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RESEARCH ARTICLE

Cardiorespiratory adjustments to chronic environmental warming improve hypoxia tolerance in European perch (*Perca fluviatilis*)

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ABSTRACT

Aquatic hypoxia will become increasingly prevalent in the future as a result of eutrophication combined with climate warming. While short-term warming typically constrains fish hypoxia tolerance, many fishes cope with warming by adjusting physiological traits through thermal acclimation. Yet, little is known about how such adjustments affect tolerance to hypoxia. We examined European perch (*Perca fluviatilis*) from the Biotest enclosure (23°C, Biotest population), a unique ∼1 km^2 ecosystem artificially warmed by cooling water from a nuclear power plant, and an adjacent reference site (16–18°C, reference population). Specifically, we evaluated how acute and chronic warming affect routine oxygen consumption rate ($\dot{M}_{O_2,routine}$) and cardiovascular performance in acute hypoxia, alongside assessment of the thermal acclimation of the aerobic contribution to hypoxia tolerance (critical O_2 tension for $\dot{M}_{O_2,routine}$: P_{crit}) and absolute hypoxia tolerance (O_2 tension at loss of equilibrium; P_{LOE}). Chronic adjustments (possibly across lifetime or generations) alleviated energetic costs of warming in Biotest perch by depressing $\dot{M}_{O_2,routine}$ and cardiac output, and by increasing blood O_2 carrying capacity relative to reference perch acutely warmed to 23°C. These adjustments were associated with improved maintenance of cardiovascular function and $\dot{M}_{O_2,routine}$ in hypoxia (i.e. reduced P_{crit}). However, while P_{crit} was only partially thermally compensated in Biotest perch, they had superior absolute hypoxia tolerance (i.e. lowest P_{LOE}) relative to reference perch irrespective of temperature. We show that European perch can thermally adjust physiological traits to safeguard and even improve hypoxia tolerance during chronic environmental warming. This points to cautious optimism that eurythermal fish species may be resilient to the imposition of impaired hypoxia tolerance with climate warming.

KEY WORDS: Cardiac performance, Global warming, Hypoxia tolerance, Teleost fish, Thermal acclimation

INTRODUCTION

Aquatic hypoxia, a condition of reduced water O_2 partial pressure ($P_{W_{O_2}}$), is a naturally occurring phenomenon in aquatic ecosystems (Diaz and Breitburg, 2009). However, hypoxic conditions have spread and become more severe in recent decades (Breitburg et al., 2018). For example, the frequency and extent of hypoxia in the Baltic Sea has increased severalfold in both the open sea and shallow coastal areas, which has led to fish kills and large uninhabitable areas (Carstensen et al., 2014; Carstensen and Conley, 2019; Casini et al., 2016). This expansion of hypoxia is mainly a result of increased anthropogenic nutrient input causing eutrophication and increased microbial O_2 consumption (Carstensen and Conley, 2019). While many water-breathing fishes have evolved a suite of physiological, biochemical and behavioral traits to cope with aquatic hypoxia (for reviews, see Farrell and Richards, 2009a; Mandic and Regan, 2018; Richards, 2011), species-specific tolerance limits are highly variable, and there is increasing concern that anthropogenically induced hypoxia may negatively impact numerous biological responses and performance traits (Sampaio et al., 2021), and even push species beyond their tolerance limits (McBryan et al., 2013). An additional problem is that elevated temperatures in a changing climate may exacerbate hypoxic conditions in the future, mainly because warming decreases the O_2 solubility of water, increases microbial O_2 consumption, and reinforces thermal stratification that prevents water mixing and re-oxygenation (Carstensen and Conley, 2019; McBryan et al., 2013; Oschlies et al., 2018; Perello et al., 2017; Shepherd et al., 2017).

If severe enough, acute hypoxia constrains blood oxygenation at the gills in fish, which may compromise tissue O_2 delivery and aerobic metabolism (Boutilier, 2001; Butler and Taylor, 1975; Chan, 1986; Holeton and Randall, 1967; Powell et al., 2000; Stecyk, 2017; Steffensen and Farrell, 1998). Most fish, however, are 'O_2 regulators', meaning that they adjust cardiorespiratory functions (cardiovascular and metabolic) to maintain a stable O_2 consumption rate ($\dot{M}_{O_2}$, a proxy for aerobic metabolic rate) over a range of species-specific hypoxic $P_{W_{O_2}}$ values (Svendsen et al., 2019). At a specific critical $P_{W_{O_2}}$ (P_{crit}), however, cardiorespiratory adjustments are no longer sufficient to compensate for increasingly lower O_2 diffusion gradients, and a transition from O_2 regulation to O_2 conformation occurs, which is marked by a progressive decline in $\dot{M}_{O_2}$ (Rogers et al., 2016; Ultsch and Regan, 2019). If low $P_{W_{O_2}}$ levels persist below P_{crit} and the animal cannot compensate with increased anaerobic metabolism and/or reduce energetic demands (e.g. through metabolic depression), a progressive mismatch between tissue ATP supply and demand develops (Richards, 2011). This leads to compromised vital functions, loss of equilibrium (LOE) and eventually death (Boutilier, 2001; Claireaux and Chabot, 2016; Farrell and Richards, 2009b; Rodnick and Gesser, 2017; Speers-Roesch et al., 2013; Wood, 2018).

In addition to exacerbating aquatic hypoxia per se, acute warming elevates the $\dot{M}_{O_2}$ of fish (Eliason and Anttila, 2017), which typically reduces the ability to maintain aerobic metabolism in hypoxia as indicated by elevated P_{crit} (Borowiec et al., 2016; Butler and Taylor, 1975; Corkum and Gamperl, 2009; Ern et al., 2016; Fry and Hart, 1948; Gehrke and Fielder, 1988; Rogers et al., 2016), as well as elevated $P_{W_{O_2}}$ at which LOE occurs (P_{LOE}) (Borowiec et al., 2016), and a reduced time to LOE in fish exposed to a constant level of

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severe hypoxia (He et al., 2015; McBryan et al., 2013, 2016). A key question is to what extent phenotypic plasticity or genetic adaptation of physiological functions following long-term (chronic) warming can alleviate the expected impaired hypoxia tolerance at elevated temperatures (McBryan et al., 2013).

The thermal sensitivity of hypoxia tolerance has so far only been studied in a limited number of fish species (Anttila et al., 2015; Chen et al., 2019; He et al., 2015; Jung et al., 2019; McBryan et al., 2016). Moreover, detailed characterizations of mechanisms underlying the thermal sensitivity of hypoxia tolerance have often been hindered by small body sizes precluding, for example, detailed cardiorespiratory measurements and repeated blood sampling. Nonetheless, available evidence suggests that warm acclimation can alleviate some of the detrimental effects of warming on hypoxia tolerance. Indeed, when fish are given time to acclimate to increased temperatures there is generally a reduction in P_{crit} and P_{LOE}, and/or an increased time to LOE in constant severe hypoxia (Anttila et al., 2015; Chen et al., 2019; He et al., 2015; McBryan et al., 2016). The mechanisms behind these responses are likely to be multi-faceted and may involve several plastic changes along the O_2 transport cascade. While warm acclimation can increase the gill surface area to improve branchial O_2 diffusion (Chen et al., 2019; McBryan et al., 2016), and increase anaerobic capacity (He et al., 2015), perhaps the most important adjustment is that the thermal sensitivity of aerobic metabolic processes is reduced, causing a reduction in animal O_2 demand (i.e. thermal compensation of $\dot{M}_{O_2}$; Seebacher et al., 2015).

In the present study, we examined thermal effects on hypoxia tolerance in European perch (*Perca fluviatilis*) subjected to long-term warming in the Biotest enclosure: a unique semi-natural ecosystem in the Baltic Sea that has been artificially warmed by ∼5–10°C for the past 40 years by cooling water effluents from a nuclear power plant (Huss et al., 2019; Sandblom et al., 2016a). This has significant relevance for this species in nature, as periods of days to months of severe (Pw_{O_2}<2 mg l^{-1}) and moderate (Pw_{O_2}=2–4 mg l^{-1}) hypoxia are well documented in shallow coastal Baltic Sea areas inhabited by perch (Conley et al., 2011). We recently showed that Biotest perch (field-acclimated to 23°C in summer) exhibit pronounced metabolic adjustments as indicated by significantly lower resting $\dot{M}_{O_2}$ relative to reference perch from an adjacent area (field-acclimated to 18°C) that were acutely warmed to 23°C (Sandblom et al., 2016a). This thermal compensation of $\dot{M}_{O_2}$ was associated with reduced cardiac output driven by depressed heart rate, the latter being a combined effect of reduced intrinsic cardiac pacemaker rate and increased autonomic inhibitory cholinergic (i.e. vagal) tone on the heart (Sandblom et al., 2016a,b). It is currently unknown, however, whether such thermal adjustments of cardiac autonomic control affect cardiovascular reflex responses to hypoxia (e.g. hypoxic bradycardia; Farrell, 2007). Moreover, very few studies have evaluated in detail how chronic warming affects integrated cardiorespiratory responses to hypoxia in fish, and how that is related to overall hypoxia tolerance.

To address these questions, we examined perch from the Biotest enclosure (∼23°C) and the adjacent reference area (∼16–18°C) to assess routine cardiovascular variables and $\dot{M}_{O_2}$ ($\dot{M}_{O_2,routine}$; Chabot et al., 2016), and define the influence of temperature on hypoxia tolerance (i.e. P_{LOE}) and cardiovascular performance. Specifically, we examined responses to acute hypoxia in perch from the two populations at their respective acclimation temperatures, as well as in reference perch acutely warmed to the Biotest temperature (∼23°C). We hypothesized that acutely warmed reference perch would exhibit elevated cardiorespiratory rates and reduced hypoxia tolerance relative to reference fish at the cooler temperature, while metabolic and cardiorespiratory thermal compensation in the chronically warmed Biotest perch would result in lower cardiorespiratory rates and mitigation of the impaired hypoxia tolerance expected to occur with acute warming. Perch are known to be O_2 regulators with a relatively low P_{crit} (Bilberg et al., 2010; Thuy et al., 2010). If Pw_{O_2} remains above P_{crit}, perch appear to be able to withstand prolonged periods of hypoxia (Horoszewicz, 1973); however, once Pw_{O_2} falls below P_{crit}, survival is very time limited (Alabaster et al., 1957). As it appears that hypoxia tolerance is reliant on the ability to maintain aerobic metabolism at low Pw_{O_2} (i.e. a low P_{crit}), we hypothesized that any thermal compensation of hypoxia tolerance during chronic warming would be linked to thermal compensation of P_{crit}.

MATERIALS AND METHODS

Study site and animal collection

We conducted this study in August and September 2018 at the Biotest enclosure, which is a man-made ∼1 km^2 artificial basin located in the Baltic Sea archipelago close to Forsmark, Sweden (60°25′41.5″N, 18°11′20.8″E) (for details, see Sandblom et al., 2016a; Huss et al., 2019). The Biotest enclosure receives heated cooling water from two reactors at the Forsmark nuclear power plant. Relative to the temperature of the surrounding Baltic Sea archipelago, this elevates the average water temperature by ∼5°C (summer) to ∼10°C (winter) (Huss et al., 2019) (see Fig. S1). The semi-natural ecosystem in the Biotest enclosure holds several species of teleost fish, including European perch (*Perca fluviatilis*, Linnaeus 1758) (Huss et al., 2019; Sandström et al., 1995).

Perch of mixed sex and age were caught by hook and line from sites close to the cooling water intake channel upstream of the power plant where the temperature is normal (reference population, ∼16°C), and from the Biotest enclosure ('Biotest population', ∼23°C; see Table 1 for fish size metrics). We then transferred the perch to 1200 l holding tanks continuously supplied with aerated seawater (∼5 ppt salinity) from the Baltic Sea or the Biotest enclosure. Thus, the temperature in the holding tanks closely matched the environmental temperature at the respective fish collection sites (see Fig. S1; Table 1). A 6 kW water heater (VB 6010L, Värmebaronen,

Table 1. Environmental and morphological characteristics of European perch (*Perca fluviatilis*) across experimental groups

	Reference (*n*=15)	Reference acutely warmed (*n*=14–16)	Biotest (*n*=14–15)
Acclimation temperature (°C)	16.8±0.8	16.8±0.8	22.5±0.4
Test temperature range (°C)	17.6–18	22.5–23.6	22.8–23.9
Body mass (g)	260±6	267±3	285±5
Body length (mm)	247±19	247±18	253±11
Condition factor	1.67±0.03	1.74±0.03	1.75±0.03
Relative spleen mass (%)	0.16±0.01	0.16±0.02	0.15±0.01
Relative ventricle mass (%)	0.057±0.002[a]	0.060±0.003[a]	0.049±0.002[b]

Dissimilar letters denote statistically significant differences (P<0.05) among experimental treatment groups. Data are presented as means±s.e.m.

Österslöv, Sweden) was used to adjust and maintain the temperature in the holding tanks, which were partly covered and located outdoors under a natural photoperiod. We acclimated the fish to the holding tanks for a minimum of 3 days prior to experimentation, during which the fish were not fed. All experimental procedures were approved by the animal ethics committee in Gothenburg (ethical permit no. 165-2015).

Surgery and instrumentation

Perch were anesthetized in aerated Baltic Sea water containing tricaine methanesulfonate (MS-222, 100 mg l^{-1}), and placed left-side up on wet foam on a surgery table. During surgery, the gills were continuously irrigated with aerated Baltic seawater (10°C) containing MS-222 (50 mg l^{-1}). The fish were then surgically instrumented with a catheter in the third afferent branchial artery to record ventral aortic blood pressure, and a Transonic blood flow probe (Transonic Systems Inc., Ithaca, NY, USA) fitted around the aorta to record cardiac output as described in detail by Ekström et al. (2016a). Care was taken not to damage the pericardium or surrounding vessels and nerves during this procedure.

The fish were then placed in one of four cylindrical transparent Perspex respirometers (3.1 l) submerged in a 160 l experimental tank continuously supplied with water at the reference or Biotest temperature (see below for details), and allowed at least 24 h of post-surgical recovery. The tank was covered with opaque drapes and the fish were monitored via a camera to minimize external disturbances.

Experimental protocols

We assessed responses to hypoxia in reference (n=15) and chronically warmed Biotest (n=15) fish at their respective environmental acclimation temperatures (i.e. 17.8±0.2°C and 23.4±0.4°C, respectively), as well as in reference fish acutely exposed (24–28 h prior to the start of the protocol) to the Biotest temperature (reference acutely warmed, n=16, 22.8–23.9°C) (see Table 1 for fish size metrics). We examined a maximum of four fish each day, and the order of experimental treatment groups was randomized.

At the start of the experimental protocol, we first took a resting blood sample (~0.3 ml) in normoxia (90–100% air saturation) from the catheter using a heparinized syringe. The collected blood volume represented less than 4% of the total blood volume (assuming a total blood volume of ~3% of body mass) and was replaced with 0.3 ml saline (0.9% NaCl). The fish were then connected to the recording equipment, and routine cardiorespiratory variables (see below for details) in normoxia were recorded for at least 2 h. We initiated hypoxia exposure by ceasing the water flow to the respirometers, whereupon the O_2 consumption of the animals caused a gradual decline in Pw_{O_2} in the respirometers. We allowed the decline in Pw_{O_2} to continue to the point at which the fish could no longer maintain an upright body position for more than 10 s, which we here define as the Pw_{O_2} at LOE (P_{LOE}). The time from the start of the protocol to P_{crit} and P_{LOE} was determined in each individual fish. From the onset of the hypoxia exposure, reference perch, chronically warmed Biotest perch and acutely warmed reference perch reached P_{crit} within 33, 17 and 14 min, respectively, and P_{LOE} within 52, 35 and 28 min, respectively (see Fig. S2). This meant that the average hypoxia induction rate during the entire hypoxia protocol was 0.33, 0.49 and 0.59 kPa min^{-1} for reference perch, chronically warmed Biotest perch and acutely warmed reference perch, respectively.

Immediately following P_{LOE}, we collected a second blood sample, and the fish were subsequently rapidly removed from the experimental chamber and euthanized by a cranial blow. If a final blood sample could not be collected from the catheter, we collected a blood sample from the euthanized fish by drawing blood from the caudal vessels using a heparinized syringe. This procedure was employed in five reference fish, four acutely warmed reference fish and five chronically warmed Biotest fish. Following termination of the experiments, the spleen and ventricle were dissected and their mass was determined. The respirometers and experimental tank were thoroughly cleaned, and the entire water volume within the experimental setup was replaced between each experimental run.

Data acquisition

The ventral aortic blood pressure and cardiac output were recorded as described by Ekström et al. (2016a). The Pw_{O_2} in the respirometers was recorded using Firesting O_2 optodes connected to a Firesting fiberoptic O_2 meter (Pyroscience, Aachen, Germany), and sensors were calibrated by two-point calibration (i.e. at 0% air saturation in water saturated with sodium sulfite and at 100% air saturation in water vigorously bubbled with air). The water temperature was recorded continuously using a custom-built temperature logger (EW 7221, Crn Tecnopart, Barcelona, Spain). Analog signals from the recording equipment were relayed to a PowerLab system (ADInstruments, Sydney, Australia) connected to a laptop computer with LabChart Pro software (version 7.3.8; ADInstruments, Bella Vista, Australia).

Data analysis

We determined the mass-specific $\dot{M}_{O_2}$ (expressed as mg O_2 h^{-1} kg^{-1}) using intermittent-closed respirometry and calculated the $\dot{M}_{O_2}$ from the decline in Pw_{O_2} inside the respirometer according to Clark et al. (2013). In normoxia (i.e. 90–100% air saturation), $\dot{M}_{O_2,routine}$ was first determined by recording $\dot{M}_{O_2}$ slopes for at least 2 h where the decline in Pw_{O_2} inside the respirometer was recorded for 5 min every 15 min between automated 10 min respirometer flush cycles. This cycle duration ensured that the Pw_{O_2} inside the respirometers did not go below 80% air saturation when the respirometers were closed and the fish remained in a quiescent state. We calculated an average $\dot{M}_{O_2}$ during this period. During the hypoxia protocol, when the respirometer remained closed, $\dot{M}_{O_2,routine}$ was assessed continuously by calculating 1 min slopes until the point at which each individual fish reached P_{LOE}. We then averaged $\dot{M}_{O_2,routine}$ values spanning ~5% air saturation sections at 90%, 80%, 70%, 60%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% and 5% air saturation (e.g. the average $\dot{M}_{O_2,routine}$ at 90% air saturation represents the average value between ~92.5% and 87.5% air saturation). We accounted for the background microbial respiration by subtracting the background $\dot{M}_{O_2}$ slopes measured in empty respirometers after each experimental run.

Cardiac output was normalized to body mass (ml min^{-1} kg^{-1}), and the recordings from the blood flow probes were corrected to account for potential deviations with changing temperature according to the manufacturer's calibration protocol (Transonic Systems). We determined heart rate from the pulsating blood flow trace, and stroke volume was calculated as:

$$\text{Stroke volume} = \text{cardiac output}/\text{heart rate}, \quad (1)$$

Cardiac power output, a proxy for cardiac O_2 demand (Farrell et al., 1996), was calculated as:

$$\text{Cardiac power output} = (\text{cardiac output} \times \text{ventral aortic blood pressure}) \times K)/m_v, \quad (2)$$

where K is a conversion factor (0.0167 min^{-1} s^{-1}) used to calculate

power in mW and m_v is the ventricle mass in g (Farrell et al., 1996). The total vascular resistance was calculated as:

$$\text{Total vascular resistance} = \text{ventral aortic blood pressure}/\text{cardiac output}. \quad (3)$$

We estimated the tissue O_2 extraction using the following equation:

$$\text{Tissue } O_2 \text{ extraction} = \dot{M}_{O_2,\text{routine}}/\text{cardiac output}. \quad (4)$$

We report mean cardiovascular values taken in normoxia during steady-state conditions as described above, and during the hypoxia protocol by taking averages spanning ∼5% air saturation sections at 90%, 80%, 70%, 60%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% and 5% air saturation. We quantified the decline in heart rate during hypoxia (i.e. the hypoxic bradycardia, Δheart rate) by calculating both the absolute change, as well as the percentage change from routine values in normoxia.

We determined the P_{crit} for each individual using a segmental linear regression analysis in GraphPad prism for Windows (version 8.0.2, San Diego, CA, USA), where the $\dot{M}_{O_2,\text{routine}}$, determined from consecutive 1 min declines in O_2 during the hypoxia protocol, was plotted against Pw_{O_2} to identify the intersection of two linear regression lines fitted to the data (Yeager and Ultsch, 1989) (Fig. S3). Similarly, segmental linear regression analyses were used to determine the Pw_{O_2} where cardiac output and heart rate started to progressively decline in hypoxia (i.e. $P_{\text{cardiac output}}$ and $P_{\text{heart rate}}$), using the average values determined at the fixed Pw_{O_2} steps as described above. In these analyses, we excluded individual data points from fish that displayed transient activity inside the respirometer and were associated with elevated cardiorespiratory activity.

We calculated the temperature coefficients (Q_{10}) for $\dot{M}_{O_2,\text{routine}}$, cardiac output and heart rate between reference (X) and Biotest or acutely warmed reference (Y) perch in normoxia according to Seebacher et al. (2015):

$$Q_{10} = X/Y^{10/T_Y - T_X}, \quad (5)$$

where T_X and T_Y represents the reference and Biotest/acutely warmed reference test temperatures, respectively.

Hematocrit (Hct) was determined using heparinized microhematocrit capillary tubes, spun at 10,000 ***g*** for 5 min. Hemoglobin concentration ([Hb]) was determined using a Hb 201^+ meter (HemoCue® AB, Ängelholm, Sweden), and the values were corrected for fish blood according to Clark et al. (2008). Mean corpuscular [Hb] (MCHC) was calculated as:

$$\text{MCHC} = [\text{Hb}]/(\text{Hct}/100). \quad (6)$$

Anemic fish (Hct<15%) were excluded from all analyses.

Statistics

We used linear mixed models to assess the effects of hypoxia exposure on cardiorespiratory variables in perch from the different temperature regimes. Individuals were set as subject variables and the fixed Pw_{O_2} steps during hypoxia as the repeated variable, excluding the values at P_{LOE}. As fixed effects, the model included Pw_{O_2}, experimental group (Exp.group; i.e. reference perch, chronically warmed Biotest perch or acutely warmed reference perch) and their interaction (Pw_{O_2}×Exp.group). We found that a first-order autoregressive covariance structure provided the best fit to these data. We further explored significant main and interaction effects with either general (across the entire hypoxia exposure) or specific (i.e. at each Pw_{O_2} level) pair-wise comparisons of the responses between experimental groups. Significant main effects of the hypoxia exposure or interaction effects were further examined by comparing the values during hypoxia with the initial values at the onset of hypoxia exposure (i.e. 90% air saturation) across or within groups.

For all other comparisons among groups, we performed one-way ANOVA or a Welch's test for data displaying heterogeneous variances (i.e. length, body mass, $P_{\text{heart rate}}$ and tissue O_2 extraction), and significant main effects were assessed by pair-wise comparisons. We used paired two-tailed *t*-tests to compare hematological variables in normoxia and after hypoxia. We transformed ($\log_{10}$) variables that failed to meet the assumption of normality (Shapiro–Wilks test) or the assumption of homogeneity of variance (Levene's test). All *P*-values were Bonferroni adjusted for multiple testing, and statistical significance was accepted at $P \leq 0.05$. Statistical analyses were performed using SPSS (v.25, SPSS Inc., Chicago, IL, USA). We present all data as means±s.e.m.

RESULTS

There was no difference in body mass, length, condition factor or relative spleen mass among experimental groups (Table 1). However, chronically warmed Biotest perch had a lower relative ventricle mass compared with both reference (P=0.018) and acutely warmed reference perch (P=0.003; Table 1).

Effects of acute warming and thermal compensation of cardiorespiratory status in normoxia

Acute warming from 18 to 23°C in normoxic reference perch resulted in increased $\dot{M}_{O_2,\text{routine}}$ (105 to 163 mg O_2 kg^{-1} h^{-1}, Q_{10}: 2.2, F_2=37.4, P<0.001; Fig. 1A), cardiac output (from 32 to 47 ml min^{-1} kg^{-1}, Q_{10}: 1.7, F_2=6.1, P=0.042; Fig. 2A) and heart rate (from 60 to 101 beats min^{-1}, Q_{10}: 2.6, F_2=28.0, P<0.001; Fig. 2B), whereas stroke volume did not change (0.58 versus 0.47 ml $beat^{-1}$ kg^{-1}; Fig. 2C).

In the chronically warmed Biotest perch, considerable metabolic and cardiovascular thermal compensation was evident because $\dot{M}_{O_2,\text{routine}}$ was only 134 mg O_2 kg^{-1} h^{-1}, which was significantly lower than in the acutely warmed reference fish (P<0.001). However, the metabolic compensation was not complete as $\dot{M}_{O_2,\text{routine}}$ was still significantly elevated relative to that of reference perch at the lower temperature (Q_{10}: 1.5, P=0.001; Fig. 1A).

The metabolic adjustments in chronically warmed Biotest perch were also reflected in pronounced thermal compensation of cardiovascular function. Routine cardiac output in Biotest perch (32 ml min^{-1} kg^{-1}) was similar to that of reference fish measured at the lower temperature (Q_{10}: 0.8; Fig. 2A). This was a combined effect of significantly lower stroke volume (0.39 versus 0.47 ml $beat^{-1}$ kg^{-1}, F_2=6.7, P=0.002; Fig. 2C) and depressed heart rate relative to the acutely warmed reference fish (84 versus 101 beats min^{-1}, F_2=28.0, P<0.001; Fig. 2B), which meant that Q_{10} for heart rate in Biotest perch was reduced to 1.6. There were no differences in ventral aortic blood pressure, cardiac power output or total vascular resistance among treatment groups in normoxia (Fig. 3). While tissue O_2 extraction was not different in normoxia (Fig. 2D), the chronically warmed Biotest perch had a higher blood oxygen carrying capacity as indicated by elevated Hct and [Hb] compared with reference perch (F_2=3.9, P=0.033 and F_2=4.8, P=0.016, respectively), but there was no difference in MCHC (Fig. 4). Acutely warmed reference perch showed an intermediate hematological response and did not differ significantly from either of the other groups (Fig. 4).

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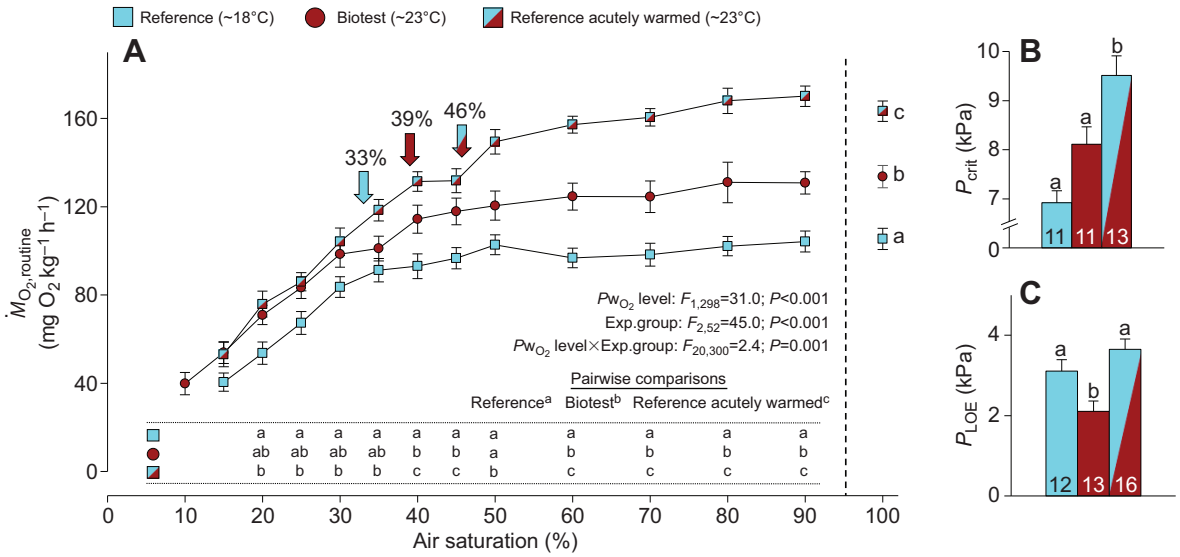

Fig. 1. Effects of gradual acute hypoxia on routine O_2 consumption rate and hypoxia tolerance metrics in European perch (*Perca fluviatilis*) at different acute and chronic temperatures. The figure shows routine O_2 consumption rate ($\dot{M}_{O_2,routine}$) in normoxia (90–100% air saturation) and during gradual hypoxia exposure (A), the critical oxygen tension for $\dot{M}_{O_2,routine}$ (P_{crit}; B) and O_2 tension at loss of equilibrium (P_{LOE}; C). Data are shown for reference perch tested at reference temperature (n=11–13), Biotest perch tested at Biotest temperature (n=9–12) and reference perch tested at Biotest temperature (i.e. acutely warmed; n=12–16). The vertical dashed line indicates the closing of the respirometers, after which the fish consumed the O_2 in the respirometer, inducing progressive hypoxia. The arrows indicate the P_{crit} (as percentage air saturation) for each respective group. The results from the repeated measures mixed model (see Materials and Methods) are displayed in A. Pw_{O_2}, water O_2 partial pressure. Differences among groups in $\dot{M}_{O_2,routine}$ in normoxia, P_{crit} and P_{LOE} were assessed with one-way ANOVA. The sample sizes for the metrics in B and C are indicated within each bar. Dissimilar letters denote statistically significant (P<0.05) differences among treatment groups. Values are means±s.e.m.

Effects of environmental warming on hypoxia tolerance and cardiorespiratory responses to hypoxia

The patterns and differences in cardiorespiratory traits observed in normoxia were generally maintained over the higher Pw_{O_2} range of the hypoxia exposure (Figs 1A and 2A–C). $\dot{M}_{O_2,routine}$ was significantly higher in chronically warmed Biotest perch compared with reference perch until 35% air saturation, and significantly lower than in acutely warmed reference perch until 30% air saturation. Importantly, however, the metabolic thermal compensation in Biotest perch was associated with an improved ability to maintain $\dot{M}_{O_2,routine}$ in hypoxia compared with the acutely warmed reference perch. Thus, P_{crit} in Biotest perch was intermediate relative to that of acutely warmed reference perch (P=0.022) and reference perch (P=0.08, F_2=14.2, P<0.001; Fig. 1B). However, this pattern was only partially reflected in the absolute hypoxia tolerance because Biotest perch had a significantly lower P_{LOE} than both the reference perch (P=0.045) and the acutely warmed reference perch (P<0.001), while there was no difference in P_{LOE} between the two reference groups (Fig. 1C).

The thermal compensation of P_{crit} in Biotest perch was associated with an improved ability to maintain cardiovascular function in hypoxia as the decline in heart rate started at a significantly lower Pw_{O_2} (i.e. lower $P_{heart\ rate}$) compared with reference and acutely warmed reference perch (Welch$_{2.18}$=14.9, P<0.001; P=0.01 and P=0.01, respectively; Fig. 2E), and there was a tendency for a lower $P_{cardiac\ output}$ compared with both reference (P=0.058) and acutely warmed reference perch (P=0.153; Fig. 2E). Even so, acute warming had no significant effect on $P_{cardiac\ output}$ and $P_{heart\ rate}$ in reference perch (Fig. 2E). If anything, there was also a tendency towards higher tissue O_2 extraction in the chronically warmed Biotest perch during hypoxia compared with both reference groups (P=0.06; Fig. 2D).

The decline in cardiac output in hypoxia were largely driven by reductions in heart rate because $P_{heart\ rate}$ always coincided with (Biotest perch) or preceded $P_{cardiac\ output}$ (both reference groups; Fig. 2E). While stroke volume generally increased from 35% air saturation to lower Pw_{O_2} across all groups, this increase did not fully compensate for the reduced heart rate (Fig. 2A–C). Nonetheless, the extent to which heart rate declined in absolute terms during hypoxia (i.e. hypoxic bradycardia) differed significantly among groups (Fig. S4A,B). The most pronounced bradycardia occurred in the acutely warmed reference perch (63 beats min^{-1}, 64% reduction), followed by chronically warmed Biotest perch (53 beats min^{-1}, 63% reduction) and, last, reference perch (40 beats min^{-1}, 65% reduction). Thus, the hypoxic bradycardia in relative terms was similar (Fig. S4B).

There were no differences among experimental groups in cardiac power output in hypoxia, which declined significantly from 40% air saturation in all groups (Fig. 3A). This was due to the reduced cardiac output because the ventral aortic blood pressure remained unchanged across intermediate Pw_{O_2} levels in all experimental groups (Fig. 3B). There was a general tendency (P=0.06) for differences amongst groups in vascular resistance, which increased across experimental groups from approximately 45% air saturation.

At the lowest Pw_{O_2} levels immediately preceding P_{LOE}, the ventral aortic blood pressure remained stable in chronically warmed Biotest perch, while both reference groups showed tendencies for a declining blood pressure.

After the hypoxia exposure, there were no significant differences among treatment groups for any of the hematological variables, but all groups showed significantly increased Hct relative to normoxic values (T=−10.5, P<0.001; T=−6.1, P<0.001; and T=−3.7, P=0.006 for reference, chronically warmed Biotest and acutely warmed reference, respectively). This was probably due to red blood cell swelling as MCHC was reduced across all experimental groups (T=4.5, P=0.003; T=8.5, P<0.001; and T=7.6, P<0.001, for reference, chronically warmed Biotest and acutely warmed

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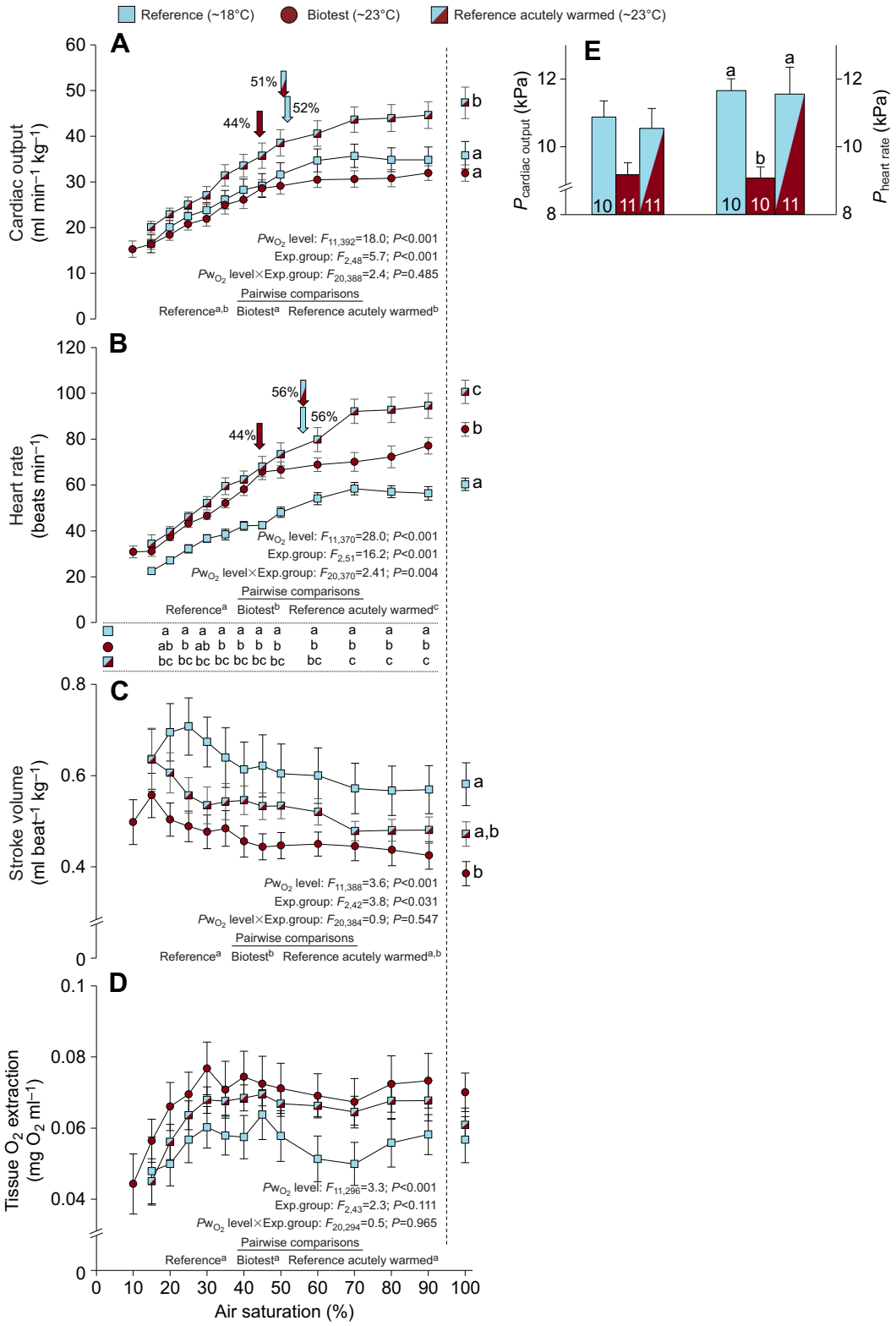

Fig. 2. Effects of gradual acute hypoxia on cardiorespiratory variables in European perch (*P. fluviatilis*) at different acute and chronic temperatures. The figure illustrates cardiac output (A), heart rate (B), stroke volume (C) and tissue oxygen extraction (D) in normoxia (90–100% air saturation) and during gradual hypoxia exposure, and the critical oxygen tension for cardiac output ($P_{cardiac\ output}$) and heart rate ($P_{heart\ rate}$; E). Data are shown for reference perch tested at reference temperature (n=9–15), chronically warmed Biotest perch tested at Biotest temperature (n=8–14) and reference perch tested at Biotest temperature (acutely warmed; n=10–14). The vertical dashed line indicates the closing of the respirometers, after which the fish consumed the O_2 in the respirometer, inducing progressive hypoxia. The arrows indicate $P_{cardiac\ output}$ and $P_{heart\ rate}$ (as percentage air saturation) for each treatment group. The results from the repeated measures mixed model (see Materials and Methods) are displayed in each panel. Differences among groups in normoxia were assessed with one-way ANOVA. The sample sizes for the metrics in E are given within each bar. Dissimilar letters denote statistically significant (P<0.05) differences between treatment groups. Values are means±s.e.m.

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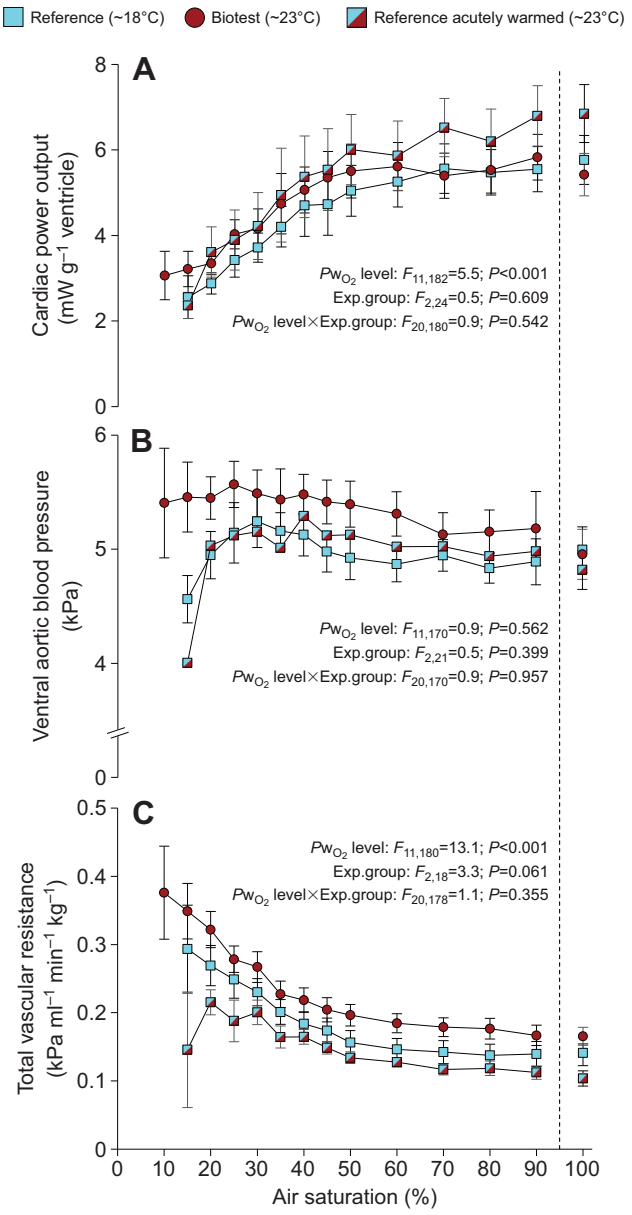

Fig. 3. Effects of gradual acute hypoxia on cardiovascular performance in European perch (*P. fluviatilis*) at different acute and chronic temperatures. The figure illustrates cardiac power output (A), ventral aortic blood pressure (B) and total vascular resistance (C) in normoxia (90–100% air saturation) and during gradual hypoxia exposure. Data are shown for reference perch tested at reference temperature (n=7), chronically warmed Biotest perch tested at Biotest temperature (n=6–7) and reference perch tested at Biotest temperature (acutely warmed; n=7–8). The vertical dashed line indicates the closing of the respirometers, after which the fish consumed the O_2 in the respirometer, inducing progressive hypoxia. The results from the repeated measures mixed model (see Materials and Methods) are displayed in each panel. Dissimilar letters denote statistically significant (P<0.05) differences among treatment groups. Values are means±s.e.m.

reference perch, respectively; Fig. 4C), while [Hb] did not consistently change in hypoxia (Fig. 4B).

DISCUSSION

We show that European perch exposed to chronic warming (possibly across lifetime or generations) in an artificially heated ecosystem display considerable physiological thermal compensation. Relative to acutely heated reference perch, this was primarily reflected in depressed $\dot{M}_{O_2,routine}$ along with improved capacity to maintain cardiovascular function and aerobic metabolism in hypoxia (i.e. reduced P_{crit}). The thermal compensation involved multiple changes along the oxygen transport cascade including elevated blood O_2 carrying capacity, reduced resting heart rate and cardiac output, as well as indications of increased tissue oxygen extraction. Interestingly, while chronic warming only elicited a partial thermal compensation of P_{crit}, Biotest perch had superior absolute hypoxia tolerance (i.e. the lowest P_{LOE}) relative to both groups of reference perch, irrespective of thermal regimes. This reveals a disconnect between P_{crit} and absolute hypoxia tolerance as measured by P_{LOE} across environmental temperatures, suggesting that factors beyond thermal compensation of $\dot{M}_{O_2,routine}$ and P_{crit} are important for safeguarding hypoxia tolerance with environmental warming. Taken together, our results suggest that European perch, and possibly other eurythermal fish species, may be relatively robust to transient hypoxic challenges in a warmer future.

Cardiorespiratory thermal compensation in normoxia is associated with improved maintenance of aerobic metabolism in moderate hypoxia

The thermal compensation of $\dot{M}_{O_2,routine}$ in normoxic Biotest perch (Q_{10}: 1.5) is identical to previous observations by Sandblom et al. (2016a), and was associated with a reduced cardiac output (Q_{10}: 0.8) resulting from lower stroke volume and smaller relative ventricle mass, along with a moderate re-setting of routine heart rate (Q_{10}: 1.8). This last response is also consistent with previous findings and probably reflects an increased inhibitory cholinergic (i.e. vagal) tone on the heart in Biotest perch (Sandblom et al., 2016a,b). Although routine cardiac output was fully compensated in Biotest perch, they still had to sustain a slightly higher $\dot{M}_{O_2,routine}$. This was achieved through elevated blood O_2 carrying capacity (i.e. higher Hct and [Hb]) and probably elevated tissue O_2 extraction.

Two previous studies have estimated P_{crit} in European perch. Thuy et al. (2010) and Bilberg et al. (2010) reported P_{crit} values of 3.4 kPa (20°C) and 4.4 kPa (16°C), respectively, in hatchery reared perch. The higher P_{crit} reported here for reference perch under similar thermal conditions (6.9 kPa at ~18°C), in part probably reflects that we defined P_{crit} as the breakpoint in $\dot{M}_{O_2,routine}$, while the previous studies estimated P_{crit} as the Pw_{O_2} where $\dot{M}_{O_2}$ declined below basal or standard $\dot{M}_{O_2}$. Another contributing factor may have been that surgical instrumentation in the current study elevated $\dot{M}_{O_2,routine}$ (previous perch P_{crit} measures were performed on uninstrumented fish). Indeed, the resting $\dot{M}_{O_2}$ of perch from the same populations as the current study were lower in a previous study using uninstrumented fish (Sandblom et al., 2016a). Thuy et al. (2010) showed a significant positive correlation between P_{crit} and resting $\dot{M}_{O_2}$ in perch, so it is entirely plausible that instrumentation and a corresponding elevated $\dot{M}_{O_2,routine}$ led to the elevated P_{crit} estimates observed here. Regardless, our experimental protocol provides a robust comparative approach to determine the influence of warming across different time scales on hypoxia tolerance, while combining assessment of hypoxia tolerance and *in vivo* cardiorespiratory responses in individual fish.

Acute warming increased P_{crit} in reference perch, which is consistent with findings in other fish species (Borowiec et al., 2016; Butler and Taylor, 1975; Corkum and Gamperl, 2009; Ern et al., 2016; Fry and Hart, 1948; Gehrke and Fielder, 1988; Nilsson et al., 2010; Rogers et al., 2016). The reduced P_{crit} in Biotest perch relative to the acutely warmed reference perch is consistent with our hypothesis that, through various metabolic and cardiorespiratory adjustments, fish subjected to long-term environmental warming have the capacity to alleviate at least some of the detrimental

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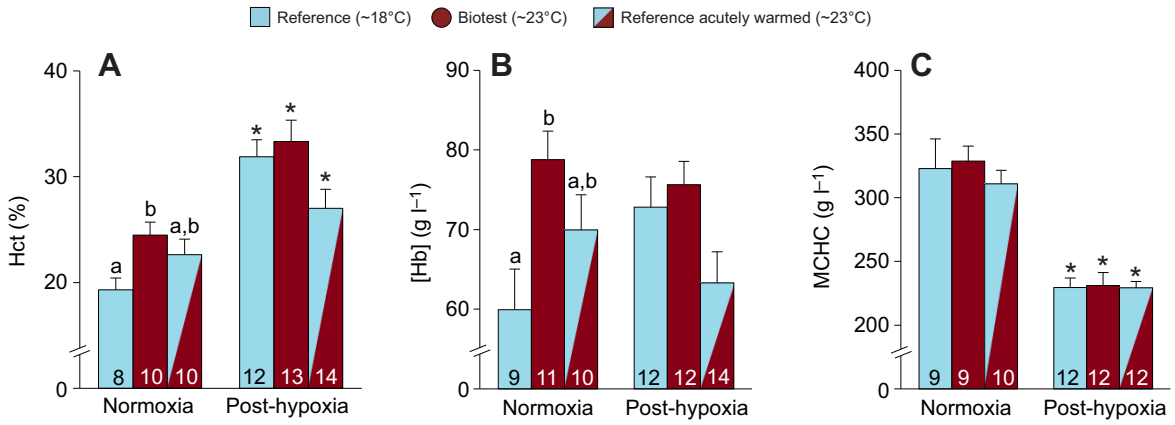

Fig. 4. Hematological variables in European perch (*P. fluviatilis*) in normoxia and after acute severe hypoxia exposure at different acute and chronic temperatures. The figure illustrates the hematocrit (Hct; A), hemoglobin concentration ([Hb]; B) and mean corpuscular hemoglobin concentration (MCHC; C) in normoxia (90–100% air saturation) and after acute hypoxia exposure (i.e. post-hypoxia). Data are shown for reference perch tested at reference temperature, chronically warmed Biotest perch tested at Biotest temperature and reference perch tested at Biotest temperature (acutely warmed). Differences among groups were assessed by one-way ANOVA, and differences within groups (i.e. normoxia versus post-hypoxia) were evaluated using paired *t*-tests. The sample size for each treatment group is given within each bar. Dissimilar letters denote statistical differences (P<0.05) among treatment groups and asterisks denote statistical differences (*P<0.05) within groups. Values are means±s.e.m.

impacts of warming on the ability to maintain aerobic metabolism during acute hypoxia exposure. It is likely that the partial thermal compensation of P_{crit} is related to the partial thermal compensation of $\dot{M}_{O_2,routine}$ in Biotest perch, because the normoxic aerobic metabolic rate is well known to be positively correlated with P_{crit} in perch (Thuy et al., 2010) and other fish species (McBryan et al., 2016; Nilsson et al., 2010; Rogers et al., 2016). Assuming that the hematological differences among groups in normoxia were also present around P_{crit}, it is also possible that an improved blood O_2 carrying capacity contributed to the lowering of P_{crit} in the chronically warmed Biotest perch.

Regardless of temperature, $P_{heart\ rate}$ and $P_{cardiac\ output}$ always occurred at higher Pw_{O_2} than P_{crit}, supporting the idea that the bradycardia and ensuing decline in cardiac output in hypoxia relate to the onset of P_{crit} as reported in other fish species (Gehrke and Fielder, 1988; McKenzie et al., 2009; Rantin et al., 1993; Speers-Roesch et al., 2010). However, reference perch at the lower temperature were better able to sustain $\dot{M}_{O_2,routine}$ at Pw_{O_2} values below $P_{cardiac\ output}$, which was probably mediated by an increase in tissue O_2 extraction as hypoxia developed. By contrast, tissue O_2 extraction was already elevated in normoxia in the acutely warmed reference perch, which probably left less scope for compensation through increased extraction in hypoxia and a more rapid decline in $\dot{M}_{O_2,routine}$ as heart rate and cardiac output started to plummet.

Biotest perch had an improved ability to maintain cardiac output in hypoxia, as $P_{heart\ rate}$ and $P_{cardiac\ output}$ occurred at lower Pw_{O_2}, whereas these breakpoints occurred at very similar Pw_{O_2} in reference perch at the two test temperatures. These observations could indicate that hypoxic bradycardia in perch is elicited by internally oriented chemoreceptors located in one or several gill arches (Milsom, 2012; Reid and Perry, 2003), which are stimulated by reduced partial pressure of O_2 in the venous blood (Pv_{O_2}) as hypoxia progresses (Perry and Reid, 1994; Steffensen and Farrell, 1998). Thus, the lower $P_{heart\ rate}$ in Biotest perch could be attributed to the fact that chronic warming elevates Pv_{O_2} relative to reference perch (Ekström et al., 2016a), and therefore postpones the decline in Pv_{O_2} and the corresponding stimulation of branchial chemoreceptors during hypoxia. This would cause the bradycardia to occur at a lower ambient Pw_{O_2} relative to that in reference perch. Moreover, it was recently shown that acute warming does not significantly lower Pv_{O_2} in reference perch over the temperature interval tested here (i.e. 18–23°C) (Ekström et al., 2016a), which could explain why the bradycardia occurred at a similar Pw_{O_2} in reference perch across test temperatures.

The increased vagal tone assumed to be associated with the thermal compensation of routine heart rate (Ekström et al., 2016b) did not interfere with the capacity to induce a vagally mediated hypoxic bradycardia in perch. The consequent depression of cardiac power output and thus cardiac O_2 demand, which was similar among experimental groups, could have aided prevention of a mismatch between cardiac O_2 supply and demand, and could thus explain how the perch heart was able to maintain an elevated stroke volume in severe hypoxia (Farrell and Stecyk, 2007; Speers-Roesch et al., 2010; Stecyk, 2017). Even while cardiac output progressively declined with bradycardia, compensatory elevations in total vascular resistance resulted in the maintenance of ventral aortic blood pressure at diminishing O_2 levels beyond P_{crit} across groups.

Absolute hypoxia tolerance of perch is resilient to acute and chronic warming

Despite a substantially elevated P_{crit}, acute warming did not affect P_{LOE} in reference perch, which contrasts with reports in other fish species (Borowiec et al., 2016). The lack of an effect of acute warming on P_{LOE} is enigmatic, but may be associated with an increased transcription of heat shock proteins during the ∼20 h acute heat exposure. For example, the induction of heat shock proteins following an acute heat shock may have contributed to an improved ability to survive subsequent hypoxia in an intertidal fish (*Oligocottus maculosus*) (Todgham et al., 2005). The maintenance of P_{LOE} in acutely warmed perch may also reflect an improved anaerobic capacity, which has been identified as a driver of hypoxia tolerance in other fish species (Borowiec et al., 2016; Mandic and Regan, 2018; McArley et al., 2019; Richards et al., 2009). Indeed, acute warming may elevate the expression and/or the activity of the anaerobic glycolytic enzyme lactate dehydrogenase in fish, including perch (Ekström et al., 2016c; Iftikar et al., 2014).

As measured by P_{LOE}, chronically warmed Biotest perch not only maintained a greater level of hypoxia tolerance than the acutely

warmed perch, which was expected, but also maintained a greater level of hypoxia tolerance than reference perch at the lower test temperature. Thus, if P_{LOE} is taken as a reliable measure of absolute hypoxia tolerance (Wood, 2018), then perch actually became more hypoxia tolerant with chronic warming. Similar to the lack of difference in P_{LOE} between acutely warmed and reference perch despite a pronounced difference in P_{crit}, the improved hypoxia tolerance (lower P_{LOE}) of Biotest perch relative to reference perch (18°C) was not impeded by a higher P_{crit}. This is not to say that thermal compensation of $\dot{M}_{O_2,routine}$ and P_{crit} is unimportant for safeguarding hypoxia tolerance with chronic warming, only that the lack of a direct association between P_{crit} and P_{LOE} across treatment groups demonstrates that other factors make an equal or more important contribution to hypoxia tolerance. This disconnect between P_{crit} and absolute hypoxia tolerance as measured by LOE is not unique to the current study, as this has been reported in several other fish species (Borowiec et al., 2020; Mandic et al., 2013; McArley et al., 2020; Speers-Roesch et al., 2013).This points to the fact that the ability to maintain aerobic ATP production at low Pw_{O_2} as measured by P_{crit}, while undoubtedly important, is only one part of a species' total hypoxia tolerance strategy, and that P_{crit} does not necessarily reflect absolute hypoxia tolerance (Mandic et al., 2013; Wood, 2018). In regards to the current study, the mechanisms by which perch are able to improve P_{LOE} with chronic warming despite a higher P_{crit} remain to be elucidated. However, one possibility could be that prolonged warming stimulates an enhanced capacity for anaerobic ATP production (e.g. through increased anaerobic fuel stores such as glycogen and/or enhanced activity and expression of glycolytic enzymes) (Mandic and Regan, 2018; Mandic et al., 2013; Speers-Roesch et al., 2013). There may also be an important behavioral component of hypoxia tolerance in perch. During 4 h exposure to severe hypoxia (~23% air saturation at 23°C), which was survivable, perch were reported to rest in a stationary position on the bottom of tanks (Douxfils et al., 2012). In the same study, despite the level of hypoxia exposure being substantially below the P_{crit} of Biotest perch (39% air saturation at 23°C) measured here, there was no evidence of lactate accumulation in white muscle following hypoxia exposure, suggesting anaerobic metabolism was not recruited. The quiescent behavior of the fish was probably important for reducing energetic demands, so that aerobic ATP production remained sufficient throughout hypoxia exposure. If there was an unmeasured difference in the behavioral response to hypoxia among the treatments in the current study, this could also have contributed to the lack of correlation between P_{crit} and P_{LOE}. Moreover, we recently showed that Biotest perch exhibited higher expression of mitochondrial genes *nd4* and *cox1*, which encode subunits for complex I and IV, respectively, in the mitochondrial respiration chain, which is indicative of molecular adaptation in the Biotest perch (Pichaud et al., 2020). It is therefore possible that the differences between Biotest and reference perch in terms of their tolerance to hypoxia may be attributable to some, as yet unknown, genetically adapted trait(s) in the Biotest perch.

A legitimate criticism of the method used in the current study to assess P_{crit} and P_{LOE} is that the rate of hypoxia induction differed between treatment groups. This was the result of using closed system respirometry to generate hypoxia: a higher $\dot{M}_{O_2}$ in the warm treatments drove a faster rate of O_2 decline in the respirometer (0.58, 0.49 and 0.33 kPa min^{-1} in the acutely warmed, Biotest and reference treatment groups, respectively). There was no evidence, however, of a faster rate of hypoxia induction relating to improved hypoxia tolerance (i.e. there was no correlation between the rate of hypoxia induction and P_{LOE} within treatment groups; Fig. S5). Thus, while we cannot rule out different findings using a common rate of hypoxia induction (i.e. through bubbling nitrogen to generate hypoxia), the unexpectedly low P_{LOE} of the acutely warmed reference and Biotest perch is not explained by the fact that these groups underwent a faster rate of hypoxia induction. Another potential caveat worth mentioning is that the closed respirometry approach to eliciting hypoxia employed here may have, to different extents, induced accumulation of metabolic by-products (e.g. CO_2) inside the respirometers (see review by Rogers et al., 2016). Whether or not this occurred here, or whether this affected the outcome of the current study is, however, unknown.

Implications of perch hypoxia tolerance in natural habitats under climate change

The hypoxia exposure used in the current study was highly acute in all treatment groups (total duration of 52, 34 and 28 min in the reference, Biotest and acutely warmed reference groups, respectively; Fig. S2). Such fast development of inescapable hypoxia would be unlikely to occur in the natural habitats of perch, if in any habitats occupied by fish. Even in shallow vegetated lakes and intertidal rock pools, both habitats with pronounced diel O_2 cycles, night-time hypoxia develops progressively over several hours (e.g. Andersen et al., 2017; McArley et al., 2019). Where tolerance to severe, acute hypoxia may be relevant to perch is when juveniles move into deeper water pelagic hypoxic zones to feed and avoid predation – a behavior known to occur in this species (Vejřík et al., 2016). Of course, when utilizing pelagic hypoxic zones, juveniles are not trapped and can migrate vertically to avoid hypoxia if it becomes too severe. Indeed, avoidance of hypoxia – moving from hypoxia to areas of higher Pw_{O_2} – has been demonstrated in perch (Alabaster and Robertson, 1961). Nevertheless, if the resilience to acute hypoxia following chronic warming in adult perch observed here is also present in juvenile perch, then this behavior may be robust to climate warming. Other than severe acute hypoxia exposure, perch could also face longer periods of severe to moderate hypoxia. In the Baltic Sea, periods of days to months of severe (Pw_{O_2}<2 mg l^{-1}) and moderate (Pw_{O_2}=2–4 mg l^{-1}) hypoxia are well documented in shallow coastal habitats important to perch (Conley et al., 2011). If such hypoxia occurs over widespread areas so that behavioral avoidance is prevented, then perch may have to tolerate prolonged episodic hypoxia exposure at warmer temperatures under climate change. We do not currently know what levels of hypoxia are survivable for Baltic Sea perch over prolonged time periods and how warming impacts tolerance to hypoxia of this nature. Furthermore, even when prolonged hypoxia is survivable, there may be important negative consequences for animal performance (e.g. growth, swimming and reproduction), which could be worsened by warming. Thus, developing a more complete picture of the resilience or susceptibility of Baltic Sea perch populations to hypoxia under climate warming will require examining responses to a range of ecologically relevant hypoxia regimes. Despite these caveats and the continued threat of worsening hypoxia due to climate change, the findings of the current study point to cautious optimism that climate warming may have somewhat less dire consequences for absolute tolerance to severe, acute hypoxia in perch and other eurythermal fishes than previous prognoses have predicted.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.E., A.G., M.H., E. Sandblom; Methodology: A.E.; Validation: A.E.; Formal analysis: A.E.; Investigation: A.E., E. Sundell, D.M., T.M.; Resources: A.G., M.H., E. Sandblom; Data curation: A.E.; Writing - original draft: A.E.; Writing - review & editing: A.E., E. Sundell, D.M., T.M., A.G., M.H., E. Sandblom; Visualization: A.E.; Supervision: A.E., E. Sandblom; Project administration: A.G., M.H., E. Sandblom; Funding acquisition: A.G., E. Sandblom.

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Data availability

Data are available from the Dryad digital repository (Ekström, 2021): dryad.tmpg4f4z3

Supplementary information

Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.241554.supplemental

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