Prevalence and mechanisms of environmental hyperoxia-induced thermal tolerance in fishes

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Recent evidence has suggested environmental hyperoxia (O₂ supersaturation) can boost cardiorespiratory performance in aquatic ectotherms, thereby increasing resilience to extreme heat waves associated with climate change. Here, using rainbow trout (Oncorhynchus mykiss) as a model species, we analysed whether improved cardiorespiratory performance can explain the increased thermal tolerance of fish in hyperoxia (200% air saturation). Moreover, we collated available literature data to assess the prevalence and magnitude of hyperoxia-induced thermal tolerance across fish species. During acute warming, O₂ consumption rate was substantially elevated under hyperoxia relative to normoxia beyond 23°C. This was partly driven by higher cardiac output resulting from improved cardiac contractility. Notably, hyperoxia mitigated the rise in plasma lactate at temperatures approaching upper limits and elevated the critical thermal maximum (+0.87°C). Together, these findings show, at least in rainbow trout, that hyperoxia-induced thermal tolerance results from expanded tissue O₂ supply capacity driven by enhanced cardiac performance. We show 50% of the fishes so far examined have increased critical thermal limits in hyperoxia (range: 0.4–1.8°C). This finding indicates environmental hyperoxia could improve the ability of a large number of fishes to cope with extreme acute warming, thereby increasing resilience to extreme heat wave events resulting from climate change.

1. Introduction

Fish living in shallow aquatic habitats, particularly those that are fully or partially enclosed (e.g. shallow lakes, ponds, streams, estuaries and intertidal rock pools), are regularly exposed to acute warming events. The most extreme of such events occur during heat wave weather conditions and are typically characterized by a daily cycle where water temperature ramps up to a peak over several hours during the day and then cools overnight or on the incoming tide. Although generally considered thermally tolerant, fishes occupying habitats where such acute thermal ramping occurs are under the threat of increasingly frequent and more extreme heatwave events due to climate change [1–3]. Compounding the problem is that behavioural avoidance of high temperatures (e.g. moving to deeper, cooler water) may not be possible in these habitats. An important feature of these environments, however, is that they often support high densities of photosynthetic aquatic plants, algae and seaweeds. Thus, when extreme high temperatures arise (i.e. on hot and sunny days), O₂ levels are likely to become hyperoxic (supersaturated with O₂) due to high rates of photosynthesis [4–6]. Indeed, in a recent review, we identified a number of examples of co-occurring acute warming and hyperoxia in shallow aquatic habitats that would support fish populations [7]. Moreover, a thorough assessment of water oxygenation and temperature in shallow Black
Sea habitats demonstrated the highest water temperatures frequently co-occur with hyperoxia [8]. Although available research is limited, there is increasing recognition that naturally occurring hyperoxia could provide an 'ecological refuge' improving thermal tolerance of fish and other aquatic ectotherms [7–10].

Beyond its ecological relevance, a physiologically based theoretical hypothesis as to why hyperoxia may affect warming tolerance in fish has been proposed. The oxygen and capacity limited thermal tolerance hypothesis places an inability of the cardiorespiratory system to meet tissue O2 demand at high temperatures as the central pillar dictating the upper thermal tolerance limits of aquatic ectotherms [11,12]. At critical thermal limits, tissue O2 demand is proposed to exceed tissue O2 supply, leading to functional tissue hypoxia and time-limited survival dependent on anaerobic metabolism [12,13]. Tissue O2 supply is commonly assessed as the rate of O2 consumption (ṀO2) which, according to the Fick principle, is a product of cardiac output (the rate of blood flow to the tissues) multiplied by the arterial–venous O2 content difference (tissue O2 extraction from the blood (A-V O2 content difference)) [14]. In fish, it is proposed that limitations of maximal cardiac output primarily dictate ceilings of tissue O2 supply capacity during warming exposure [15,16]. A prediction stemming from this is that environmental conditions that increase maximal cardiac output during warming will also improve tissue O2 supply capacity and may lead to better thermal tolerance. Hyperoxia is one such environmental condition that facilitates improved tissue O2 supply (i.e. higher ṀO2) at temperatures approaching critical thermal limits in fish [9,17], and there is evidence this is driven by increased cardiac output [18]. While this may explain why hyperoxia improves acute thermal tolerance in some fish [8], a direct link between increased tissue O2 supply capacity, higher cardiac output, mitigation of anaerobiosis and improved upper thermal tolerance is yet to be demonstrated.

In the first part of this study, using rainbow trout (Oncorhynchus mykiss) as a model species, we aimed to establish the mechanistic basis of a putative improvement of acute upper thermal limits of fish in hyperoxia. We predicted that thermal tolerance improve with hyperoxia, this would be associated with greater scope to increase ṀO2 during thermal ramping and a consequent mitigation of anaerobiosis. While environmental hyperoxia increases arterial O2 partial pressure (PaO2) in fish [7], haemoglobin is normally fully saturated in normoxia [19], theoretically leaving little scope for environmental hyperoxia to increase arterial O2 content further. Moreover, hyperoxia typically increases venous O2 partial pressure (PvO2) [7,18], which would tend to increase venous blood O2 content. Thus, in the context of the Fick principle, we predicted that increased ṀO2 with environmental hyperoxia would primarily result from increased cardiac output rather than a higher A-V O2 content difference. In turn, we predicted that improved cardiac output would be driven by increased cardiac contractility (stroke volume), and that this would be associated with elevated PaO2 and therefore an enhanced cardiac O2 supply due to a steeper O2 diffusion gradient between the returning venous blood and heart tissue [20]. To assess these predictions, we fitted rainbow trout with a ventral aortic blood flow probe and a venous cannula to allow simultaneous measurements of ṀO2, cardiac function, venous blood oxygenation and blood parameters (lactate and haematology) during thermal ramping to the critical thermal maximum (CTmax) under hyperoxia (200% air saturation) or normoxia.

Hyperoxia does not always increase the thermal tolerance of fish examined under controlled laboratory conditions [9,17]. Thus, it is unclear whether a recently proposed idea that natural environmental hyperoxia can enhance the resilience of aquatic ectotherms to more extreme acute warming [8] is relevant in a broad range of fishes and environments. In the second part of this study, we therefore synthesized the existing literature concerning the impact of hyperoxia on acute upper thermal tolerance limits in fish. In doing so, we aimed to assess: (i) whether improved thermal tolerance in hyperoxia is a general response observed in a broad range of fishes and (ii) the magnitude of environmental hyperoxia-induced increases in thermal tolerance in fishes.

2. Methods

(a) Experimental animals and holding conditions

The rainbow trout (mean body mass of 896.5 ± 47.5 g and 901.8 ± 73.9 g at the time of experimentation in the normoxia and hyperoxia treatments, respectively) used in this study were of mixed sex and obtained from a commercial trout farm (Vännäs Fiskodling AB, Halland, Sweden). Prior to experimentation, they were held in two 400 l tanks supplied with recirculated freshwater (air saturated, approx. 10°C and 12:12 h light cycle) for a period of at least four weeks of laboratory acclimation. They were fed commercial aquaculture feed (7 mm, Protec Trout pellets, Skretting, Norway) twice a week, but food was withheld for a period of 3 days prior to experimentation.

(b) Surgery and instrumentation

To measure cardiac output, heart rate and stroke volume, a 2.5 mm Transonic transit-time blood flow probe (L type; Transonic Systems, Ithaca, NY) was placed around the ventral aorta to allow recordings of blood flow. Anaesthesia and surgical methods for fitting the flow probe were identical to McArley et al. [21]. The ducts of Cuvier were then cannulated with a PE50 catheter to allow venous blood sampling as previously described by Sandblom et al. [22].

(c) Experimental protocol prior to thermal ramping

Following surgery, individual fish were placed into respirometers held in 120 l aquariums receiving a constant flow of approximately 10°C recirculated freshwater from the main holding tank supply. After the fish was placed in the respirometer, an O2 level of approximately 200% air saturation was established for the hyperoxic treatment by bubbling water with O2, while the normoxia treatment (approx. 100% air saturation) was maintained by bubbling air. These O2 treatment conditions were then maintained for the remainder of the protocol. Fish recovered from surgery for approximately 22 h, at which point they were removed from the respirometers and exhaustively exercised under normoxia or hyperoxia by manual chassing for a period of 5 min. They were then returned to the respirometers and allowed to recover for 21 h prior to the onset of thermal ramping. During the time prior to thermal ramping, five approximately 250 µl venous blood samples (approx. 1.25 ml total and approx. 3.3% of total blood volume) had been drawn from which the physiological parameters measured are not reported in the current study; the data pertaining to these samples are reported in McArley et al. [21]. The blood sample drawn (sixth sample) and the ṀO2 and cardiac function measured at the end of the
post-exhaustive exercise recovery period are used as routine values at 10°C in the current study (see electronic supplementary material, figure S1 for a visual outline of the experimental protocol). At this point, following exhaustive exercise, O₂ cardiac output and all blood parameters measured had recovered to pre-exhaustive resting levels under both normoxia and hyperoxia [21]. Thus, although previously exposed to exhaustive exercise, fish in both O₂ treatments were in a similarly well-rested and recovered state prior to thermal ramping. This allowed us to reduce the total number of research animals used, while maximizing the data collected, in accordance with 3R principles.

(d) Thermal ramping protocol
Thermal ramping involved step-wise increases in temperature, which were achieved by heating the water supply to the respirometers housing fish with a water heater controlled with a thermostat. The mean water O₂ level inside the respirometer throughout the entire thermal ramping protocol was 96.9 ± 0.2% air saturation and 208.8 ± 2% air saturation in the normoxia and hyperoxia treatment, respectively. These are referred as normoxia (approx. 100% air saturation) and hyperoxia (approx. 200% air saturation) for the remainder of this paper. Initially, temperature was increased from 10°C to 15°C and then 15°C to 20°C at a rate of 5°C h⁻¹. At 15°C and 20°C, O₂ and cardiac variables were measured for approximately 20 min once temperature stabilized (e.g. temperature was increased from 10°C to 15°C in 40 min; then measurements were taken at 15°C for 20 min). A venous blood sample (approx. 250 µl) was also drawn at the end of the 20°C measurement period. From 20°C, the rate of heating was reduced to 2°C h⁻¹. O₂ and cardiac variables were measured for a period of approximately 20 min at 20°C and then for 20 min with every 1°C increase in temperature beyond 22°C. A venous blood sample was also taken at the end of the 24°C and 26°C temperature steps. Thermal ramping continued until the loss of equilibrium (i.e. an inability to maintain a stable, upright body position) occurred for a period of 10 s, which was defined as the critical thermal maximum (CTmax) [23]. A final blood sample was taken at CTmax prior to the fish being removed from the respirometer and euthanized with a concussive blow to the head.

(e) Respirometry for O₂ measurement and data acquisition for cardiac variables
O₂ was measured using intermittent stop-flow respirometry [24]. Respirometer design, respirometric data acquisition equipment and software, and calculation of O₂ were identical to McArley et al. [21]. Briefly, in the ‘closed’ measurement phase, the linear decline in water O₂ level (sampled at 10 Hz with a fibre optic probe) within a sealed 10 l PVC respirometer was used to calculate O₂ at each thermal ramping temperature step. The R² for the slope of the linear decline in water O₂ level within the respirometer was greater than 0.98 for the majority of measurement cycles and never below 0.95. Three ‘closed’ phase measurement cycles (2–5 min) interspersed with a ‘flushing’ period (5–8 min) were run at each temperature. Background O₂ consumption was assessed at 10°C at the start of the protocol and at the temperature of CTmax. At 10°C, a positive background slope, which likely related to a small increase in temperature (approx. 0.15°C) within the sealed respirometer during ‘closed’ measurement cycles, was detected. The source of this heat was almost certainly the mixing pump connected to the respirometer. This positive slope, however, was reduced in a linear fashion as temperature increased during thermal ramping, and it often became slightly negative at the highest temperatures. Thus, to estimate background O₂ consumption, a linear regression between temperature and the background slope measured at the start (10°C) and end (CTmax temperature) of the protocol was used to calculate the background slope at each thermal ramping temperature. These slopes were then added (positive slope) or subtracted (negative slope) from the measurement cycle slopes used to calculate O₂.

The signal from the Transonic blood flow probe was sampled at 10 Hz using identical equipment and software as McArley et al. [21], and the probe was bench calibrated between temperatures of 10°C to 26°C according to the manufacturer’s instructions (see Morgenroth et al. [25] for a detailed description of the calibration set-up). The flow probe signal was recorded continuously throughout thermal ramping, but only data pertaining to periods of O₂ measurement, once temperature had stabilized at each thermal ramping step, was used to assess cardiac parameters.

(f) Calculation of cardiorespiratory variables
Cardiac output was determined from blood flow data and normalized to body mass (ml min⁻¹ kg⁻¹), and heart rate was determined from the pulsatile blood flow measurements. Cardiac stroke volume (ml heart beat⁻¹) was calculated by dividing cardiac output by heart rate. Routine and maximal values for cardiorespiratory variables are reported in this study. Routine values for MO₂ (MO₂-ROU) are the mean of three measurements taken at each thermal ramping temperature step. For cardiac variables, routine values are determined from the mean of three sections of flow trace recorded at the same time as MO₂-ROU (i.e. routine cardiac variables are tied to MO₂-ROU). Maximal MO₂-ROU during thermal ramping was taken as the highest MO₂-ROU (mean of three MO₂ values) recorded at any temperature. In all fish, this occurred at temperatures of 24°C or higher. Like routine cardiac variables, maximal cardiac variables were tied to MO₂ such that the maximum values for cardiac output, heart rate and stroke volume reported pertain to the same time when maximal MO₂-ROU was recorded. Cardiac variables were tied to MO₂ because a main focus of the experiment was to determine whether predicted differences in MO₂ between normoxia and hyperoxia were driven by differences in cardiac function. Using the tied MO₂ and cardiac output measurements, routine and maximal A-V O₂ content difference was estimated by rearrangement of the Fick equation:

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A-V \text{ O}_2 \text{ content difference} = \frac{\text{MO}_2}{\text{cardiac output}}
\]  

(g) Blood analysis
Venous blood samples (approx. 250 µl) were drawn prior to thermal ramping at 10°C and during thermal ramping at 20°C, 24°C and 26°C. In each sample, P₅₀, haemoglobin concentration ([Hb]), haematocrit (Hct) and plasma lactate were assessed using identical equipment and protocols to McArley et al. [21].

(h) Statistics
All analyses were performed using GraphPad Prism (version 9.10), with statistical significance accepted at p < 0.05. For repeated measures analyses, a violation of sphericity was assumed and Geisser–Greenhouse adjusted p-values and F-tests are reported. Cardiorespiratory variables (MO₂-ROU, cardiac output, heart rate, stroke volume and A-V O₂ content difference) were analysed in two ways. First, routine responses were compared between normoxia and hyperoxia up to a temperature of 25°C (i.e. prior to any fish reaching CTmax) using mixed two-way analysis of variance (ANOVA). For cardiac variables and A-V O₂ content difference, the findings of these analyses are...
Figure 1. Thermal tolerance and cardiorespiratory performance of rainbow trout (Oncorhynchus mykiss) facing acute warming under hyperoxia (200% air saturation) or normoxia. All values are means ± s.e.m. (n = 9 unless indicated by bracketed numbers). (a) Routine mass-specific \( \dot{O}_2 \) consumption rate (\( \dot{M}_{O_2-RQ} \)), with critical thermal maximum (\( CT_{\text{max}} \); the temperature at which fish could no longer maintain a stable, upright body orientation) shown in the insert. The bubbles in (a) indicate a significant difference (\( p < 0.05 \)) in \( \dot{M}_{O_2-RQ} \) at 25°C as assessed by mixed two-way ANOVA (see electronic supplementary material, figure S1 for statistical results); (b–e) show variables at maximal \( \dot{M}_{O_2-RQ} \), which occurred at 24.9 ± 0.26 and 25.7 ± 0.24°C in normoxia and hyperoxia, respectively. (b) Cardiac output (CO); (c) arterial-venous O\(_2\) content difference (A-V O\(_2\)) estimated by the Fick equation; (d) cardiac stroke volume (SV) and (e) heart rate (HR); (f–g) show venous O\(_2\) partial pressure (P\(_{VO_2}\)) and haematocrit (Hct) in blood samples drawn via a cannula at 26°C. For the normoxia treatment, seven out of nine blood samples for the 26°C comparison were drawn immediately upon reaching \( CT_{\text{max}} \). In the hyperoxia treatment, the eight fish included in the 26°C comparison did not reach \( CT_{\text{max}} \) for the entire temperature step. The sample size of 8 at 26°C reflects the fact that one fish in hyperoxia reached \( CT_{\text{max}} \) at 25°C. (Online version in colour.)

Figure 2. Plasma lactate concentration in rainbow trout (Oncorhynchus mykiss) facing acute warming under hyperoxia (200% air saturation) or normoxia. All values are means ± s.e.m. (n = 9 unless indicated by bracketed numbers). Over the 10–24°C range, letters represent significant differences (\( p < 0.05 \)) between temperatures across O\(_2\) treatments as assessed by mixed two-way ANOVA (temperature: \( F_{1,15} = 30.95, p < 0.001 \)). At 26°C, bubbles represent a significant difference (\( p < 0.05 \)) between O\(_2\) levels as assessed by an independent-sample t-test, with the exception of \( CT_{\text{max}} \) and heart rate for which comparisons were made using a Mann–Whitney U test (see electronic supplementary material, table S1 for statistical results). (Online version in colour.)
3. Results and discussion

(a) Hyperoxia increases thermal tolerance through boosting maximal tissue O2 supply capacity

The upper acute thermal limit ($CT_{\text{max}}$) was measured to determine if rainbow trout gain a thermal tolerance advantage of hyperoxic (200% air saturation) water oxygenation. In the case of improved $CT_{\text{max}}$ with hyperoxia, we predicted a corresponding increase in tissue O$_2$ supply capacity. To assess this prediction, MO$_2$-ROU (an estimate of whole-animal tissue O$_2$ supply capacity) was measured in normoxia and hyperoxia during acute thermal ramping (approx. 2°C h$^{-1}$) to the temperature at which fish could no longer maintain equilibrium (i.e. a stable, upright body orientation; $CT_{\text{max}}$). In hyperoxia, the $CT_{\text{max}}$ of rainbow trout was significantly higher than in normoxia (figure 1a). As predicted, the elevation of $CT_{\text{max}}$ with hyperoxia was associated with a greater tissue O$_2$ supply capacity. Indeed, there was a striking difference in the ability of normoxia and hyperoxia-exposed rainbow trout to increase MO$_2$-ROU beyond 23°C (figure 1a). This was reflected by hyperoxia-treated fish having a 52% higher MO$_2$-ROU at 25°C (figure 1a). Moreover, the maximal MO$_2$-ROU during thermal ramping, which was observed at 24.9 ± 0.26 and 25.7 ± 0.24°C in normoxia and hyperoxia, respectively, was 58% higher in hyperoxia (292.8 ± 6.6 mg O$_2$ kg$^{-1}$ h$^{-1}$ in normoxia versus 462.1 ± 21.6 mg O$_2$ kg$^{-1}$ h$^{-1}$ in hyperoxia; $p < 0.001$, see electronic supplementary material, table S1 for statistical results). It is proposed that acute warming limits in aquatic ectotherms are set by temperature-dependent performance ceilings of maximum tissue O$_2$ supply capacity, and that when this ceiling is reached, survival becomes time limited and increasingly reliant on unsustainable anaerobic ATP production [11,12]. Here, we show that the substantial benefit of hyperoxia to tissue O$_2$ supply capacity mitigated anaerobiosis at high temperatures. Indeed, at 26°C, a temperature where seven out of nine fish in normoxia but only one of nine fish in hyperoxia had reached $CT_{\text{max}}$, plasma lactate levels—a by-product of anaerobic metabolism—were significantly lower in hyperoxia than normoxia (figure 2). This finding indicates that higher $CT_{\text{max}}$ in hyperoxia may have resulted from improved O$_2$ supply capacity shifting the point at which anaerobic ATP production became unsustainable to a higher temperature. As $CT_{\text{max}}$ is marked by a loss of coordination, which likely involves some form of neural impairment, severe anaerobiosis in brain tissue may be a candidate for the proximate cause of loss of equilibrium at high temperatures. In support of this, unsustainable anaerobic respiration (i.e. exhaustion of ATP, depletion of glycogen and marked increases in lactate in brain tissue was identified as a key characteristic at the loss of equilibrium in hypoxia exposed sculpin species [26].

(b) Hyperoxia boosts heart blood pumping capacity

Limits on the maximum blood pumping capacity of the heart have been proposed as the primary determinant of ceilings in tissue O$_2$ supply during warming exposure in fish [16]. Moreover, as haemoglobin is normally fully saturated in normoxia [19], hyperoxia is unlikely to increase arterial O$_2$ content and therefore should not influence A-V O$_2$ content difference [27]. Thus, we predicted that any expansion of tissue O$_2$ supply capacity (i.e. higher MO$_2$) in hyperoxia during thermal ramping would be driven by higher cardiac output. Confirming this prediction, higher MO$_2$-ROU at 25°C in hyperoxia occurred alongside a 29% elevation of cardiac output at the same temperature ($p < 0.05$; electronic supplementary material, figure S2). This also occurred at maximal MO$_2$-ROU, where cardiac output was 33% higher with hyperoxia ($p < 0.05$; figure 1b). Because heart rate was similar between treatment groups across temperatures (electronic supplementary material, figure S1) and at maximal MO$_2$-ROU (figure 1c), the higher cardiac output with hyperoxia likely reflected an increased cardiac contractility. Indeed, a strong trend for higher stroke volume with hyperoxia existed at temperatures beyond 24°C (electronic supplementary material, figure S2), and stroke volume was 46% higher with hyperoxia at maximal MO$_2$-ROU ($p < 0.05$; figure 1d). Increased cardiac contractility with hyperoxia may have been the result of an improved cardiac O$_2$ supply. The partial pressure of O$_2$ in venous blood was elevated in hyperoxia across thermal ramping temperatures (electronic supplementary material, figure S3) and was approximately 1.2 kPa higher at 26°C (figure 1f). Although the difference in P$_2$O$_2$ between normoxia and hyperoxia was relatively small, it existed in a range (normoxia approx. 2 kPa and hyperoxia approx. 3.2 kPa; figure 1f) below the threshold (approx. 6 kPa) where progressive declines in maximal cardiac output with falling perfusate O$_2$ partial pressure begin in rainbow trout perfused heart preparations [28]. Thus, as P$_2$O$_2$ was below the threshold known to impair maximal cardiac output in situ, it is plausible that the higher in vivo P$_2$O$_2$ in hyperoxia could have contributed to improved cardiac performance by steepening the O$_2$ diffusion between blood entering the heart lumen and the spongy myocardium. Elevated P$_2$O$_2$ in hyperoxia was also observed alongside improved stroke volume during thermal ramping in European perch (Perca fluviatilis) [18].

The interpretation made here that enhanced cardiac contractility with hyperoxia is driven by higher P$_2$O$_2$ is complicated by two factors. First, rainbow trout also have a coronary circulation, which supplies oxygenated arterial blood directly from the gills to the outer compact myocardium [29]. We have recently observed that arterial O$_2$ partial pressure (P$_{2O_2}$) is approximately 16 kPa higher under hyperoxia (200% air saturation) relative to normoxia following exhaustive exercise in rainbow trout (T.J.M. 2022, unpublished data). Moreover, elevated P$_2$O$_2$ is a common response to hyperoxia in almost all fish so far examined [7]. Thus, it is likely that hyperoxia also results in increased P$_2$O$_2$ during thermal ramping and that improved contractility may be due to a steepened O$_2$ diffusion gradient between the coronary blood and the compact myocardium of the heart. The second complicating factor is that hyperoxia also reduced Hct in the current study (from 32% to 25% at 24°C, and from 37% to 29% at 26°C; electronic supplementary material, figure S2; figure 1g). In European seabass (Dicentrarchus labrax), experimental anaemia that reduced Hct from 42% to 20% increased peak cardiac output during thermal ramping by 42% [30]. Thus, some of the approximately 33% increase in peak cardiac output observed here with hyperoxia may have been the result of the lower Hct rather than being solely related to improved cardiac O$_2$ supply directly influencing contractility. In hyperoxia, due to the
mitigating influence of increased blood $\text{PO}_2$ it may be that an active reduction in Hct can take place without compromising aerobic performance. The potential benefit of this is that lower Hct reduces blood viscosity and could therefore lower the energetic costs of the heart [31].

A somewhat perplexing finding of our study is that, despite lower [Hb] and higher $P_{\text{O}_2}$ (electronic supplementary material, figure S3), there was a trend for a 19% higher A-V $O_2$ content difference (Fick estimated) with hyperoxia at maximal $MO_{2,\text{ROU}}$ (figure 1c). If haemoglobin were fully saturated in arterial blood under both $O_2$ levels, this finding would be inexplicable. Our working hypothesis is that this is not necessarily the case. In rainbow trout exposed to thermal ramping under normoxia, it is known that haemoglobin $O_2$ saturation can fall to approximately 75% at high temperatures [32]. Moreover, although haemoglobin $O_2$ saturation was unaffected, $P_{\text{O}_2}$ fell from approximately 18.6 kPa to approximately 9.5 kPa in heat shocked (13°C to 25°C in 4 h) rainbow trout [33]. As noted earlier, we now know that hyperoxia drastically increases $P_{\text{O}_2}$ relative to normoxia following exhaustive exercise in rainbow trout (T.J.M. 2022, unpublished data). If this also occurs during thermal ramping, it may afford protection against collapsing haemoglobin $O_2$ saturation and arterial $O_2$ content as $CT_{\text{max}}$ is approached. This hypothesis remains speculative, however, and follow-up studies measuring arterial oxygenation under normoxia and hyperoxia during thermal ramping are required.

(c) Prevalence and magnitude of hyperoxia-induced thermal tolerance in fishes

Recent evidence, as was the case in the current study, has shown hyperoxia can increase the critical upper thermal limits of fish inhabiting shallow, tropical coastal environments, suggesting photosynthetically driven $O_2$ supersaturation could increase the resilience of fishes living in such habitats to more extreme heat waves associated with climate change [8]. In our own past work, however, hyperoxia has failed to influence upper critical thermal limits [9,17], indicating hyperoxia-induced thermal tolerance may be species and context specific. To understand the generality of the phenomenon of hyperoxia-induced thermal tolerance, we collated existing literature data from studies that examined the influence of hyperoxia on upper critical limits in fish. Ten publications (present study included) were identified (table 1). These studies included 20 species ranging from exclusively tropical to Antarctic climatic regions. Of the 20 species examined, a significant elevation of $CT_{\text{max}}$ with hyperoxia has been demonstrated in nine species (table 1). In one further species, hyperoxia appeared to increase upper thermal tolerance (Carrisius auratus +1°C ) but no statistical comparison could be made due to experimental design (table 1). The magnitude of improvement in thermal tolerance with hyperoxia ranged from +0.4°C to +1.8°C (table 1). This finding indicates naturally occurring hyperoxia could benefit thermal tolerance in a large number of fishes and potentially improve resilience to more extreme heat wave events due to climate change. A caveat of this conclusion, however, is that almost all studies have failed to replicate naturally occurring hyperoxic episodes. The reason for this is that most have been performed in a purely mechanistic rather than ecological context, where replicating naturally occurring $O_2$ levels and heating rates have not been a priority. The best effort so far has been that of Giomi et al. [8] who matched experimental $O_2$ levels and heating rates to extensive monitoring data from relevant ecosystems. These authors’ study demonstrates the largest benefits of environmental hyperoxia to critical thermal limits among the available literature.

In addition to improving upper critical thermal limits in fish, other benefits of environmental hyperoxia may exist at elevated but sub-lethal temperatures. In the current study, the large increase in maximal $MO_{2,\text{ROU}}$ at elevated temperatures with hyperoxia, probably means that hyperoxia also increases aerobic scope (the difference between resting $M_O_2$ and maximal $M_O_2$) at elevated temperatures. The same expansion of $MO_2$ with hyperoxia at acutely elevated temperatures has also been observed in European perch and two triplefin fishes [9,17]. Aerobic scope is proposed to represent the metabolic performance window within which fish can perform aerobically demanding activities [38]. The basic principle is that constraint or expansion of aerobic scope by a given environmental factor corresponds to a constraint or expansion of the capacity of an organism to perform aerobically demanding activities such as swimming, feeding, digestion, growth and reproduction [39]. In this context, we propose the apparent expansion of aerobic scope in fish facing acute warming exposure under hyperoxia may represent a sub-lethal metabolic refuge that mitigates severe constraints on aerobic performance that would otherwise occur with acute warming under normoxia. A possible trade-off to the proposed benefit of hyperoxia to aerobic performance, however, could be increased levels of oxidative stress. Indeed, it is known that hyperoxia can increase $O_2$ free radical production and cause oxidative damage to tissues in fish (see [7] for a detailed review of this topic). Future studies assessing the benefits of hyperoxia to aerobic performance and thermal tolerance should also consider whether co-occurring hyperoxia and acute warming also impose harmful oxidative stress.

(d) Conclusions

Recent evidence has demonstrated that naturally occurring environmental hyperoxia can improve upper critical thermal limits in fish and therefore may increase resilience of fish living in heat-vulnerable habitats (e.g. rock pools, shallow estuaries and shallow lakes and ponds) to more extreme acute warming events occurring with climate change. Here, we show hyperoxia also increases $CT_{\text{max}}$ in rainbow trout. We demonstrate hyperoxia substantially increases maximal tissue $O_2$ supply capacity at elevated temperatures approaching upper critical limits and mitigates anaerobiosis. Moreover, the blood pumping capacity of the heart is boosted with hyperoxia as evidenced by increased cardiac output and stroke volume. Together these findings indicate that hyperoxia can benefit acute thermal tolerance in fish through expanding cardiorespiratory performance and improving tissue $O_2$ supply capacity. Our literature review found that environmental hyperoxia increases upper critical thermal limits in half of the fishes so far examined. Thus, naturally occurring environmental hyperoxia could improve upper acute thermal tolerance limits and increase resilience to extreme heat wave events resulting from climate change in a large number of fishes.
Table 1. The effect of environmental hyperoxia on upper thermal tolerance limits in fish. CT\textsubscript{max} difference = CT\textsubscript{max} in hyperoxia - CT\textsubscript{max} in normoxia (a positive number shows higher CT\textsubscript{max} in hyperoxia), na = no statistical comparison available, ns = not stated. Note: Gomi et al. [8] reported the temperature at which 50% of fish became unresponsive (LT50) as a measure of thermal tolerance. As LT50 was determined by sigmoidal regression, the separation of 95% confidence intervals between the regressions in normoxia and hyperoxia was taken as a statistically significant difference. All other studies reported CT\textsubscript{max} (i.e. the temperature of loss of equilibrium). Ecotype: FW = freshwater, M = marine, BW = backish water. Climatic region was determined from the latitudinal distribution listed for each species on FishBase (https://www.fishbase.de/).

| species                   | ecotype                        | climatic region               | acclimation temperature (°C) | heating rate (°C h\(^{-1}\)) | O\textsubscript{2} level (% air saturation) | CT\textsubscript{max} difference (°C) | \(p < 0.05\) | reference |
|---------------------------|--------------------------------|--------------------------------|-----------------------------|-------------------------------|------------------------------------------|---------------------------------------|----------------|-----------|
| Carassius auratus         | benthopelagic; FW/BW           | subtropical-temperate         | 17                          | 109                           | 200                                      | +0.9                                  | na             | [13]      |
| C. auratus                |                                |                                | 17                          | 109                           | 450                                      | +1                                    | na             |           |
| C. auratus                |                                |                                | 27                          | 73                            | 200                                      | +0.21                                 | na             |           |
| C. auratus                |                                |                                | 27                          | 73                            | 450                                      | +0.81                                 | na             |           |
| Fundulus notatus          | benthopelagic, FW              | subtropical-temperate         | 30                          | 20                            | 160                                      | −0.01                                 | no             | [34]      |
| Notropis lutrensis        | benthopelagic, FW              | subtropical-temperate         | 30                          | 20                            | 160                                      | −0.53                                 | no             |           |
| Amphiphas vigilax         | benthopelagic, FW              | subtropical-temperate         | 30                          | 20                            | 160                                      | −0.16                                 | no             |           |
| Fundulus heteroclitus     | benthopelagic, FW/M/BW         | subtropical-temperate         | 15                          | 18                            | ns                                       | +0.3                                  | no             | [35]      |
| Percula fluviatilis       | benthopelagic (Biotest population), FW/BW | temperate                     | 23                          | 2                             | 200                                      | +0.6                                  | no             |           |
| P. fluviatilis            | benthopelagic, FW/BW           | temperate                     | 17                          | 2                             | 200                                      | +1.1                                  | yes            | [18]      |
| Chaenophthalmus aceratus  | benthopelagic, M               | Antarctic                      | 0.5                         | 4                             | 240                                      | +0.12                                 | no             | [36]      |
| Nototheria coriceps       | benthopelagic, M               | Antarctic                      | 0.5                         | 4                             | 240                                      | +0.74                                 | no             |           |
| Bellapiscis medius        | benthic (intertidal), M        | temperate                     | 21                          | 2                             | 200                                      | +0.13                                 | no             | [9]       |
| Forsterygion lapillum     | benthopelagic (intertidal/subtidal), M | temperate                     | 21                          | 2                             | 200                                      | +0.43                                 | yes            |           |
| Atherinomorus sp.         | pelagic, M                     | tropical                      | 20                          | 2                             | 140                                      | +1.4                                  | yes            | [8]       |
| Dasyllus sp.              | reef associated, M             | tropical                      | 20                          | 2                             | 140                                      | +1.8                                  | yes            |           |
| Apistogramma borellii     | benthopelagic, FW              | tropical-subtropical          | 31                          | 12                            | 200                                      | +0.66                                 | no             | [37]      |
| Brycon amazonicus        | benthopelagic, FW              | tropical                      | 31                          | 12                            | 200                                      | +1.4                                  | yes            |           |
| Carnejillosa striatula    | pelagic, FW                    | tropical                      | 31                          | 12                            | 200                                      | +0.51                                 | yes            |           |
| Colossoma macropomum      | benthopelagic, FW              | tropical                      | 31                          | 12                            | 200                                      | +0.08                                 | no             |           |
| Corydoras pulcher         | benthopelagic, FW              | tropical                      | 31                          | 12                            | 200                                      | +0.81                                 | no             |           |
| Corydoras schwartzii      | benthopelagic, FW              | tropical                      | 31                          | 12                            | 200                                      | +0.48                                 | yes            |           |
| Paracheirodon axelrodii   | pelagic, FW                    | tropical                      | 31                          | 12                            | 200                                      | +0.41                                 | yes            |           |
| Oncorhynchus mykiss       | benthopelagic, FW/M/BW         | temperate                     | 10                          | 2                             | 200                                      | +0.87                                 | yes            | current study |

\(^{a}\)Weatherley [13] did not test statistical significance when comparing CT\textsubscript{max} but did show a significant increase in the time fish survived exposure to 40°C under hyperoxia relative to normoxia.
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