimeter is unlikely to impact on \( CO_2 \) excretion by fishes significantly, given that they normally exhibit a blood partial pressure of \( CO_2 (P_{CO_2}) \) of around 2–4 mmHg, much higher than normal ambient levels (Ishimatsu et al., 2005; Nilsson et al., 2007a). However, a precedent has been set, albeit at more severe levels of hypercarbia (2.25–20 mmHg), to show that elevated \( P_{CO_2} \) can increase \( P_{\text{crit}} \) in European eels \((Anguilla anguilla); Cruz-Neto and Steffensen, 1997\), although no effect on \( P_{\text{crit}} \) was observed when eels were given enough time to acclimate fully in terms of acid–base regulation (McKenzie et al., 2003), or in spot fish \((Leiostomus xanthurus)\) and mummichog \((Fundulus heteroclitus)\) (Cochran and Burnett, 1996). Given the potential influence of hypercarbia, it would be prudent to report any change in water \( P_{CO_2} \) alongside values for \( P_{\text{crit}} \) that have been determined through closed respirimetry, but this has rarely been the case throughout the existing literature. A single study so far has evaluated this potential confounding factor in determining \( P_{\text{crit}} \) but in this unusual oxyconforming species \( (inanga, Galaxias maculatus) \) elevated \( P_{CO_2} \) had no effect on oxygen uptake rate at any level of ambient oxygen (Urbina et al., 2012). Furthermore, the authors pointed out that the effect of \( CO_2 \) on \( M_O \) in fishes appears to be species specific (Gilmour, 2001; Ishimatsu et al., 2008).

An important issue that does not appear to have been considered previously is that the extent to which \( P_{CO_2} \) increases within a closed respirometer will be highly dependent on the starting water chemistry, in particular \( pH \) and salinity (Fig. 2). A higher seawater \( pH \) indicates a greater total alkalinity (TA). In turn, this gives increased capacity for buffering added \( CO_2 \) and limiting the increase in \( P_{CO_2} \) for a given increase in total \( CO_2 \) attributable to net excretion by the fish in a respirometer. Therefore, the lower the starting water \( pH \), the larger the

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**Figure 2:** Model of the estimated partial pressure of carbon dioxide \((P_{CO_2})\) reached, in water of different salinities and starting \( pH \) values, after the addition of 140 \( \mu M \) \( CO_2 \). The value of 140 \( \mu M \) approximates the increase in total \( CO_2 \) attributable to excretion by a fish at 15°C during a closed respirimetry experiment. In this theoretical example, the oxygen level is allowed to decline as a result of respiration from a normoxic partial pressure of \( \sim 20 \) kPa \((-245 \mu M)\) to a common \( P_{\text{crit}} \) value of \(-6 \) kPa \((-74 \mu M)\), and we have assumed a respiratory quotient \((CO_2 \text{ excreted} \div O_2 \text{ consumed})\) of 0.85 for fish (Kieffer et al., 1998). At each starting \( pH \), the total alkalinity (TA) and total \( CO_2 \) were calculated from the \( pH \) and assuming equilibration with atmospheric \( CO_2 \). When excreted \( CO_2 \) is dissolved in water, the total \( CO_2 \) increases accordingly (in this case, by 140 \( \mu M \)) but TA remains unchanged (Riebesell et al., 2010). For each starting \( pH \), we therefore used the CO2ys program (for the national bureau of standards \( pH \) scale) to calculate the final \( P_{CO_2} \) that would result from increasing total \( CO_2 \) by 140 \( \mu M \) while TA remained constant. This was repeated for salinities of 20, 25, 30, 35 and 40 practical salinity units (PSU) and starting \( pH \) values of 7.5–8.5 to cover ranges experienced in many marine laboratories.
overall change in $P_{\text{CO}_2}$ over the course of the $P_{\text{crit}}$ measurement. From the models shown in Figure 2, it is clear that pH has a massive influence on the ambient $P_{\text{CO}_2}$ reached within such a closed respirometry scenario, with final $P_{\text{CO}_2}$ values ranging by 5-fold, from ~650 μatm (0.49 mmHg) to ~3300 μatm (2.66 mmHg) at the highest (8.5) and lowest (7.5) starting pH values shown, respectively. Note that even the lowest of these final $P_{\text{CO}_2}$ values has been shown (in experiments designed to mimic future ‘ocean acidification’ scenarios) to have significant detrimental effects in fishes (Munday et al., 2009). When the starting pH is low, the highest $P_{\text{CO}_2}$ values of ~3500 μatm occur, which are more than 3.5 times higher than the ‘business as usual’ for end-of-century global CO2 projections (representative concentration pathway scenario 8.5; Meinshausen et al., 2011). It is also relevant to note that salinity has a major modulating effect, in particular within the middle of the range of starting pH values. For example, at a starting pH of 8.0, the final $P_{\text{CO}_2}$ will vary from slightly <1500 μatm (1.14 mmHg) at the highest salinity (40 PSU) to >2500 μatm (1.90 mmHg) at the lowest salinity (20 PSU).

The larger ambient $P_{\text{CO}_2}$ values indicated above would certainly be expected to cause significant blood acid–base disturbance during the time scale of a typical closed respirometry experiment (minutes to hours) and thus have the potential to influence $P_{\text{crit}}$ via alterations in the oxygen binding affinity of haemoglobin. It is therefore important to recognize this variability in $P_{\text{CO}_2}$ when conducting closed respirometry experiments to determine hypoxia tolerance, and particularly, when interpreting $P_{\text{crit}}$ measurements.

Flow-through respirometry is a technique whereby oxygen content of the inflowing ($O_{2,\text{in}}$) and outflowing ($O_{2,\text{out}}$) water is continuously measured at a fixed water flow rate through the respirometer ($F_w$). By application of the Fick principle, oxygen uptake ($M_{O_2}$) is determined by:

$$M_{O_2} = F_w(O_{2,\text{in}} - O_{2,\text{out}}) + \text{bw}.$$ 

Although flow-through respirometry avoids the accumulation of metabolites in the chamber, it suffers from problems primarily related to the ‘wash-out’ effect, whereby a significant lag can develop between changes in the fish’s real $M_{O_2}$ and changes in observed $O_{2,\text{out}}$. The degree of wash-out depends on the dilution factor, which is a function of water mixing, volume and flow rate (Steffensen, 1989).

Intermittent flow-through respirometry is generally considered the ideal method of $M_{O_2}$ determination in fishes because it involves none of the problems associated with closed or flow-through techniques (Steffensen, 1989; Clark et al., 2013). The term ‘intermittent’ or ‘semi-closed’ in this context refers to the transitioning between a closed phase for determination of $M_{O_2}$ and a flush phase for restoring $O_2$ to a set level and removing metabolites from the respirometer. As the equipment and software for automating flush–recirculation cycles and simultaneous data acquisition from multiple chambers have become more sophisticated and widely available, intermittent flow-through respirometry has been increasingly used (Svendsen et al., 2016). However, $P_{\text{crit}}$ measurements via this preferred technique account for only 20% of values incorporated into the present database.

Flow-through techniques allow for the supply of hypoxic water to the respirometry chamber. This hypoxic water can be produced by bubbling with N2 via a solenoid valve linked to an O2 probe (Schurmann and Steffensen, 1997) or by bubbling with set gas mixtures of variable O2 and N2 content. Both methods allow for finer control of the hypoxic exposure compared with allowing the fish to deplete ambient oxygen levels dependent on its own $M_{O_2}$. Progressive hypoxia can be generated in a stepwise fashion such that multiple $M_{O_2}$ measurements can be made at a specific $P_{\text{O}_2}$, thereby increasing the likelihood of determining an $M_{O_2}$ that is representative of true SMR or RMR (Rantin et al., 1993).

Using the present database, we were able to explore differences in respirometry methods within three species, Atlantic salmon (Salmo salar), common carp (Cyprinus carpio) and Nile tilapia (Oreochromis niloticus), for which the sample size for at least two methods was greater than $n > 2$. Between closed static or closed flow-through respirometers, there was no difference in $P_{\text{crit}}$ of common carp (Student’s unpaired t-test, $t = 1.429$, d.f. = 6, $P = 0.203$). Likewise, between closed, static respirometers (individual fish) and open flow respirometry (with grouped fish), there was no difference in $P_{\text{crit}}$ in Atlantic salmon (Student’s unpaired t-test, $t = -0.678$, d.f. = 8, $P = 0.517$). There was no difference in $P_{\text{crit}}$ between closed, flow-through or intermittent flow-through respirometry within Nile tilapia (Student’s unpaired t-test, $t = -0.644$, d.f. = 6, $P = 0.543$). In both Atlantic salmon and common carp, oxygen levels were reduced by the respiration of the fish, whereas in Nile tilapia the oxygen was reduced by nitrogen equilibration. A direct comparison in the shiner perch (Cymatogaster aggregata) found, however, that $P_{\text{crit}}$ measured by intermittent flow-through respirometry was significantly lower than that measured by closed respirometry (Snyder et al., 2016). Thus, more direct comparisons are needed to investigate whether the two most common methodologies might provide different estimates of $P_{\text{crit}}$.

To determine $P_{\text{crit}}$, $M_{O_2}$ is plotted against ambient $P_{O_2}$ in order to identify the inflection point at which $M_{O_2}$ changes from being independent of ambient oxygen to dependent on ambient oxygen. Within this procedure, a great deal of subtle variation exists among studies. Most obvious is the differential use of SMR or RMR, with the majority (84%) of studies reporting a $P_{\text{crit}}$ for RMR. Arguably, the $P_{\text{crit}}$ exhibited for RMR is more ecologically relevant, given that this level of $M_{O_2}$ is likely to be exhibited most of the time in the field (Ultisch et al., 1978; Pörtner, 2010). Indeed, for some highly active species, such as salmonids, $P_{\text{crit}}$ determined during active swimming may be most useful in considering the ecological implications of hypoxia (Fry, 1957). Activity level may affect $P_{\text{crit}}$ in unexpected ways, such as in the Adriatic sturgeon (Acipenser naccarii), which exhibits a well-developed ability to...
oxyregulate ($P_{\text{crit}} = 4.9 \pm 0.5$ kPa) when permitted to swim at a low sustained speed but oxyconforms across the entire range of declining ambient oxygen when its activity is restricted in a static respirometer (McKenzie et al., 2007). Some species exhibit a relatively high $P_{\text{crit}}$ for RMR at a $P_{O_2}$ that is well above the $P_{100}$ (half of the hemoglobin oxygen binding sites are saturated with oxygen) of their haemoglobin. In these instances, $P_{\text{crit}}$ may indicate a behavioural change and not simply a physical limitation of oxygen supply (McBryan et al., 2013).

Of the studies that determine the $P_{\text{crit}}$ for SMR, the methods used for quantifying SMR vary considerably. Some studies use the single lowest $M_{O_2}$ value recorded at normoxia, whereas others take the average of a set number of the lowest $M_{O_2}$ values (Iversen et al., 2010). More sophisticated and robust methods involve extrapolating the average $M_{O_2}$ measured at specified swimming speeds back to zero activity (Wilson et al., 1994; Cook et al., 2014) or the use of percentiles and frequency distributions to assess all normoxic $M_{O_2}$ data (Dupont-Prinet et al., 2013). As the critical level for basal metabolism, $P_{\text{crit}}$ determinations based on SMR should theoretically reflect a true physiological limitation of oxygen extraction capacity (McBryan et al., 2013), although this may not be true in species for which metabolic depression below $P_{\text{crit}}$ has a facultative component. Given that the $P_{\text{crit}}$ for RMR is likely to be encountered at higher $P_{O_2}$ than that for SMR (Fig. 1), intra- or inter-species comparisons among studies reporting different levels of $P_{\text{crit}}$ may not be entirely valid. Whether SMR or RMR measurements are used to reflect normoxic $M_{O_2}$, it is essential that sufficient time is allowed for the fish to acclimate to the respirometry chamber; otherwise, apparent reductions in $M_{O_2}$ as hypoxia develops may be an artefact of increasing habituation rather than true oxyconforming (Nilsson et al., 2004).

The method used to establish the point of intersection between continuous oxyregulation and oxyconforming $M_{O_2}$ data is also inconsistent among studies. The slope of these lines will determine the $P_{\text{crit}}$ and vice versa; therefore, determining which data points should be included within each line is critical to establishing an accurate estimate of $P_{\text{crit}}$ (Yeager and Ultsch, 1989). This can be achieved graphically by fitting a least-squares linear regression through data points that show a progressive decline in $M_{O_2}$, such that it intersects with a regression line fitted through normoxic $M_{O_2}$ data (Monteiro et al., 2013). A number of mathematical methods for performing so-called piece-wise or segmented linear regression analyses are available, which provide greater robustness to estimates of $P_{\text{crit}}$ and are used in the majority of studies incorporated into the present database (Nickerson et al., 1989; Yeager and Ultsch, 1989; Leiva et al., 2015). These approaches assume that the response of $M_{O_2}$ to declining $P_{O_2}$ is biphasic and consists of two entirely linear elements, with an abrupt transition between the two. Such assumptions are not necessarily met by real-world data, and indeed, concentration-dependent reaction kinetics make truly linear relationships between $M_{O_2}$ and $P_{O_2}$ unlikely (Marshall et al., 2013). Recent developments in non-linear regression techniques are now being promoted as a more accurate approach to determining biological thresholds such as $P_{\text{crit}}$ (Stinchcombe and Kirkpatrick, 2012; Marshall et al., 2013).

### Critical oxygen level as a hypoxia tolerance trait

A low $P_{\text{crit}}$ is generally associated with greater hypoxia tolerance because it indicates a higher capacity for oxygen extraction and tissue delivery at low $P_{O_2}$ (Mandic et al., 2009). Maintaining aerobic metabolism during hypoxia is advantageous because it is up to 30-fold more efficient than anaerobic ATP production (per unit substrate consumed) and avoids accumulation of the deleterious by-products (e.g. $H^+$) of anaerobic metabolism (Richards, 2009). Hypoxia-induced physiological modifications that increase oxygen extraction capacity, such as increased gill surface area (Nilsson, 2007) and haemoglobin–$O_2$ binding (Brix et al., 1999), are observed in fishes that frequently encounter hypoxia, suggesting that maintaining aerobic metabolism is a primary hypoxia survival strategy (Mandic et al., 2009). However, when ambient $P_{O_2}$ declines below $P_{\text{crit}}$, survival depends on the availability of substrate for $O_2$-independent ATP production (primarily glycolysis) and the ability to reduce metabolic demand (Richards, 2009).

How long a fish can maintain a balance between ATP demand and supply below its $P_{\text{crit}}$, and thus delay the onset of cellular dysfunction, necrosis and subsequent death, is a key component of hypoxia tolerance (Nilsson and Östlund-Nilsson, 2008; Urbina and Glover, 2012; Speers-Roesh et al., 2013). Speers-Roesh et al. (2013) showed that $P_{\text{crit}}$ does not entirely predict hypoxia tolerance at lower oxygen levels. The authors used three species of sculpin ($Blepsias cirrhosis$, $Leptocottus armatus$ and $Oligocottus maculosus$), which exhibit different $P_{\text{crit}}$ values (1.76, 1.48 and 1.03 kPa, respectively), and exposed them to hypoxia levels that were 30% below each of their respective $P_{\text{crit}}$ values while recording the time to loss of equilibrium. The loss of equilibrium was consistent between only two of the three species ($L. armatus$ and $O. maculosus$). Similar relative hypoxia exposures in the eel pout ($Hemiscyllium ocellatum$) and shovel-nose ray ($Aptechotrema rostratum$) revealed lower lactate accumulation in eel pout, indicating enhanced metabolic depression in this species (Speers-Roesh et al., 2012). Furthermore, Nilsson and Östlund-Nilsson (2008) showed that $P_{\text{crit}}$ did not correlate with body mass in juvenile and adult damselfish (Pomacentridae) ranging between 10 mg and 40 g but that smaller fish were much less tolerant to hypoxia below $P_{\text{crit}}$, owing to their limited capacity for meeting ATP demand through anaerobic metabolism. These findings were further supported in $G. maculatus$ (Urbina and Glover, 2013). These results illustrate the benefit of considering $P_{\text{crit}}$ alongside other methods of determining hypoxia tolerance, such as measurements of tissue-specific lactate accumulation and determinations of the loss of equilibrium of 50% of the fish, in order to assess overall hypoxia tolerance (Urbina and Glover, 2013; Speers-Roesh et al., 2013; Claireaux and Chabot, 2016).
A recent review by Salin et al. (2015) argues that whole-animal oxygen consumption measurements may provide only a partial proxy for energy metabolism because of variation, within and between individuals, in the amount of ATP produced per molecule of oxygen consumed by mitochondria (P/O ratio). Environmental factors such as ambient temperature, food intake and diet composition have been shown both to increase and to decrease P/O ratios in the mitochondria of a variety of organisms (Salin et al., 2015). Hence, conclusions based on oxygen consumption rate alone could lead to misleading conclusions regarding respiratory performance during environmental changes. To our knowledge, the effect of hypoxia on P/O ratios in fish has yet to be investigated, and as such, provides an interesting avenue for further research.

As a hypoxia-tolerance trait, low \( P_{\text{crit}} \) can often, but not always, indicate an ability to survive in hypoxic water. It does not consider the use of hypoxia-avoidance strategies, such as adaptations for emersion, aquatic surface respiration and air breathing (Chapman and McKenzie, 2009). The inanga (\( G. \) maculatus), which inhabits lowland streams prone to severe hypoxia, is a rare example of a fish species that appears to be an entirely obligate oxyconformer and thus demonstrates no discernible \( P_{\text{crit}} \) (Urbina et al., 2012). Likewise, several species of Gymnotiform electric fishes from South America, which inhabit naturally hypoxic floodplain pools, also appear to be obligate oxyconformers with no \( P_{\text{crit}} \) (Reardon E. E., personal communication), an observation that is also anatomically supported in \( Brachyhypopomus brevirostris \) (Crampton, 1998). In some of these species, such as the inanga, a lack of scales and a large surface area-to-volume ratio indicate a high capacity for cutaneous \( O_2 \) uptake whilst emersed, and hence, provide a short-term means to escape aquatic hypoxia (Urbina et al., 2011). The oxygen thresholds for aquatic surface respiration, air breathing and emergence were incorporated into the database, but only where they have been reported alongside \( P_{\text{crit}} \) measurements. Such examples demonstrate the limitation of \( P_{\text{crit}} \) as a universal and comparative measure of hypoxia tolerance between species and emphasize the benefit of multi-trait-based approaches.

**Biotic and abiotic interactions**

Environmental stressors, such as hypoxia, rarely occur in isolation, and the interaction between stressors is of key concern in the context of predicting the ecological impacts of future environmental change (Crair et al., 2008). As a typical threshold effect, the response of fish to hypoxia is likely to result in ‘ecological surprises’, whereby seemingly resilient populations suddenly collapse once a critical threshold is crossed (McBryan et al., 2013). Additive or synergistic interactions with hypoxia could hasten the arrival of such thresholds, meaning that small environmental shifts could result in large effects on the performance of a population. Theoretically, any abiotic or biotic factor that affects either oxygen supply (cardiorespiratory capacity) or oxygen demand (metabolic rate) of an individual, and the balance therein, will have implications for its hypoxia tolerance. As an indicator of hypoxia tolerance, the effects of a wide range of abiotic and biotic interactions on \( P_{\text{crit}} \) in fish have been published (Table 3).

The stepwise multiple linear regression found that biotic (body mass, RMR) and abiotic (temperature, salinity) variables were highly correlated with \( P_{\text{crit}} \) (see Table 4). A significant regression \( F_{6,114} = 10.565, P < 0.001 \) predicted 19.5% of the variation in the data, based on an adjusted \( R^2 \) (multiple linear regression). Predicted \( P_{\text{crit}} \) is equal to \( 5.689 + 0.047 \) (salinity) – \( 0.083 \) (temperature) + \( 1.931 \) (body mass) + 0.001 (RMR), where salinity is measured in practical salinity units, temperature in degrees Celsius, body mass in kilograms, and RMR in milligrams of oxygen per litre. All four variables were significant predictors of \( P_{\text{crit}} \) in the full model (Table 4).

Temperature is by far the most widely studied abiotic factor potentially interacting with hypoxia (reported in 30 species) and is particularly relevant, given ongoing global climate change (Ficke et al., 2007; Pörtner, 2010). As ectotherms, oxygen demand in fishes increases in a roughly exponential manner with temperature (inter-species mean \( Q_{10} \) of 1.83; Clarke and Johnston, 1999), and the intrinsic link between temperature and environmental hypoxia has become the basis of an overarching concept termed ‘oxygen and capacity limitation of thermal tolerance’ (Pörtner, 2001, 2010). Essentially, this concept suggests that the thermal tolerance of ectotherms is dictated by their capacity to meet the oxygen demands of aerobic metabolism. Increased temperature both elevates basal oxygen demand (SMR) and reduces oxygen supply (via its effect on oxygen solubility), whereas hypoxia reduces the oxygen supply. Hence, temperature and hypoxia are likely to act synergistically in fishes. Within species, increasing temperature generally results in a higher \( P_{\text{crit}} \) but among species, the slope of the relationship between temperature and \( P_{\text{crit}} \) is highly variable (Fig. 3). For example, the Atlantic salmon (\( S. \) salar) exhibits a steep linear increase of \( P_{\text{crit}} \) in comparison to the shallower slope seen in the common carp (\( C. \) carpio) across a similar temperature range (Ott et al., 1980; Remen et al., 2013). A surprising exception to the generally positive intra-species correlation between temperature and \( P_{\text{crit}} \) was observed in four out of six species of darter (\( Etheostoma \)), for which \( P_{\text{crit}} \) was lower at 20 than 10°C (Ultsch et al., 1978). Variation in the sensitivity of species to temperature in terms of hypoxia tolerance may arise because of differences in their potential for thermal acclimation. Explanations for this variation may include reducing the metabolic impact of increased temperature or enhancing oxygen extraction capacity (Ott et al., 1980; Pörtner, 2010). Species exhibit highly contrasting capacities for plastic acclimation responses. At opposite ends of this spectrum, crucian carp (\( C. \) carassius) can dramatically increase respiratory surface area through gill remodelling in response to temperature and hypoxia (Solliid et al., 2005), whereas certain tropical reef fish species (\( Ostorhinchus doederleini \) and \( Pomacentrus moluccensis \)) demonstrate no thermal acclimation ability even over a relatively modest temperature range (29–32°C; Nilsson et al., 2010).
Table 3: Summary of biotic and abiotic factors and their interactions with the intra-species critical oxygen level as reported by studies included in the database

<table>
<thead>
<tr>
<th>Variable</th>
<th>Species</th>
<th>Effect on $P_{crit}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing temperature</td>
<td><em>Gadus morhua</em></td>
<td>Increase</td>
<td>Schurmann and Steffensen (1997)</td>
</tr>
<tr>
<td></td>
<td><em>Late calcarifer</em></td>
<td>Increase</td>
<td>Collins et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>Scyliorhinus canicula</em></td>
<td>Increase</td>
<td>Butler and Taylor (1975)</td>
</tr>
<tr>
<td></td>
<td><em>Salmo salar</em></td>
<td>Increase</td>
<td>Barnes et al. (2011)</td>
</tr>
<tr>
<td></td>
<td><em>S. salar</em></td>
<td>Increase</td>
<td>Remen et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>Dentex dentex</em></td>
<td>Increase</td>
<td>Cerezo Valverde et al. (2006)</td>
</tr>
<tr>
<td></td>
<td><em>Tautogolabrus adspersus</em></td>
<td>Increase</td>
<td>Corkum and Gamperl (2009)</td>
</tr>
<tr>
<td></td>
<td><em>Gadus ogac</em></td>
<td>Increase</td>
<td>Corkum and Gamperl (2009)</td>
</tr>
<tr>
<td></td>
<td><em>Bellapiscis medius</em></td>
<td>Increase</td>
<td>Hilton et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>Bellapiscis lesleyae</em></td>
<td>Increase</td>
<td>Hilton et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>Morone saxatilis</em></td>
<td>Increase</td>
<td>Lapointe et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>Carassius carassius</em></td>
<td>Increase</td>
<td>Solli et al. (2005)</td>
</tr>
<tr>
<td></td>
<td><em>Gobiodon histrio</em></td>
<td>Increase</td>
<td>Sørensen et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>Gobiodon erythrosplius</em></td>
<td>Increase</td>
<td>Sørensen et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>Oreochromis niloticus</em></td>
<td>Increase</td>
<td>Fernandes and Rantin (1989)</td>
</tr>
<tr>
<td></td>
<td><em>Cyprinus carpio</em></td>
<td>Increase</td>
<td>Ott et al. (1980)</td>
</tr>
<tr>
<td></td>
<td><em>Oncorhynchus mykiss</em></td>
<td>Increase</td>
<td>Ott et al. (1980)</td>
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<tr>
<td></td>
<td><em>Pomacentrus moluccensis</em></td>
<td>Increase</td>
<td>Nilsson et al. (2010)</td>
</tr>
<tr>
<td></td>
<td><em>Ostorhinchus doederleini</em></td>
<td>Increase</td>
<td>Nilsson et al. (2010)</td>
</tr>
<tr>
<td></td>
<td><em>Carassius auratus grandoculis</em></td>
<td>No effect</td>
<td>Yamanaka et al. (2013)</td>
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<td></td>
<td><em>Etheostoma boschungi</em></td>
<td>Decrease</td>
<td>Ultsch et al. (1978)</td>
</tr>
<tr>
<td></td>
<td><em>Etheostoma fusiforme</em></td>
<td>Decrease</td>
<td>Ultsch et al. (1978)</td>
</tr>
<tr>
<td></td>
<td><em>Etheostoma flabellare</em></td>
<td>Decrease</td>
<td>Ultsch et al. (1978)</td>
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<tr>
<td></td>
<td><em>Etheostoma rufilineatum</em></td>
<td>Decrease</td>
<td>Ultsch et al. (1978)</td>
</tr>
<tr>
<td>Increasing salinity</td>
<td><em>Cottus asper</em></td>
<td>Decrease</td>
<td>Henriksson et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>Leptocottus armatus</em></td>
<td>No effect</td>
<td>Henriksson et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>Cyprinus carpio</em></td>
<td>Increase</td>
<td>De Boeck et al. (2000)</td>
</tr>
<tr>
<td></td>
<td><em>Cyprinodon ariegatus</em></td>
<td>Increase</td>
<td>Haney and Nordlie (1997)</td>
</tr>
<tr>
<td>Increased $P_{O_2}$</td>
<td><em>Fundulus heteroclitus</em></td>
<td>No effect</td>
<td>Cochran and Burnett (1996)</td>
</tr>
<tr>
<td></td>
<td><em>Leiostomus xanthurus</em></td>
<td>No effect</td>
<td>Cochran and Burnett (1996)</td>
</tr>
<tr>
<td></td>
<td><em>Anguilla anguilla</em></td>
<td>Increase</td>
<td>Cruz-Neto and Steffensen (1997)</td>
</tr>
<tr>
<td></td>
<td><em>Platichthys flesus</em></td>
<td>Increase</td>
<td>Rogers (2015)</td>
</tr>
<tr>
<td>Hypoxic acclimation</td>
<td><em>Pagrus auratus</em></td>
<td>No effect</td>
<td>Cook et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>S. salar</em></td>
<td>No effect</td>
<td>Remen et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>Hemiscyllium ocellatum</em></td>
<td>Decrease</td>
<td>Routley et al. (2002)</td>
</tr>
<tr>
<td></td>
<td><em>Spinibarbus sinensis</em></td>
<td>Decrease</td>
<td>Dan et al. (2014)</td>
</tr>
<tr>
<td></td>
<td><em>C. auratus</em></td>
<td>Decrease</td>
<td>Fu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td><em>Poecilia latipinna</em></td>
<td>Decrease</td>
<td>Timmerman and Chapman (2004 a,b)</td>
</tr>
</tbody>
</table>

(Continued)
Table 3: continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>Species</th>
<th>Effect on $P_{\text{crit}}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reared in hypoxic environment</td>
<td><em>Pseudocrenilabrus multicolor</em></td>
<td>Decrease</td>
<td>Reardon and Chapman (2010)</td>
</tr>
<tr>
<td>Exercise pre-conditioning</td>
<td><em>C. auratus</em></td>
<td>Decrease</td>
<td>Fu et al. (2011)</td>
</tr>
<tr>
<td>Fed</td>
<td><em>Astronotus ocellatus</em></td>
<td>Increase</td>
<td>De Boeck et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>Oreochromis niloticus</em></td>
<td>Increase</td>
<td>Mamun et al. (2013)</td>
</tr>
<tr>
<td></td>
<td><em>Perca fluviatilis</em></td>
<td>Increase</td>
<td>Thuy et al. (2010)</td>
</tr>
<tr>
<td>Fatty acid-enriched diet</td>
<td><em>Solea solea</em> (larvae)</td>
<td>Decrease</td>
<td>McKenzie et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>S. solea</em> (juveniles)</td>
<td>Decrease</td>
<td>McKenzie et al. (2008)</td>
</tr>
<tr>
<td>Increasing body mass</td>
<td><em>Hypostomus plecostomus</em></td>
<td>Decrease</td>
<td>Perna and Fernandes (1996)</td>
</tr>
<tr>
<td></td>
<td><em>Astronotus ocellatus</em></td>
<td>Decrease</td>
<td>Sloman et al. (2006)</td>
</tr>
<tr>
<td>Pre- to post-settlement (larvae)</td>
<td><em>Chromis atripectoralis</em></td>
<td>Decrease</td>
<td>Nilsson et al. (2007a,b)</td>
</tr>
<tr>
<td></td>
<td><em>Pomacentrus amboinensis</em></td>
<td>Decrease</td>
<td>Nilsson et al. (2007a,b)</td>
</tr>
<tr>
<td>Larvae to juveniles</td>
<td><em>C. auratus grandoculis</em></td>
<td>Decrease</td>
<td>Yamanaka et al. (2013)</td>
</tr>
<tr>
<td>Juveniles to adults</td>
<td><em>Reinhardtius hippoglossoides</em></td>
<td>Decrease</td>
<td>Dupont-Prinet et al. (2013)</td>
</tr>
<tr>
<td>Mycobacteriosis infection</td>
<td><em>Morone saxatilis</em></td>
<td>Increase</td>
<td>Lapointe et al. (2014)</td>
</tr>
<tr>
<td>Acidified water</td>
<td><em>Salmo gairdneri</em></td>
<td>Increase</td>
<td>Ultsch et al. (1980)</td>
</tr>
<tr>
<td></td>
<td><em>Cyprinus carpio</em></td>
<td>Increase</td>
<td>Ultsch et al. (1980)</td>
</tr>
<tr>
<td>Metal exposure</td>
<td><em>Brycon amazonicus</em></td>
<td>Increase</td>
<td>Monteiro et al. (2013) (Hg$^{2+}$)</td>
</tr>
<tr>
<td></td>
<td><em>C. carassius</em></td>
<td>Increase</td>
<td>Schjolden et al. (2007) (Cu$^{2+}$)</td>
</tr>
<tr>
<td></td>
<td><em>Perca fluviatilis</em></td>
<td>Increase</td>
<td>Bilberg et al. (2010) (AgNO$_3$)</td>
</tr>
<tr>
<td></td>
<td><em>P. fluviatilis</em></td>
<td>Increase</td>
<td>Bilberg et al. (2010) (nano-Ag)</td>
</tr>
<tr>
<td>Organophosphate exposure</td>
<td><em>Oreochromis niloticus</em></td>
<td>Increase</td>
<td>Thomaz et al. (2009)</td>
</tr>
<tr>
<td>Anaemia</td>
<td><em>Pagrus auratus</em></td>
<td>Increase</td>
<td>Cook et al. (2011)</td>
</tr>
</tbody>
</table>

Abbreviations: $P_{\text{CO}_2}$, partial pressure of carbon dioxide; $P_{\text{crit}}$, critical oxygen level.
Unlike intra-species $P_{\text{crit}}$, there is no apparent relationship between temperature and inter-species $P_{\text{crit}}$ (Fig. 3), suggesting that evolution may have nullified the thermal sensitivity of hypoxia tolerance across species. It has been shown that the difference in RMR between a typical cold-water and warm-water fish is less than expected, given the thermal sensitivity of RMR within individual species (intra-species median $Q_{10} = 2.4$; Clarke and Johnston, 1999). In addition, gill surface area appears to scale in a linear manner with metabolic rate, implying that natural selection equips fishes with the oxygen extraction capacity required to match demand at higher temperatures (Nilsson and Östlund-Nilsson, 2008). Selective pressures for small gills, such as the osmorespiratory compromise (Nilsson, 1986; Gonzalez and McDonald, 1992; Urbina and Glover, 2015), gill parasites and risks associated with gill injury, are likely to limit respiratory surface area so that oxygen extraction capacity does not exceed that required by a particular species for survival in its natural range (Nilsson, 2007). Thus, generalizations regarding hypoxia tolerance across temperatures cannot be established firmly at the inter-species level.

Although salinity has long been recognized as a key environmental factor, studies evaluating the effects of salinity on $P_{\text{crit}}$ are scarce. A previous study in the euryhaline sheephead minnow (Ciprinodon variegatus), acclimated to salinities from freshwater (0 PSU) to hypersaline waters (100 PSU), showed a marked effect on $P_{\text{crit}}$ (Haney and Nordlie 1997) as environmental salinity rose. Inter-specific comparisons in the database agree with this previous intra-specific finding; that is, salinity had a significant influence on $P_{\text{crit}}$, whereby freshwater

### Table 4: Results of the stepwise linear regression analysis where salinity, body mass, routine metabolic rate (RMR) and temperature had zero-order $r$ correlations with $P_{\text{crit}}$ ($P < 0.05$) and with each other, where values were reported

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salinity (psu)</th>
<th>Temperature (°C)</th>
<th>Body mass (kg)</th>
<th>RMR (mg O$_2$ l$^{-1}$)</th>
<th>$P_{\text{crit}}$ (kPa)</th>
<th>$\beta$</th>
<th>$s^2$</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>0.317</td>
<td>−0.165</td>
<td>0.354</td>
<td>0.279</td>
<td>0.346</td>
<td>0.099</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.366</td>
<td>−0.141</td>
<td>−0.166</td>
<td>0.166</td>
<td>−0.314</td>
<td>0.081</td>
<td>−0.083</td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>−0.166</td>
<td>0.166</td>
<td>0.242</td>
<td>0.056</td>
<td>1.931</td>
<td>0.032</td>
<td>1.931</td>
<td></td>
</tr>
<tr>
<td>RMR</td>
<td>0.17</td>
<td>0.202</td>
<td>0.202</td>
<td>0.032</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23.54</td>
<td>23.1</td>
<td>0.1</td>
<td>323.84</td>
<td>5.4</td>
<td>Intercept = 4.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>15.36</td>
<td>7.9</td>
<td>0.3</td>
<td>434.04</td>
<td>2.1</td>
<td>Adjusted $r^2 = 0.195$</td>
<td>$P &lt; 0.001$</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: $P_{\text{crit}}$, critical oxygen level; RMR, routine metabolic rate. In the full model, all four variables were significant predictors of $P_{\text{crit}}$.

![Figure 3: The effect of temperature on inter-species critical oxygen level ($P_{\text{crit}}$, black dashed line) and intra-species $P_{\text{crit}}$ (continuous lines).](https://academic.oup.com/conphys/article-lookup/doi/10.1093/conphys/cow012)
Oncorhynchus mykiss; Ultsch, 1996

ences in of osmoregulation may explain, in part, some of these differences. To pump ions against a concentration gradient, increased costs to the action of ATP-driven pumps (i.e. Na+K+ATPase) in order to maintain internal homeostasis (efforts to keep constant the ions concentration is more relevant when considering species; Fig. 4A; P ≤ 0.001).

Figure 4: The effect of environmental salinity on inter-species critical oxygen level (Pcrit), expressed as partial pressure of oxygen (in kilopascals: A) and concentration of oxygen (in milligrams per litre; B). Data are shown as means + SEM, including data from 82 species in seawater and 50 species in freshwater. *Unpaired t-test, significant when P < 0.05.

As explained in earlier sections, any factor influencing the oxygen demand (metabolic rate) of an individual will be likely to have implications for its hypoxia tolerance. Given that teleost fishes must maintain a tight regulation of their internal salts and water composition (osmolality), as external salinity changes or becomes extreme, fishes must expend increased efforts to maintain internal homeostasis (Urbina and Glover, 2015). As many of the mechanisms of osmoregulation involve the action of ATP-driven pumps (i.e. Na+K+ATPase) in order to pump ions against a concentration gradient, increased costs of osmoregulation may explain, in part, some of these differences in Pcrit, at least for intra-specific comparisons. However, from our database (inter-specific), where more freshwater vs. seawater species comparison are presented, it is likely that other mechanisms are explaining differences in Pcrit. Given that seawater species separated million years ago from a freshwater ancestor (actinopterygians, 300–180 million years ago; Vega and Wiens, 2012), both fresh- and seawater species have adapted to their respective environments, and therefore, have also optimized their energy allocated to osmoregulation. Thus, the differences in Pcrit found in the present study, rather than being explained by energy-related/oxygen demand issues, could be associated with intrinsic characteristics of both media (freshwater vs. seawater). Owing to differences in size, organic matter load and stability, hypoxia is much more prevalent and common in freshwater than in seawater environments. As such, the driver for an enhanced hypoxia tolerance (lower Pcrit) could potentially explain the lower Pcrit found in freshwater species. A future phylogenetic analysis might contribute to test this hypothesis.

It is also worth noting that the difference found in Pcrit when presented as the partial pressure of oxygen (in kilopascals) was no longer found when Pcrit was calculated as the concentration (in milligrams per litre; Fig. 4B; P > 0.05). This could potentially highlight the importance of working with partial pressure, because this is what drives diffusion when considering gases. Alternatively, it could indicate that the oxygen concentration is more relevant when considering Pcrit values, because it determines the total amount of oxygen that is potentially available for diffusion as water flows over the gills, i.e. for the same oxygen uptake, salinity (through its effect on solubility) will have a bigger effect on the difference between inspired and expired PO2.

The biological processes that consume O2 also produce CO2; therefore, hypoxia and hypercarbia can often co-occur in aquatic environments (Ultsch, 1996; Cruz-Neto and Steffensen, 1997; Gilmour, 2001). Despite this, the interactive effect of environmental hypercarbia on hypoxia tolerance has been relatively understudied. As previously discussed (Table 3), there are conflicting reports within the available literature regarding to the effect of hypercarbia on the Pcrit of fishes (Cochran and Burnett, 1996; Cruz-Neto and Steffensen, 1997; McKenzie et al., 2003). The most likely mechanism by which hypercarbia could negatively impact hypoxia tolerance is through respiratory acidosis, leading to Bohr/Root effects on haemoglobin and reduced oxygen transport capacity (Jensen et al., 1993; Cruz-Neto and Steffensen, 1997). In this respect, hypercarbia is partly akin to the far more extreme acidosis that can occur in poorly buffered freshwater environments subjected to acid precipitation or drainage. Acidification of the surrounding water by addition of sulphuric acid (water pH range 7.4–4.0, at constant atmospheric PCO2) increases Pcrit in both rainbow trout (Oncorhynchus mykiss) and common carp (Cyprinus carpio; Ultsch et al., 1980). The time required to compensate for acid–base disturbance is highly variable among species (10–24 h during moderate hypercarbia; Melzer et al., 2009), and as such, the effect of hypercarbia and acidification on hypoxia tolerance is likely to be dependent.
largely on the species in question as well as the severity and duration of the hypercarbic or acid exposure (Jensen et al., 1993).

Exposure to toxicants, such as trace metal contamination, appears to reduce hypoxia tolerance in fishes. Specifically, exposure to elevated concentrations of copper (300 µg l⁻¹), mercury (150 µg l⁻¹) and silver (63 µg l⁻¹) have been demonstrated to increase Pₐₐ in various species (Table 3). The accumulation of toxic metals on the gills can stimulate the hypersecretion of mucus, which acts as a barrier to diffusion of external toxicants into the blood (McDonald and Wood, 1993; Wilson et al., 1994). In addition, some trace metals cause hyperplasia and hypertrophy of gill epithelia cells that result in the fusing and thickening of gill lamellae (Schiolden et al., 2007; Bilberg et al., 2010). As a consequence, respiratory function is compromised as a result of reduced diffusion area and increased diffusion distance (McDonald and Wood, 1993). The organophosphate insecticide trichlorfon has been shown to increase Pₐₐ by inducing similar changes in gill morphology as well as by promoting vasoconstriction that reduces lamellar blood flow in Nile tilapia (Oreochromis niloticus; Thomaz et al., 2009). These potential interactions between toxic contaminants and hypoxia in fishes are clearly of concern, particularly given that both stressors predominantly threaten freshwater and coastal marine systems and are therefore likely to coincide (McDonald and Wood, 1993; Diaz and Rosenberg, 2008).

Determinations of Pₐₐ in fishes have almost universally been made in unfed, post-absorptive individuals which, although providing a useful basis for comparing absolute hypoxia tolerance among species and individuals, does not fully account for the digestive state typical of fishes in their natural setting. An increase in oxygen uptake following ingestion of food, termed specific dynamic action (SDA), is required in order to meet the energetic costs associated with mechanical and biochemical digestion and assimilation (Jobling, 1993). Shortly after a meal, oxygen uptake in fish typically rises rapidly, reaching a peak two to three times higher than pre-fed levels within a few hours. The shape and duration of the SDA is highly dependent on the species in question as well as the meal size and composition (Secor, 2009). Measurements of Pₐₐ in fishes undergoing SDA have revealed significant increases in Pₐₐ compared with unfed control fishes, showing that increased aerobic demand during digestion has negative consequences for hypoxia tolerance (Table 3). In common perch (Perca fluviatilis) force-fed a 5% body mass ration, Pₐₐ at 20 h post-feeding was increased by 1.44-fold compared with sham-fed individuals (Thuy et al., 2010). Likewise, oscars (Astronotus ocellatus) fasted for 14 days showed a 1.6-fold lower Pₐₐ than individuals fed a daily 1% body mass ration up to 24 h prior to Pₐₐ determination (De Boeck et al., 2013). In such experiments, the requirement for a stable M₀, on which to base a determination of Pₐₐ means that measurements at peak SDA are not feasible, and thus, are likely to underestimate the effect of digestion on hypoxia tolerance (Thuy et al., 2010).

Several studies have investigated the effect of hypoxia acclimation on Pₐₐ (Table 3). Broadly, short-term physiological acclimation to hypoxia appears to be achieved through either enhanced O₂ extraction capacity or metabolic depression. In goldfish (Carassius auratus), 48 h of severe (0.63 kPa) hypoxia induced dramatic increases in both lamellar surface area and blood haemoglobin content, leading to a 49% reduction in Pₐₐ compared with individuals held at normoxia (Fu et al., 2011). Likewise, sailfin molly (Poeclia latipinnia) demonstrated increased haemoglobin and red blood cell concentrations and a reduced Pₐₐ following a 6 week exposure to severe hypoxia (Timmerman and Chapman, 2004a). Depression of RMR at normoxia and a subsequent reduction in Pₐₐ following chronic hypoxic exposure has been observed in the epaulette shark (H. ocellatum; Routley et al., 2002) and qingbo (Spinibarbus sinensis; Dan et al., 2014). However, some less hypoxia-tolerant species appear to demonstrate no physiological acclimation potential through hypoxic pre-conditioning. Daily exposure to 6 h of moderate hypoxia (10.5 kPa) for 33 days had no effect on Pₐₐ in post-smolt Atlantic salmon (S. salar; Remen et al., 2013). Additionally, chronic (6 week) moderate hypoxia produced no change in the Pₐₐ of juvenile snapping (P. auratus; Cook et al., 2013).

As hypoxia is likely to become an increasingly predominant aquatic perturbation in the future (Vaquer-Sunyer and Duarte, 2008; Keeling et al., 2009), the degree of physiological plasticity for hypoxia tolerance will be a key determinant of species performance. The potential for long-term and trans-generational hypoxia acclimation with respect to Pₐₐ has been largely unstudied. A trans-generational transfer of hypoxia tolerance has been demonstrated in zebrafish (Danio rerio) larvae after 2–4 weeks of parental hypoxia exposure, but this was based on determinations of time to loss of equilibrium (4 kPa O₂) rather than through measurement of Pₐₐ in post-smolt Atlantic salmon (S. salar; Burggren, 2012). Reardon and Chapman (2010) demonstrated a strong element of developmental plasticity in the Pₐₐ of the Egyptian mouthbrooder (Pseudocrenilabrus multicolar) when reared in hypoxic conditions. In addition, intraspecies population effects on Pₐₐ across habitats of differing O₂ regimes have been observed in several species, indicating that a high degree of phenotypic plasticity for Pₐₐ exists within these populations (Timmerman and Chapman, 2004b; Reardon and Chapman 2010; Fu et al., 2011).

Future applications

The comprehensive Pₐₐ database presented here provides the opportunity for a variety of further analyses with potential to offer fundamental physiological, as well as wider ecological, insights. For example, further analyses could involve comparing species Pₐₐ values within a phylogenetic context as a means to investigate the evolutionary relationships of hypoxia tolerance among species (Mandic et al., 2009). Likewise, combining species Pₐₐ data with information on the spatial distribution of populations would help to refine our understanding of the ecological relevance of Pₐₐ as a physiological trait. Such an analysis would be particularly relevant to predicting the
impacts on fish populations likely to arise from the increasingly widespread occurrence of hypoxic zones in aquatic environments around the globe (Friedrich et al., 2014). Given the variability found in the reported $P_{\text{crit}}$ for different fish species, it is likely that hypoxic events will have consequences that are very dependent on individual species. This highlights the complexity of predicting the effects that hypoxia will have at community and ecosystem levels, and the potential for hypoxia to have differential effects on predator-prey interactions, migrations, and ultimately, global fisheries.

The integration of the present database with similar databases of other widely measured physiological parameters in fishes should offer useful insights into interactions among traits. Such physiological data are of great value for improving the predictive capacity of models as an aid to the management and conservation of aquatic systems (Jørgensen et al., 2012; Cooke et al., 2013). Traits for which databases are currently under construction include the metabolic response to feeding (SDA), aerobic scope, growth rate and critical temperature. On completion, the combined data set will be made widely accessible via an online data repository facility, such as that provided by Dryad (http://datadryad.org/). Thus, it is envisaged that these data will prove to be a tangible link between the field of fish physiology and future studies of ecology, conservation and management.

**Supplementary material**

Supplementary material is available at *Conservation Physiology* online.

**Acknowledgements**

The authors wish to thank Silvana Birchennenough and Julian Metcalfe (Cefas, Lowestoft, UK) for their mentoring and encouragement in the creation of the $P_{\text{crit}}$ database.

**Funding**

This work was supported by a Natural Environment Research Council (NERC, UK) PhD studentship awarded to N.J.R./R.W.W. and NERC and Biotechnology and Biological Sciences Research Council (BBSRC) research grants (NE/H010041/1, BB/D005108/1 and BB/J00913X/1) awarded to R.W.W. The physiological database is a contribution of the European Union Cooperation in Science and Technology (COST) Action (FA1004) on the ‘Conservation Physiology of Marine Fishes’. The same EU COST Action supported this work as a Short Term Scientific Mission (STSM). For more information, see: http://fish-conservation.nu/.

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Research article


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