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REVIEW

Do aquatic ectotherms perform better under hypoxia after warm acclimation?

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ABSTRACT

Aquatic animals increasingly encounter environmental hypoxia due to climate-related warming and/or eutrophication. Although acute warming typically reduces performance under hypoxia, the ability of organisms to modulate hypoxic performance via thermal acclimation is less understood. Here, we review the literature and ask whether hypoxic performance of aquatic ectotherms improves following warm acclimation. Interpretation of thermal acclimation effects is limited by reliance on data from experiments that are not designed to directly test for beneficial or detrimental effects on hypoxic performance. Most studies have tested hypoxic responses exclusively at test temperatures matching organisms' acclimation temperatures, precluding the possibility of distinguishing between acclimation and acute thermal effects. Only a few studies have applied appropriate methodology to identify beneficial thermal acclimation effects on hypoxic performance, i.e. acclimation to different temperatures prior to determining hypoxic responses at standardised test temperatures. These studies reveal that acute warming predominantly impairs hypoxic performance, whereas warm acclimation tends to be either beneficial or have no effect. If this generalises, we predict that warm-acclimated individuals in some species should outperform non-acclimated individuals under hypoxia. However, acclimation seems to only partially offset acute warming effects; therefore, aquatic ectotherms will probably display overall reduced hypoxic performance in the long term. Drawing on the appropriate methodology, future studies can quantify the ability of organisms to modulate hypoxic performance via (reversible) thermal acclimation and unravel the underlying mechanisms. Testing whether developmental acclimation and multigenerational effects allow for a more complete compensation is essential to allow us to predict species' resilience to chronically warmer, hypoxic environments.

KEY WORDS: Dissolved oxygen, Critical oxygen tension, Thermal acclimation, OCLTT, Climate change, Metabolic rate

Introduction

Oxygen is essential to almost all animal life to support aerobic metabolism and meet the energetic costs of living (Semenza, 2007; Willmer et al., 2004). That said, many aquatic organisms are physiologically capable of inhabiting environments that naturally undergo variation in dissolved oxygen levels, such as diurnally hypoxic tidal pools and eutrophic ponds, and seasonally hypoxic estuaries and fjords; some species even make a living in the almost permanently hypoxic areas of the deep sea (Childress and Seibel, 1998; Harrison et al., 2018; Jenny et al., 2016; Levin et al., 2009; Spicer, 2014).

Despite natural fluctuations in dissolved oxygen levels, all aquatic systems, from shallow freshwater environments to the deep ocean, are affected by the increasing frequency of hypoxic events and prevalence of prolonged, more severe hypoxia (Breitburg et al., 2018; Diaz and Rosenberg, 2008; Jenny et al., 2016) – phenomena that can drive reductions in biodiversity (Diaz and Rosenberg, 2008). Key factors driving the increase in hypoxia in freshwater and coastal environments include not only anthropogenic nutrient input but also climate change (Laffoley and Baxter, 2019). Global warming could drive a general reduction in oxygen levels across aquatic ecosystems through a combination of enhanced stratification, disrupted oxygen circulation by current systems, reduced oxygen solubility and enhanced rates of biological oxygen consumption (Altieri and Gedan, 2015; Breitburg et al., 2018; Rabalais et al., 2009). In marine systems, elevated water temperatures over the past 50 years have already driven a ∼2% decline in ocean oxygen levels (Schmidtko et al., 2017). The Intergovernmental Panel on Climate Change (IPCC) predicts a future temperature rise of ∼2°C by 2100 (Pörtner et al., 2014), which may contribute to further predicted average decline in oceanic oxygen of up to ∼7% (Keeling et al., 2010). Although these changes may seem modest, any temperature-driven decline in average oxygen levels may exacerbate hypoxic episodes within ecosystems that already experience variability in oxygen levels (Breitburg et al., 2018; Rabalais et al., 2014).

The effects of temperature and low oxygen, singly and in combination, on the physiological performance of aquatic life have received considerable attention (Ern, 2019; Fry, 1971; Grieshaber et al., 1994; Hoefnagel and Verberk, 2015; Pörtner et al., 2017; Precht et al., 1973; Seibel and Deutsch, 2020). However, most studies of their interactive effects are relatively short term (McBryan et al., 2013). In experimental work, acute warming (i.e. increased test temperature, T_t; see Glossary) typically drives reductions in hypoxic performance (see Glossary) in fish and invertebrates, including reduced survival time and a lower capacity to maintain aerobic metabolism (raised P_{crit}; see Glossary) (Herreid, 1980; McBryan et al., 2013). Whether acclimation to warming could modify the physiological responses to hypoxia in a 'beneficial' manner is less well understood (Gunderson et al., 2016; Huey and Berrigan, 1996; McBryan et al., 2016). Yet, understanding these longer-term stressor interactions will be key to predicting how life will respond to an increasingly warm, oxygen-depleted aquatic environment.

It has long been recognised that prolonged exposure to elevated temperatures may result in thermal acclimation, i.e. physiological changes that alter the way organisms respond to temperature (Prosser, 1973). Thermal acclimation has been studied extensively under normoxic conditions by both thermal and evolutionary biologists, and these studies have demonstrated that acclimation needs to be considered when assessing the consequences of

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Glossary

Acclimation temperature (T_{acc})
Temperature at which individuals are incubated for an extended period prior to experimentation.

Aerobic scope (AS)
The difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR). Can be measured as absolute aerobic scope (AAS=MMR−SMR) or factorial aerobic scope (FAS=MMR/SMR).

Hypoxic performance
Any physiological metric/indicator of an individual's capacity to deal with hypoxia, e.g. metabolic performance (critical partial pressure, P_{crit}; regulation value, R) or tolerance (loss of equilibrium, LOE; survival).

Loss of equilibrium (LOE)
The inability of an organism to maintain an upright position within the water column. The P_{O_2} at LOE and/or time to LOE is used as a measure of hypoxia tolerance in fish.

Maximum metabolic rate (MMR)
The maximal oxygen consumption ($\dot{M}_{O_2}$) of an organism.

P_{crit}
Critical partial pressure of oxygen/critical oxygen tension below which SMR can no longer be sustained and individuals typically resort to anaerobiosis and metabolic suppression.

$P_{crit,max}$
Critical partial pressure of oxygen below which MMR can no longer be sustained.

Standard metabolic rate (SMR)
The oxygen consumption ($\dot{M}_{O_2}$) of a post-absorptive organism where activity is reduced as much as possible.

Regulation index (RI)
The area encompassed by an individual's $\dot{M}_{O_2}$–P_{O_2} curve and derived oxyconformity line as a proportion of the area encompassed by this oxyconformity line and a hypothetical 'perfect' oxyregulatory response, i.e. where the individual shows no change in SMR over a range of oxygen tensions.

Regulation values (R)
The entire area under the $\dot{M}_{O_2}$–P_{O_2} curve as a proportion of the hypothetical response of an individual that shows no change in SMR over a range of oxygen tensions.

Test temperature (T_t)
The acute test temperature at which responses are measured.

environmental warming (Angiletta, 2009; Precht et al., 1973; Prosser, 1973; Schulte et al., 2011; Seebacher et al., 2015; Somero, 2010). It is also essential to understand what role thermal acclimation will play in determining hypoxic performance in a warming world (McBryan et al., 2013). Broadly, effects of thermal acclimation on hypoxic performance could arise through shared physiological mechanisms that underpin responses to warming and hypoxia. This makes metabolism, or effects on oxygen supply and demand or anaerobic capacity, a promising avenue to explore (Fry, 1971; Harrison et al., 2018; Herreid, 1980; Kielland et al., 2019; McBryan et al., 2013; Pörtner, 2010; Seibel and Deutsch, 2020; Spicer, 2014).

Therefore, in this Review, we ask whether warm acclimation is beneficial for hypoxic performance in aquatic ectotherms. We first explain the methodological framework to test for beneficial acclimation, before comparing hypoxic performance at ambient and warm temperatures between (warm-) acclimated and non-acclimated individuals. We use this overview of the literature to answer our question and highlight directions for future research.

Testing for beneficial effects of thermal acclimation on hypoxic performance

Beneficial (or detrimental) acclimation can be identified by comparing the physiological responses between individuals incubated at different temperatures (acclimation temperature, T_{acc}; see Glossary) for a given period at standardised acute T_t using a well-established methodological framework (Huey and Berrigan, 1996; Huey et al., 1999; Precht et al., 1973; Prosser, 1973). If thermal acclimation takes place, the thermal sensitivity of a given physiological process is altered (i.e. T_{acc} modifies the effect of T_t on a physiological response) (Angiletta, 2009; Precht et al., 1973; Prosser, 1973; Schulte et al., 2011). T_{acc} can increase, have no effect or compensate (partially, fully or overcompensate) for T_t effects on a physiological trait (Fig. 1A), but T_{acc} effects may not be uniform across the thermal range because of interactions between T_{acc} and T_t (Cossins and Bowler, 1987; Precht et al., 1973).

In the context of hypoxic responses, identifying beneficial effects of warm acclimation would involve exposure of individuals to multiple T_{acc} before hypoxic responses are then assayed at one or more standardised T_t (Fig. 1B). We refer to those individuals exposed to increased T_{acc} as (warm) 'acclimated' (Fig. 1C, red line) with the caveat that, for some species, being kept at increased T_{acc} does not necessarily guarantee acclimation to that T_{acc} (Fig. 1A, 'none'). We compare responses of 'acclimated' individuals against those that have not been exposed to increased T_{acc}. The latter are referred to as 'non-acclimated' for brevity (Fig. 1C, blue line), but may represent the control group or those individuals kept at lower T_{acc}. Because of a paucity of data on responses to chronic hypoxia, when referring to 'acclimation' throughout this Review, we refer only to temperature and not to hypoxic acclimation. We discuss only the consequences of thermal acclimation for measures of physiological performance made under short-term hypoxic exposure, concentrating on oxyregulation of aerobic metabolism [specifically P_{crit} of standard metabolic rate (SMR; see Glossary) and regulation values (R; see Glossary)] and hypoxia tolerance [such as loss of equilibrium (LOE; see Glossary) or survival], for which there are sufficient data to compare responses across the various combinations of hypoxia, acute warming and (thermal) acclimation.

Using this methodological framework (Fig. 1C), we begin by reviewing hypoxic performance of non-acclimated individuals at ambient temperature (Fig. 1Ci). Essentially, this group represents the responses to hypoxia in isolation. Next, hypoxic performance of non-acclimated individuals exposed to acute warming is examined (Fig. 1Ci versus ii). As temperature increases oxygen demand, and hypoxia decreases oxygen supply, the general prediction is for poorer hypoxic performance following an acute temperature increase (Fig. 1Cii performs worse than i). We then consider studies that investigate how acclimated individuals respond to subsequent hypoxia. Testing for beneficial effects of thermal acclimation on hypoxic performance requires comparison of responses of acclimated and non-acclimated individuals at standardised T_t (Fig. 1Ci versus iii and/or ii versus iv). However, most studies were not designed to address this aim and have measured hypoxic responses solely at the respective temperature to which individuals have been acclimated (Fig. 1Ci versus iv). We review these studies before critically analysing other studies, which have directly identified beneficial/detrimental effects (Fig. 1C, if acclimation is beneficial, iii should outperform i and/or iv should outperform ii). Finally, having tested the predictions presented in Fig. 1C, we discuss the extent to which hypoxic performance will be improved or impaired via acclimation in a chronically warmer, more hypoxic environment.

Hypoxic performance of non-acclimated individuals

Non-acclimated individuals at ambient temperature

Numerous studies have investigated the responses to hypoxia in isolation (Fig. 1Ci), particularly the capacity of individuals to

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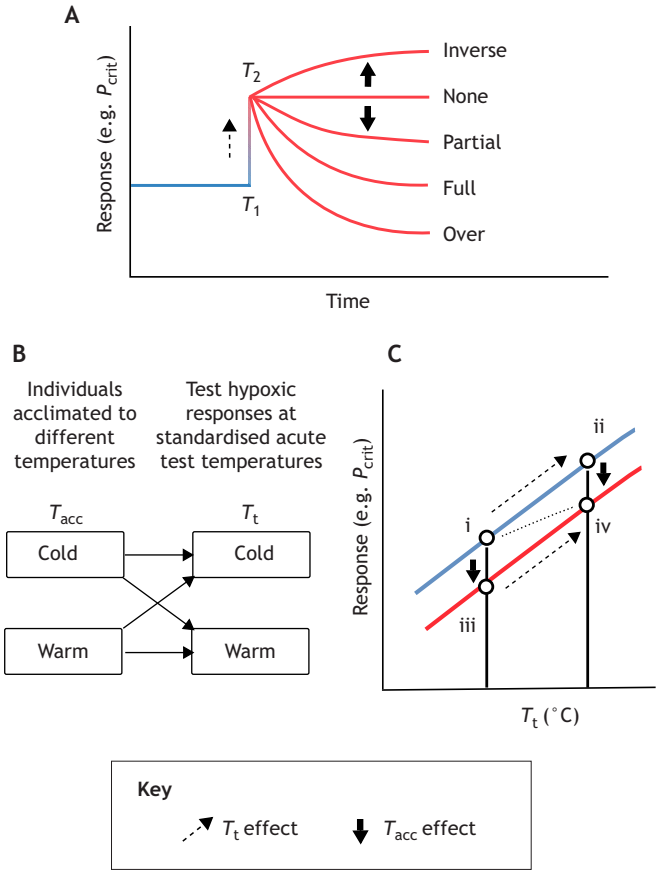

Fig. 1. Testing for beneficial effects of thermal acclimation on hypoxic performance. (A) Following an acute temperature increase (T_1 to T_2, dashed arrow), individuals may be able to acclimate a physiological response upon prolonged exposure to T_2 (e.g. P_{crit}) but to differing degrees (Huey and Berrigan, 1996). Acclimation effects (indicated by solid arrows) may either increase ('inverse') or compensate for ('partial', 'full' or 'overcompensated') the effects of the acute temperature increase. Individuals may also display no ability to acclimate a physiological response to increased temperature ('none'). (B) Acclimation can be tested using classic methodology, where individuals acclimated to different temperatures (T_{acc}) have hypoxic responses measured at standardised test temperatures (T_t) (a 2×2 design is depicted for clarity but could include a range of different T_{acc} or T_t). (C) A thermal reaction norm displaying the effects of T_t and T_{acc} on a physiological response (e.g. P_{crit}). This facilitates comparison of hypoxic performance between non-acclimated (blue) and acclimated individuals (red) at each T_t, either ambient or warm T_t. The graph shows hypoxic performance measured in (i) non-acclimated individuals at ambient temperature; (ii) non-acclimated individuals exposed to warming; (iii) acclimated individuals at ambient temperature; and (iv) acclimated individuals exposed to warming. Note that this is a simplified diagram displaying a singular acclimation response (partial). A full thermal performance curve would vary markedly in its shape and slope depending upon the metric of performance and variation between individuals and species. T_{acc} effects may not be uniform across T_t. Whatever effects are elicited, whether they are beneficial or detrimental can be interpreted by comparing effects between T_{acc} at the T_t of interest.

sustain SMR under (commonly acutely) declining environmental partial pressures of oxygen (P_{O_2}) (Burnett and Stickle, 2001; Farrell and Richards, 2009; Grieshaber et al., 1994; Harrison et al., 2018; Herreid, 1980; Mangum and Van Winkle, 1973; McMahon, 2001; Spicer, 2016; Wu, 2002). In response to declining P_{O_2}, SMR can display a spectrum of responses, with most responses typically falling between (1) oxyconformity, where SMR declines linearly with decreasing P_{O_2}, and (2) oxyregulation, where SMR appears to remain independent of environmental P_{O_2}, via alterations to ventilation and/or circulation, down to a critical P_{O_2} value (P_{crit}) (Fig. 2A). Below P_{crit}, individuals display oxyconformity, and SMR declines with decreasing P_{O_2} (Grieshaber et al., 1994).

P_{crit} is viewed as a key indicator of hypoxia tolerance because exposure to levels of 'severe' hypoxia below P_{crit} results in time-limited survival (Boutilier and St-Pierre, 2000; Seibel, 2011). Below P_{crit}, several mechanisms may become important in prolonging survival, including (1) anaerobic capacity/'anaerobic scope' (Sørensen et al., 2014), which involves the availability of energy reserves and the capacity of glycolytic enzymes to produce sufficient ATP, (2) metabolic suppression through reductions in energetically costly cellular processes such as protein synthesis and ion pumping to reduce ATP demand, and (3) the ability to deal with cellular damage and toxic anaerobic end products (Boutilier and St-Pierre, 2000; Mandic et al., 2009; Sørensen et al., 2014; Speers-Roesch et al., 2013). The interpretation of P_{crit} and methodology used to define it is a continually evolving field (Reemeyer and Rees, 2019; Regan et al., 2019; Ultsch and Regan, 2019; Wood, 2018), and a recent meta-analysis offers a new interpretation of P_{crit} as a corollary of aerobic scope (AS; see Glossary) rather than an indicator of tolerance per se (Seibel and Deutsch, 2020). It has long been recognised that maximum metabolic rate (MMR; see Glossary), like SMR, may also become limited by hypoxia, but at a higher oxygen tension (termed $P_{crit,max}$; see Glossary) below which AS declines (Fry, 1971; Pörtner and Grieshaber, 1993) (Fig. 2A). However, the paucity of direct tests of the oxyregulation of MMR makes it difficult to quantify $P_{crit,max}$ and its thermal dependency in great detail; hence, whether oxygen limitation lowers AS at elevated temperatures is an area of ongoing debate (see Boxes 1 and 2).

Developments in the methodology to measure oxyregulatory capacity have occurred for species that display a degree of regulation but not a distinct P_{crit} (Alexander and McMahon, 2004; Mueller and Seymour, 2011; Wood, 2018). For these types of species where the oxygen consumption rate ($\dot{M}_{O_2}$) responds gradually to declining P_{O_2} with no distinct breakpoint, an interesting question exists of whether the notion of SMR, as a level of $\dot{M}_{O_2}$ of no excess costs, truly exists. Models such as the 'regulation values' (R) (Alexander and McMahon, 2004) or 'regulation index' (RI; see Glossary) (Mueller and Seymour, 2011) have been developed to attempt to quantify oxyregulatory capacity. The two methods use a broadly similar approach: the oxyregulatory ability is expressed as the calculated area below the $\dot{M}_{O_2}$–P_{O_2} curve for an individual as a proportion of the area that would be observed for a perfect oxyregulatory response. However, there are differences between the two models. RI quantifies oxyregulatory capacity based upon the area encompassed by a perfect oxyregulatory response and oxyconformity line (Mueller and Seymour, 2011). R makes no assumption that oxyconformity represents the lowest limit of oxyregulatory capacity under declining P_{O_2} (Alexander and McMahon, 2004). Interestingly, the R model has explicitly extended the range of possible metabolic responses to hypoxia, going beyond oxyconformity and allowing the characterisation of 'hypoxia-sensitive' individuals. Such individuals display a large decrease in SMR at a comparatively small P_{O_2} reduction, a response that remains largely unexamined (Alexander and McMahon, 2004; Leiva et al., 2018) (Fig. 2B). These types of methods seem to be particularly suited to those aquatic invertebrate species that display curvilinear or sigmoidal relationships between SMR and P_{O_2} (Alexander and McMahon, 2004; Mangum and Van Winkle, 1973; Spicer and Morley, 2019; Sutcliffe, 1984). R- or RI-type approaches have been criticised as they may not necessarily provide

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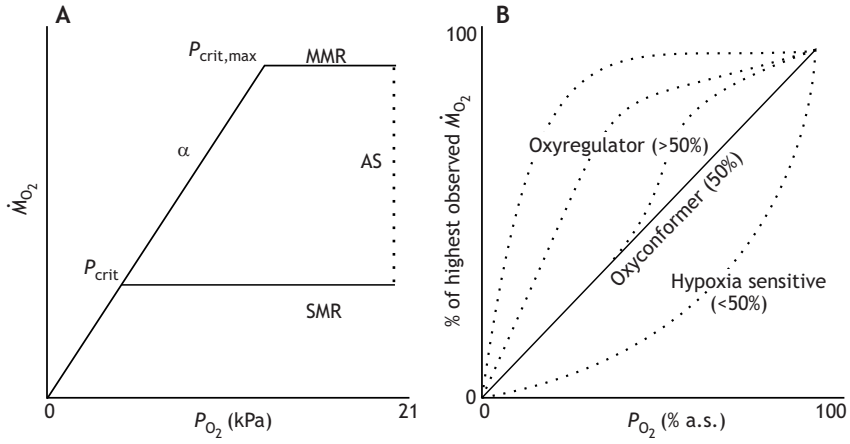

Fig. 2. Measures of aerobic metabolic regulation in response to declining P_{O_2}. (A) Oxygen consumption rate ($\dot{M}_{O_2}$) against partial pressure of oxygen (P_{O_2}). Critical oxygen tension (P_{crit}) is the most commonly used metric of hypoxic performance. Standard metabolic rate (SMR) can be sustained down to P_{crit} before a transition to anaerobic and/or hypometabolism occurs. Maximum metabolic rate (MMR) is less well maintained, and $P_{crit,max}$ occurs at much higher P_{O_2}. Aerobic scope (AS) represents the difference between MMR and SMR. α represents the oxygen-supply capacity (Seibel and Deutsch, 2020; Box 2). (B) Regulation values have been proposed to characterise the degree of oxyregulation displayed by different species. Regulation values equal the proportion represented by the area under the $\dot{M}_{O_2}$–P_{O_2} curve, relative to the area displayed by a perfect oxyregulator. $\dot{M}_{O_2}$ is standardised against the highest $\dot{M}_{O_2}$ observed regardless of where it occurs across the P_{O_2} range of 0–100% air saturation (% a.s.). Oxyregulators display values >50%, oxyconformity lies at 50% and hypoxia-sensitive individuals display values <50% (adapted from Leiva et al., 2018).

a clear threshold P_{O_2} (Regan et al., 2019), but this could make them more suitable for organisms that do not show a clear threshold (see above). In any case, the two methods will overlap somewhat in that individuals with lower P_{crit} will tend to have a greater area under the $\dot{M}_{O_2}$–P_{O_2} curve and thus greater oxyregulatory capacity (Regan et al., 2019).

Non-acclimated individuals exposed to warming

The responses of non-acclimated individuals to acute warming (Fig. 1Ci versus ii) are relatively well characterised, and typically include an increase in P_{crit} (Dupont-Prinet et al., 2013; Herreid, 1980) and a reduction in survival time under low oxygen (Semsar-kazerouni et al., 2020; Vaquer-Sunyer and Duarte, 2011). Classic models attribute the increase in P_{crit} to a rise in oxygen demand at higher temperatures, which shifts the point at which SMR can still be sustained (P_{crit}) to a higher external P_{O_2} (Fry, 1971; Herreid, 1980). This increase in P_{crit} can be offset somewhat by temperature driving concomitant increases in oxygen supply capacity, resulting in stronger increases in SMR relative to P_{crit} (Fig. 3; Kielland et al., 2019; Seibel and Deutsch, 2020; Verberk et al., 2011). Several more-recent models such as the 'oxygen- and capacity-limited thermal tolerance' (OCLTT) hypothesis (Pörtner, 2010; Pörtner et al., 2017), 'oxygen- and temperature-limited metabolic niche framework' (Ern, 2019), and Seibel and Deutsch's (2020) model of oxygen-supply capacity have expanded on these classic models, integrating other important metabolic traits such as MMR, $P_{crit,max}$ and AS (see Boxes 1 and 2). Here, we focus on P_{crit} of SMR, because this is what the majority of acclimation studies have measured. Irrespective of the precise model that predicts raised P_{crit} of SMR, not allowing for acclimation, aquatic organisms will probably perform worse under warming and hypoxia (Deutsch et al., 2020; Verberk et al., 2016a,b).

Hypoxic performance of acclimated individuals

Warm acclimation could be predicted to affect hypoxic responses such as P_{crit} by reducing the thermal sensitivity of oxygen demand (Seebacher et al., 2015) or increasing the capacity for oxygen supply (Sollid et al., 2005). Enhanced capacity for extracting and delivering oxygen could potentially be achieved by a number of mechanisms, such as increased respiratory surface area, ventilation rates or circulation rates or changes to the affinity for oxygen of respiratory pigments (Anttila et al., 2015; Hilton et al., 2008; McBryan et al., 2013; Sollid et al., 2005). Additionally, warm acclimation could be predicted to affect hypoxia tolerance by modulating anaerobic capacity, such as anaerobic enzyme activity and/or the ability to deal with toxic anaerobic end products (Matthews and McMahon, 1999; Seebacher et al., 2015).

However, most thermal acclimation–hypoxia studies conducted to date were not designed to explicitly address whether thermal acclimation is beneficial for hypoxic performance. Hypoxic responses have primarily been measured 'at different acclimation temperatures', i.e. responses are only measured at the respective temperature to which individuals have been acclimated (T_{acc}=T_t; Fig. 1Ci versus iv). Essentially, these types of study capture the outcome of a T_{acc}×T_t interaction without an indication of the relative contributions of T_{acc} or T_t, which would be required to explicitly test for beneficial or detrimental changes to performance (Havird et al., 2020). As these investigations (Fig. 1Ci versus iv) make up the majority of acclimation–hypoxia studies, here, we assess the degree to which they can inform whether acclimation is beneficial. We then consider those few studies where hypoxic responses have been directly compared between acclimated and non-acclimated individuals at standardised T_t (Fig. 1Ci versus iii and/or ii versus iv).

Hypoxic performance at different acclimation temperatures

A number of studies, primarily involving fish and crustaceans, have investigated acute hypoxic performance following chronic incubation (weeks to months) at different acclimation temperatures (T_{acc}) (Fig. 1Ci versus iv). These longer term studies are often carried out in the pursuit of greater ecological realism, e.g. investigating temperature differences that may occur in nature (Taylor et al., 1977; Barnes et al., 2011; Butler and Taylor, 1975; Collins et al., 2013; Schurmann and Steffensen, 1997). When testing hypoxic responses solely at the temperature to which

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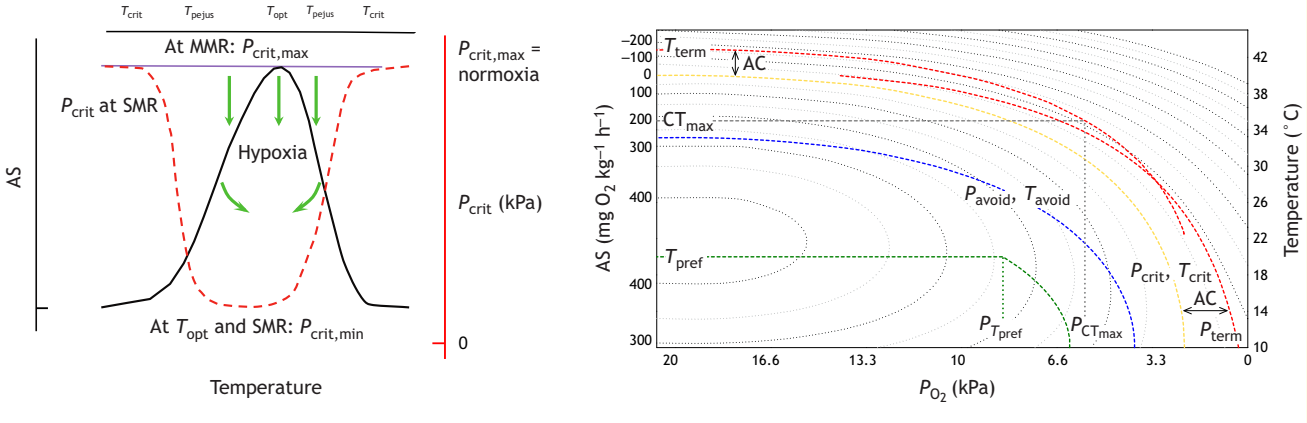
Box 1. Beyond P_{crit} of SMR: (i) OCLTT and metabolic niche framework

The 'oxygen- and capacity-limited thermal tolerance' (OCLTT) hypothesis (Pörtner, 2010) (left) suggests an optimal thermal range (T_{opt}) exists where aerobic performance (solid black line) is maximised. At both higher and lower temperatures, aerobic performance declines; these temperatures are referred to as 'pejus' temperatures (T_{pejus}). Oxygen limitation is hypothesised to cause a decline in maximum metabolic rate (MMR) and aerobic scope (AS), which approaches zero at critical temperatures (T_{crit}). Critical oxygen tension of SMR (P_{crit}; dashed red line) is proposed to mirror the pattern for AS, being lowest at T_{opt} ($P_{crit,min}$) and increasing at T_{pejus}. At T_{crit}, P_{crit} equals normoxia and beyond T_{crit}, individuals increasingly rely on anaerobic metabolism. Critical oxygen tension of MMR ($P_{crit,max}$) approximates normoxia across the thermal range. Few studies have focused on predictions from the OCLTT hypothesis at temperatures below T_{opt}, and there is little evidence for cold-induced oxygen limitation (Verberk et al., 2016a) or increasing P_{crit}. With warming, there is support that P_{crit} and AS vary in tandem along thermal clines, but P_{crit} can increase while absolute aerobic scope (AAS) is increasing or constant, as accounted for by recent models (Ern, 2019; Seibel and Deutsch, 2020). Studies so far demonstrate that, except for eelpout (Pörtner and Knust, 2007), P_{crit} tends not to reach normoxia at temperatures where individuals are alive (i.e. P_{crit} only reaches normoxia at T_{crit}>CT_{max}) (Seibel and Deutsch, 2020). Given the mixed evidence for OCLTT (Jutfelt et al., 2018; Pörtner et al., 2017; Verberk et al., 2016a), the 'oxygen- and temperature-limited metabolic niche framework' (Ern, 2019) has been proposed (right). Here, P_{crit}=T_{crit} (yellow line) at equivalent temperature and P_{O_2}. A zone of hypoxic insensitivity is assumed near normoxia above ∼19 kPa (i.e. we assume $P_{crit,max}$≈19 kPa). At temperatures >T_{crit} and when P_{O_2}<P_{crit}, survival becomes dependent upon anaerobic capacity (AC, black arrows) until terminal temperature or P_{O_2} (T_{term} or P_{term}, red line). For oxygen-limited species: CT_{max} (grey line)=T_{term} under normoxia, and thermal tolerance will decline with declining P_{O_2}. With increasing temperature, P_{crit} and AAS (dotted isopleths) rise in tandem until preferred temperature (T_{pref}, green line), after which P_{crit} keeps increasing, while AAS declines. P_{crit} approximates normoxia where AAS=0 at temperatures <T_{term}. For non-oxygen-limited species, CT_{max}<T_{term} under normoxia and thermal tolerance does not decrease with hypoxia until $P_{CT_{max}}$, the oxygen limit for thermal tolerance. Similar to oxygen-limited organisms, P_{crit} rises with increased temperature and approximates normoxia where AAS=0. However, P_{crit} approaches normoxia and AAS=0 at a temperature >CT_{max}. Thus, for non-oxygen-limited species, P_{crit} for an organism across its thermal range will never approximate normoxia. The framework also integrates behavioural responses by considering how P_{O_2} may limit T_{pref}, termed $P_{T_{pref}}$, and the possible causes of avoidance behaviour at sub-optimal temperature and P_{O_2} (T_{avoid} and P_{avoid}, blue line).



individuals are acclimated (T_{acc}=T_t), the majority of studies have identified raised P_{crit} associated with long-term incubation at a warm T_{acc} (Taylor et al., 1977; Barnes et al., 2011; Butler and Taylor, 1975; Collins et al., 2013; Schurmann and Steffensen, 1997; Rogers et al., 2016; Kielland et al., 2019). However, in a smaller number of species, there is potential for long-term warming and associated effects on oxygen supply and demand to lead to P_{crit} reaching a plateau. In such cases, P_{crit} does not increase with increased T_{acc}, but either remains stable (Fry and Hart, 1948; Sollid et al., 2005; Yamanaka et al., 2013) or decreases (Ultsch et al., 1978). This suggests that enhancements in oxygen-supply capacity following warm acclimation compensate for the increased oxygen demand at higher temperatures. In conclusion, these studies demonstrate that a complete recovery of hypoxic performance can occur when individuals are exposed to chronic warming, but only rarely.

Tests of beneficial acclimation reveal warm acclimation improves hypoxic performance in some species

Few studies follow the classic methodology (Huey et al., 1999; Precht et al., 1973; Prosser, 1973) of determining hypoxic responses at standardised T_t post-acclimation (Fig. 1Ci versus iii and/or ii versus iv), which would allow the direct elucidation of acclimation effects on hypoxia thresholds. To our knowledge, these studies are restricted to those in Table 1, and, in general, T_t and T_{acc} appear to differ in their effect on metabolic performance and hypoxia tolerance. This further corroborates the supposition that effects of thermal acclimation need to be taken into account when assessing how species will perform in warmer, hypoxic waters.

The effect of thermal acclimation on oxyregulation varies across species. In the triplefin fish *Bellapiscis lesleyae*, warm-acclimated individuals outperform non-acclimated individuals at raised T_t, displaying a lower P_{crit} (Hilton et al., 2008). The mechanism governing this reduction in P_{crit} was not determined but supply capacity is likely to be involved, as non-acclimated and acclimated individuals do not differ in SMR. However, warm acclimation has no effect on P_{crit} in the sister species *Bellapiscis medius* (Hilton et al., 2008) nor in the black sea bass *Centropristis striata* (Slesinger et al., 2019) or two tropical fish species, where increased T_t increases P_{crit} irrespective of T_{acc} (Nilsson et al., 2010; Slesinger et al., 2019). Notably, if the study on one of these two tropical fish species, *Ostorhinchus doederleini* (Nilsson et al., 2010), had only measured P_{crit} at T_{acc}=T_t, the results could have implied that individuals perform worse at warm T_{acc} (Fig. 4A). However, this study made the comparison between acclimated and non-acclimated individuals, demonstrating that raised P_{crit} was entirely attributable to T_t whereas T_{acc} had no significant effect (Fig. 4B).

In molluscs, there is less evidence for improvements of metabolic performance in acclimated individuals, based upon regulation

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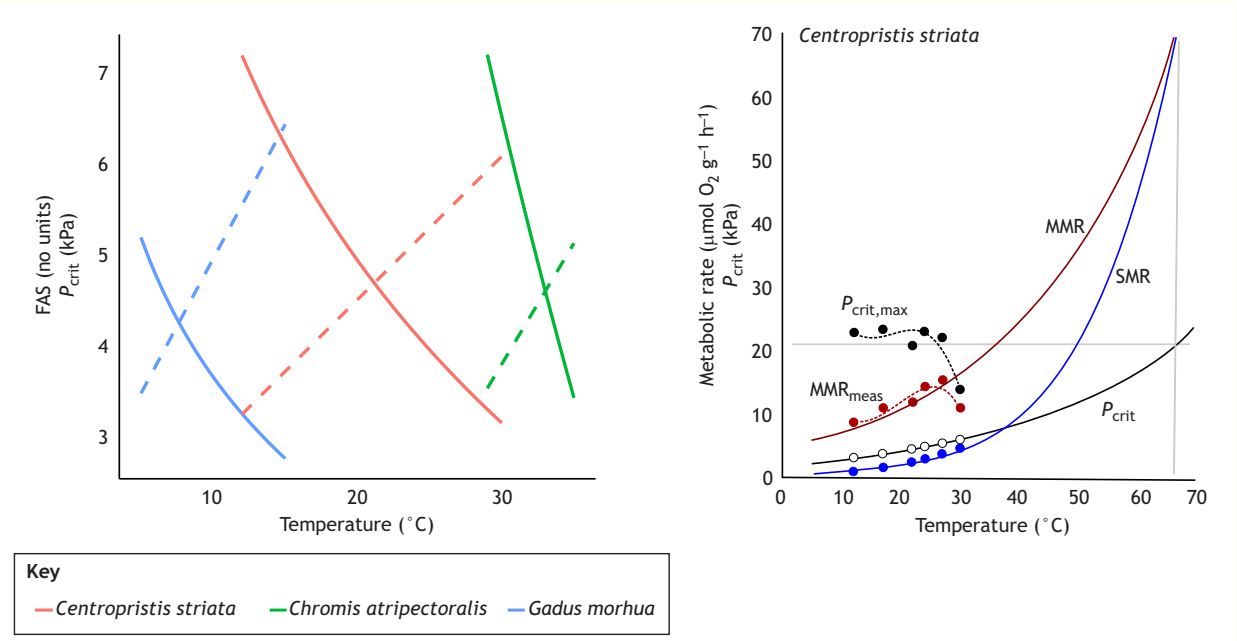
Box 2. Beyond P_{crit} of SMR: (ii) oxygen supply capacity model

Seibel and Deutsch (2020) propose a quantitative model based upon a meta-analysis of P_{crit}, SMR and MMR, with a view that oxygen-supply capacity has evolved to meet maximum demand. In their hypoxia model, they propose a novel metric, the oxygen supply capacity (α) (Fig. 2A), which is the rate at which metabolic rate increases with P_{O_2} below a critical oxygen tension. At a given temperature, α is constant so that SMR/P_{crit}=MMR/$P_{crit,max}$. $P_{crit,max}$ is modelled to be ~21 kPa for most species, except those that experience persistent hypoxia. From this, it follows that MMR should decline proportionally with P_{O_2} by 4.7% kPa^{-1} for normoxic species. Furthermore, factorial aerobic scope (FAS) should be inversely correlated with P_{crit} across species and temperatures, and this was supported by data on species where P_{crit} and FAS were measured (left), leading to the notion that P_{crit} may be an adaptation for AS. In *Centropristis striata* (right; Seibel and Deutsch, 2020; Slesinger et al., 2019), P_{crit} increases with temperature as a result of the faster rise in SMR compared with MMR, equalling normoxic oxygen tension when SMR has caught up with MMR. In *C. striata*, P_{crit} increases with temperature but would not reach normoxia at temperatures where individuals can still live. Similarly, extrapolation of the temperature coefficients for SMR and MMR suggests they would become equal at extremely high temperatures (>60°C) where individuals clearly cannot survive. The authors reinterpret declining MMR (MMR_{meas}) beyond a certain temperature as thermal limitation, rather than oxygen related, as organisms are modelled to still have a functional oxygen-supply capacity. Previous measurements also show declines in MMR and AAS, without an increase in P_{crit} (see Slesinger et al., 2019). The model opens up novel avenues of research, as its hypotheses are quantitative, making them testable/falsifiable. For example, $P_{crit,max}$ still awaits widespread direct measurement, as the authors note, but the notions that (1) $P_{crit,max}$ is constrained near normoxia regardless of temperature (for normoxic species) and that (2) $P_{crit,max}$ matches the prevailing environmental P_{O_2}, are both readily testable ideas.



values, with no reported beneficial effects of T_{acc} (Alexander and McMahon, 2004; Hicks and McMahon, 2002). Warm T_{acc} reduces oxyregulatory capacity (regulation values) across all T_t in the zebra mussel, *Dreissena polymorpha*, a response that may be detrimental in a chronically warming aquatic environment undergoing hypoxia (Alexander and McMahon, 2004). However, the authors suggested that a better oxyregulatory capacity following cold acclimation could be beneficial with regards to the particular ecology of the species, allowing it to overwinter under ice sheets, where chronic cold and hypoxia co-occur (Alexander and McMahon, 2004). It would be interesting to test whether variation across species in how warm acclimation affects oxyregulation is related to acclimation effects on SMR. None of the fish species tested so far appear to have the capacity to reduce SMR via acclimation (Hilton et al., 2008; Nilsson et al., 2010; Slesinger et al., 2019), something that might reduce P_{crit}.

In terms of hypoxia tolerance, there is evidence of beneficial effects of thermal acclimation for both fish and molluscs. Warm acclimation increases time to LOE in killifish (McBryan et al., 2016) and time to LOE in salmon (Anttila et al., 2015) compared with non-acclimated individuals. This phenomenon was associated with gill and cardiac remodelling in warm-acclimated killifish and salmon, respectively (Anttila et al., 2015; McBryan et al., 2016).

Hypoxia tolerance also improves with warm acclimation in the zebra mussel, *D. polymorpha* (Matthews and McMahon, 1999), and this perhaps provides the most convincing support for the adoption of a beneficial acclimation framework to understand acclimation effects on hypoxic responses. Zebra mussels were acclimated to three temperatures (T_{acc}=5, 15 and 25°C) and exposed to severe hypoxia at three acute test temperatures (T_t=5, 15 and 25°C) in a fully factorial experimental design (Matthews and McMahon, 1999). This species experiences these temperatures frequently under normoxic conditions and can survive up to 45°C. Survival time under hypoxia decreased with increasing temperature when individuals were tested at their acclimation temperature (T_{acc}=T_t) (Fig. 4C). However, when comparing individuals acclimated to different T_{acc} at a given T_t, it was clear that warm acclimation was beneficial, leading to an increase in hypoxic survival time compared with that of cold-acclimated individuals (for example, when comparing T_{acc}=25°C against T_{acc}=5°C: warm-acclimated individuals survived 1.9 times longer at T_t=15°C and 1.6 times longer at T_t=25°C). No effect of acclimation on survival under hypoxia was observed at T_t=5°C and this was attributed to potential

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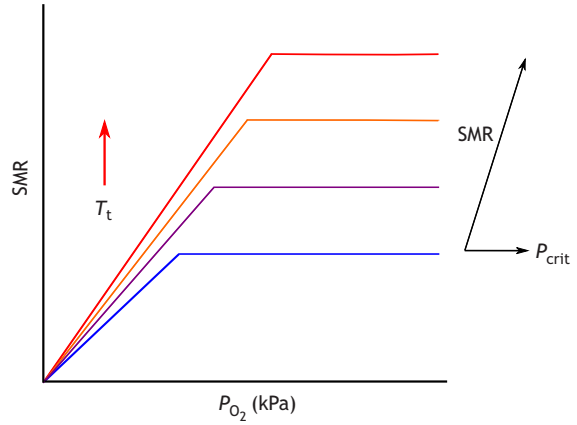

Fig. 3. Temperature sensitivity of P_{crit}. Increased T_t (red arrow) typically increases SMR and leads to an increase in P_{crit}. The increase in P_{crit} is offset by temperature-driven increases in oxygen-supply capacity and, as a result, the lines do not overlap and the ascending part becomes steeper (indicative of a higher oxygen-supply capacity) at higher temperatures (Kielland et al., 2019; Seibel and Deutsch, 2020). Colours indicate temperatures increasing from cold (blue) to warm (red).

re-acclimation of individuals to cold T_t given the long survival time of ∼40 days (Fig. 4D). The underpinning mechanism is unclear but it was suggested that individuals from warm T_{acc} have reduced energy demands that could be sustained with lower rates of anaerobic metabolism and lower concomitant production of harmful end products (Matthews and McMahon, 1999).

This study exemplifies how the effects of warm T_{acc} could be misinterpreted as being detrimental when acclimation actually buffers against the detrimental effects of acute warming. The interaction between T_{acc} and T_t in this species, where improvements in hypoxia tolerance were observed at some but not all T_t, may also have ecological significance. Organisms may not always experience hypoxia at the temperature to which they have been acclimated. The responses to hypoxia at any given time in nature may be a complex combination of current thermal conditions (T_t) and previous thermal history (T_{acc}), which has rarely been taken into account.

Will thermal acclimation prevent reductions in hypoxic performance of aquatic ectotherms in a chronically warming world?

Given the paucity of data and studies using appropriate experimental designs, it is currently not possible to draw definitive conclusions on the effects of thermal acclimation on physiological responses to hypoxia or the extent to which acclimation can compensate for effects of raised acute temperatures. The studies reviewed here support the idea that some fish species show beneficial effects of warm acclimation on oxyregulatory capacity and hypoxia tolerance. However, no crustacean studies to date have used the appropriate methodological framework to explicitly test for beneficial/detrimental effects of thermal acclimation on hypoxic performance. For molluscs, there is weak evidence for beneficial acclimation of metabolic performance and mixed evidence for hypoxia tolerance (Table 1).

Improvements to hypoxia tolerance due to increased T_{acc} (i.e. beneficial acclimation) may be mediated through changes to enhance oxygen uptake and circulation or to reduce the thermal sensitivity of oxygen demand. Alternatively, beneficial acclimation may increase the capacity for anaerobic metabolism or act through other, as yet unknown, mechanisms. Which of the above mechanisms are important awaits empirical testing (Matthews and McMahon,

Table 1. Studies investigating beneficial acclimation of hypoxic performance

Species	Performance	T_{acc} (°C)	Standard T_t (°C)	Warm T_t effect?	Warm T_{acc} effect?	Interaction?	Response type (see Fig. 1A)	Reference
Fish								
Bellapiscis lesleyae	P_{crit}	15, 20	25	–	+	n.a.	Partial	Hilton et al. (2008)
Bellapiscis medius	P_{crit}	15, 20	25	–	No effect	n.a.	None	Hilton et al. (2008)
Fundulus heteroclitus	Time to LOE	15, 20, 25, 30	20, 25, 30	–	+	No	Partial	McBryan et al. (2016)
Pomacentrus moluccensis	P_{crit}	29, 32	32	–	No effect	n.a.	None	Nilsson et al. (2010)
Ostorhinchus doederleini	P_{crit}	29, 32	32	–	No effect	n.a.	None	Nilsson et al. (2010)
Centropristis striata	P_{crit}	22, 30	30	–	No effect	n.a.	None	Slesinger et al. (2019)
Salmo salar	Time to LOE	7.7, 14.9	12	n.a.	+	n.a	n.a.	*Anttila et al. (2015)
Salvelinus alpinus	Critical P_{O_2} for LOE	7.7, 14.9	12	n.a.	No effect	n.a.	n.a.	*Anttila et al. (2015)
Mollusca								
Dreissena polymorpha	Survival time	5, 15, 25	5, 15, 25	–	+ at T_t=15, 25 No effect at T_t=5	Yes	Partial	Matthews and McMahon (1999)
Dreissena polymorpha	Regulation values	5, 15, 25	5, 15, 25	+	–	No	Over-compensated	Alexander and McMahon (2004)
Corbicula fluminea	Survival time	5, 15, 25	5, 25	–	No effect	No	None	Matthews and McMahon (1999)
Perna perna	Regulation values	15, 20, 25	10, 15, 20, 25, 30	+	No effect	No	None	Hicks and McMahon (2002)

T_{acc}, acclimation temperature; T_t, test temperature; P_{crit}, critical partial pressure of oxygen for standard metabolic rate; LOE, loss of equilibrium; n.a., not applicable. *Focusing only upon prior thermal acclimation conducted under normoxic conditions before hypoxia tolerance was determined.

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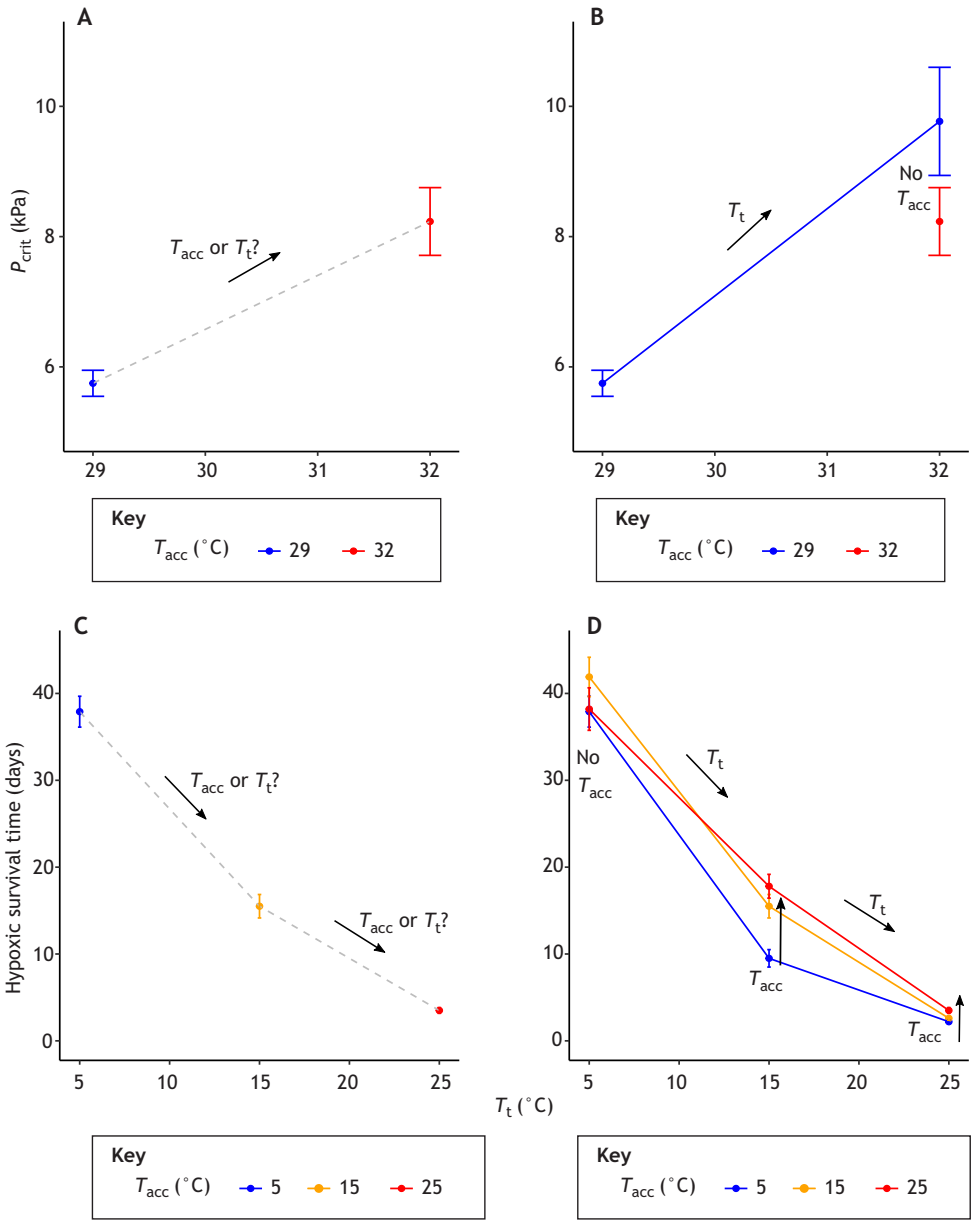

Fig. 4. Effects of temperature acclimation (T_{acc}) and acute test temperature (T_t) on hypoxic responses. (A,B) Individuals of the tropical fish *Ostorhinchus doederleini* were acclimated to either T_{acc}=29 or 32°C for 7 days prior to determination of P_{crit} under acutely declining oxygen tensions at T_t=29 or 32°C (mean±s.e.m.) (data from Nilsson et al., 2010). (A) When T_{acc}=T_t, it appears that warm acclimation temperatures are detrimental to P_{crit} but this is not the case. (B) Using a classic methodological approach, the effects of T_{acc} can be disentangled from those of T_t. Increased T_t is detrimental and raises P_{crit} in non-acclimated individuals. Comparing responses within T_t=32°C between different T_{acc} showed warm acclimation was not detrimental and had no significant effect on P_{crit}. (C,D) Hypoxia tolerance in the zebra mussel, *Dreissena polymorpha*. Individuals acclimated to T_{acc}=5, 15 and 25°C were exposed to severe hypoxia (<3% a.s.) at T_t=5, 15 and 25°C in a fully factorial experiment and survival time was measured (mean±s.e.m.). (C) When T_{acc}=T_t, it appears that warm acclimation temperatures are detrimental to hypoxia tolerance. (D) Increased T_t is detrimental to hypoxic survival in individuals acclimated to the same T_{acc}. Comparing responses within T_t between different T_{acc} shows that warm acclimation is beneficial and significantly increases hypoxic survival time at T_t=15 and 25°C (1.9- and 1.6-fold increase in survival time, respectively) but has no significant effect at T_t=5°C (black arrows indicate direction of T_{acc} and T_t effects) (data from Matthews and McMahon, 1999).

1999). Despite a relatively good understanding of the physiological and biochemical mechanisms of acclimation under normoxic conditions (Prosser, 1973; Seebacher et al., 2015), empirical evidence is still required to understand how these mechanisms subsequently affect performance under hypoxia when tested at standardised T_t post-acclimation. Such an understanding will aid prediction of whether warm acclimation will positively or negatively affect hypoxic performance for a given species.

It is clear that thermal acclimation can be beneficial for hypoxic performance in some species (Fig. 1Civ outperforms ii), in contrast to the relatively consistent detrimental effects of acute warming (such as raised P_{crit} and reduced tolerance) on non-acclimated individuals (Herreid, 1980; Vaquer-Sunyer and Duarte, 2011) (Fig. 1Cii performs worse than i). The studies that have only investigated responses where T_{acc}=T_t (Fig. 1Ci versus iv) do not facilitate direct identification of beneficial acclimation, but highlight that P_{crit} remains raised in many cases under chronic warming. Thus, reversible acclimation may only partially compensate for the detrimental effects of raised acute thermal conditions on hypoxic performance (Fig. 1Civ still performs worse than i), mirroring normoxic conditions, where physiological rates are also typically only partially compensated by acclimation (Seebacher et al., 2015).

Future directions

More studies applying the beneficial acclimation framework to hypoxic responses are needed. Studies where T_{acc}=T_t should use the term ‘acclimation temperature’ with caution, as the effects of acclimation cannot be distinguished without non-acclimated individuals for comparison (Havird et al., 2020). We suggest that studies applying this type of design instead refer only to ‘measurement temperature’. From the few beneficial acclimation studies that are available so far, there appears to be considerable variation between species in their capacity to thermally acclimate their hypoxic performance, which may have significant fitness implications in determining so-called ‘winners’ and ‘losers’ (Somero, 2010). Future studies need to investigate a greater number of species in order to identify the sources of this variation, be it methodological (e.g. duration of acclimation,

duration of P_{crit} experiments), biological (e.g. mass effects on the speed and extent of acclimation, capacity to modulate oxygen supply via ventilation and circulation, oxygen demand, anaerobic capacity) or ecological (e.g. freshwater versus marine, temperature and oxygen levels experienced in the wild, latitude). In addition, future studies would preferably measure not only hypoxic performance but also putative mechanisms by which thermal acclimation can improve hypoxic performance (for example, does acclimation lower oxygen demand or increase the capacity for oxygen supply or anaerobic metabolism?). Finally, in this Review, we have focused on reversible acclimation, which shows partial compensation, whereas developmental acclimation and transgenerational effects could allow for a more complete compensation. Understanding the physiological diversity, the mechanisms and time scales involved is essential if we wish to be able to assess the vulnerability of aquatic life to both predicted expansions in hypoxic regions under future climate change (Breitburg et al., 2018) and the widespread deoxygenation that is predicted during the Anthropocene (Laffoley and Baxter, 2019).

Conclusions

There is a reasonably good understanding of the hypoxic responses of non-acclimated individuals at ambient temperature. In non-acclimated individuals, the effects of acute warming are fairly consistent, tending to raise P_{crit} and reduce the hypoxia tolerance of many organisms (Herreid, 1980). However, the effects of warm acclimation on performance under hypoxic conditions remain unclear because of a paucity of experimental work with an appropriate experimental design. From the limited evidence to date, acclimation to increased temperature may partially improve the hypoxic performance of some, but not all, species. Drawing on the appropriate methodology, future studies can quantify the ability of organisms to modulate hypoxic performance via (reversible) thermal acclimation and unravel the underlying mechanisms. Thermal acclimation needs to be considered if we are ever to accurately predict species' performance in a warmer, hypoxic world.

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Competing interests

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