Cardiac oxygen limitation during an acute thermal challenge in the European perch: Effects of chronic environmental warming and experimental hyperoxia.

Running head: Oxygen limitation of cardiac thermal tolerance in perch

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Abstract

Oxygen supply to the heart has been hypothesized to limit cardiac performance and whole animal acute thermal tolerance (CTmax) in fish. We tested these hypotheses by continuously measuring venous oxygen tension (PVO2) and cardiovascular variables in vivo during acute warming in European perch (Perca fluviatilis) from a reference area during summer (18°C) and a chronically heated area (Biotest enclosure) that receives warm effluent water from a nuclear power plant and is normally 5-10°C above ambient (24°C at the time of experiments). While CTmax was 2.2°C higher in Biotest compared to reference perch, the peaks in cardiac output and heart rate prior to CTmax occurred at statistically similar PVO2 values (2.3 – 4.0 kPa), suggesting that cardiac failure occurred at a common critical PVO2 threshold.

Environmental hyperoxia (200% air saturation) increased PVO2 across temperatures in reference fish, but heart rate still declined at a similar temperature. CTmax of reference fish increased slightly (by 0.9°C) in hyperoxia, but remained significantly lower than in Biotest fish despite an improved cardiac output due to an elevated stroke volume. Thus, while cardiac oxygen supply appears critical to elevate stroke volume at high temperatures, oxygen limitation may not explain the bradycardia and arrhythmia that occur prior to CTmax. Acute thermal tolerance and its thermal plasticity can therefore only be partially attributed to cardiac failure from myocardial oxygen limitations, and likely involves limiting factors on multiple organizational levels.

Keywords: cardiac performance, myocardial oxygenation, PVO2 threshold, thermal acclimation, teleost
Introduction

Models of future climate change predict a warming of average global temperatures and an increase in the frequency, duration and intensity of transient summer heat waves (32). Such acute thermal events may represent an important selective force as they expose ectothermic animals such as fish to temperatures outside their thermal tolerance range. Indeed, tolerance to extreme thermal events has been suggested to constitute an important determinant for species geographical distribution (62) and a key trait subjected to natural selection (see 11).

Although the influence of temperature on the biology of fish has been studied for well over a century, the physiological mechanisms determining acute thermal tolerance (termed the critical thermal maximum, $CT_{\text{max}}$) are still not fully understood (4, 9). Heart failure has been proposed as a possible factor governing thermal tolerance in fish and other ectotherms (8, 21, 41). This idea builds on the observation that heart rate ($f_H$) and cardiac output ($Q$) initially rise exponentially with acute warming, plateau, and then plummet at high temperatures preceding $CT_{\text{max}}$, indicating the onset of cardiac failure. Indeed, such patterns have been demonstrated in vivo in numerous ectothermic animals including fish (8, 18, 21, 34, 43, 52, 56), as well as with anaesthetised fish pharmacologically treated to elicit maximal heart rate (1, 6). It has been hypothesized that limitations in cardiac oxygen supply constrain myocardial aerobic ATP production and lead to the observed heart failure (8, 21, 41).

This hypothesis is related to the fact that about two thirds of all teleost species lack coronary arteries and only possess trabecular (spongy) myocardium, while the other third have coronaries supplying oxygenated arterial blood to the compact layer of the myocardium (2, 14, 65). This differs from mammals, for example, where the vast majority of the adult heart is composed of compact myocardium supplied by an extensive coronary circulation (54). Thus, the most important (and often exclusive) oxygen source for the heart in most fishes is from the luminal venous blood returning to the heart from the systemic circulation.
(14). As increasing temperature results in increased tissue oxygen extraction due to the elevated oxygen demand of the tissues, warming progressively lowers the partial pressure of oxygen in the returning venous blood ($P_{\text{VO}_2}$) (8, 37, 41). It has therefore been proposed that at a certain species-specific critical $P_{\text{VO}_2}$ threshold, myocardial oxygen diffusion becomes insufficient and leads to a deterioration of cardiac performance (8, 37, 41), which inevitably causes further reductions in tissue and cardiac oxygen supply (14, 24, 26). However, as most of the current information stems from studies measuring $P_{\text{VO}_2}$ in extracted venous blood (8, 37) (but see Farrell and Clutterham (24)), pinpointing an exact $P_{\text{VO}_2}$ threshold value for cardiac collapse at high temperature is difficult. Additionally, the majority of previous studies have been conducted on salmonids, which have a well-developed coronary supply, and thus an alternative route for myocardial oxygenation (14).

As far as we are aware, the only study evaluating the relationship between cardiac oxygenation and performance in a species lacking coronaries, the Atlantic cod (Gadus morhua), used surgically implanted oxygen optodes for on-line recordings of venous oxygen tension and magnetic resonance imaging to determine relative changes in blood flow (41). While that study reported that oxygen availability limited $f_{Hi}$ and $Q$ at high temperatures, absolute blood flow and blood pressure were not determined and thus prevented a more detailed analysis of the underlying hemodynamic mechanisms. Thus, there is a need for further detailed evaluations of the relationship between cardiac oxygenation and cardiovascular performance in fish at critically high temperatures. Moreover, previous studies attempting to elucidate the limiting factors for cardiac performance at elevated temperatures have been carried out under normoxic conditions, making it difficult to discriminate between the indirect effects of temperature on cardiac oxygen supply and the direct effects of temperature *per se* on cardiovascular function. Manipulations of oxygen availability (e.g. ambient environmental hyperoxia) may aid in distinguishing between these factors and their
effects on cardiovascular function and thermal tolerance. In fact, thermal tolerance has been shown to increase with ambient hyperoxia in some studies (67), perhaps as a consequence of improved cardiac function. Yet, to our knowledge this possibility has never been thoroughly investigated in fish.

Furthermore, acute thermal tolerance is known to increase with warm acclimation in fish (3, 12, 52, 60, 61), but the mechanistic basis for this is not clear. As $P_{\text{VO}_2}$ increases with warm acclimation (24, 46), it is possible that improved cardiac oxygenation due to elevated $P_{\text{VO}_2}$ may explain the beneficial effects of warm acclimation on $CT_{\text{max}}$ and cardiac thermal tolerance. Moreover, a decrease in resting cardiac output following warm acclimation (35, 48, 49), would presumably lead to reduced oxygen demand of the heart and further improve function at high temperatures. Nonetheless, a key limitation in our general understanding of physiological responses to warm acclimation in fish, including the implications of global warming, stems from a lack of appropriate systems where fish have been warm-acclimated for durations that are representative of progressive climate warming scenarios.

In the present study we examined wild European perch (*Perca fluviatilis*) from inside (24°C) and outside (18°C) the ‘Biotest enclosure’, which is an enclosure in the Baltic Sea that has been warmed by effluent water from a nuclear power plant for over 30 years (see Materials and methods). Morphological examinations indicate that the European perch lacks coronary vessels, and is therefore exclusively dependent on luminal oxygen supply for myocardial oxygenation (AE unpublished observation and (51)). This is also supported by recent molecular phylogenetic analyses suggesting that perciforms lack a coronary vasculature (27). Specifically, we used surgically implanted oxygen optodes and blood flow probes to study the relationship between cardiac oxygenation, cardiovascular performance and $CT_{\text{max}}$ during a thermal ramping protocol. By using warm-acclimated fish from inside the Biotest enclosure and reference fish from the adjacent Baltic Sea, we were able to test the
hypothesis that increases in $CT_{\text{max}}$ with warm acclimation can be explained by elevated $P_{\text{VO}_2}$ improving myocardial oxygenation and cardiac thermal tolerance. To further test the hypothesis that cardiac oxygen supply constitutes an important limiting factor for thermal tolerance and cardiac performance at temperatures approaching $CT_{\text{max}}$, an additional group of reference perch (18°C) was tested under ambient hyperoxia (200% air saturation), with the expectation that this treatment would increase $P_{\text{VO}_2}$ and lead to elevated $CT_{\text{max}}$ and improved cardiac thermal tolerance.

Materials and methods

Study area and experimental animals

The study was conducted in August 2013 at the Biotest enclosure, which is an artificial enclosure located in the Baltic Sea near Forsmark in Sweden (60°25'41.5"N 18°11'20.8"E). The Biotest enclosure is about 1 km² and was constructed in the late 1970s by building dikes in between a series of natural islands in the archipelago, thus creating an enclosure within the surrounding Baltic Sea with the natural ecosystem including a population of European perch ($\textit{Perca fluviatilis}$, Linneaus) remaining in place (50). The Biotest enclosure has received warmed cooling water from two of the reactors at the Forsmark nuclear power plant since 1980, which increases the average water temperature of the Biotest enclosure by approximately 5°C in summer and approximately 10°C in winter compared with the temperature of the surrounding Baltic Sea (38, 48, 50).

European perch were caught by hook and line. Reference fish were collected from the cooling water intake channel upstream of the power plant, where thermal conditions are natural. Chronically heated fish (Biotest fish) were caught at the cooling water outlet where it flows into the Biotest enclosure downstream of the power plant. Information on fish body characteristics and environmental temperatures for the capture sites are presented in Table 1.
Following capture, the fish were transferred to 1200 l holding tanks continuously supplied with a flow-through of aerated water from either the Baltic Sea (~17°C) or from the Biotest enclosure (~23°C) (both ~5 ppt salinity). The temperatures in the holding tanks were maintained at the reference and Biotest thermal conditions as appropriate (see acclimation temperatures, Table 1) using 6 kW water heaters (VB 6010 L, Värmebaronen, Österslöv, Sweden). Fish were held in the holding tanks for at least three days prior to experimentation and were not fed. All procedures were approved by ethical permit 65-2012 issued from the ethical committee of Gothenburg.

Surgery and instrumentation
Fish were anaesthetized in water containing tricaine methanesulfonate (MS-222, 100 mg l\(^{-1}\), Western Chemical Inc., Ferndale, WA, USA), and placed left side up on wet foam on a surgery table. The gills were continuously irrigated with aerated water (8-10°C) containing MS-222 (50 mg l\(^{-1}\)). To record mean ventral aortic blood pressure (P\(_{Va}\)), the third afferent branchial artery was cannulated. A 4-0 silk ligature was placed around the gill arch and the artery was then punctured, upstream from the ligation at the base of the gill filaments. A PE-31 catheter (Natsume, Tokyo, Japan) filled with heparinized saline (25 IU ml\(^{-1}\)) was advanced into the vessel towards the ventral aorta (66), hence occluding 1/8 of the total gill surface. The catheter was secured using the ligation suture and an additional suture placed around the gill arch approximately 5 mm below the point of insertion, as well as with skin sutures.

To record P\(_{VO2}\), a 1mm (Ø) fiberoptic Firesting O\(_2\) optode (Pyroscience, Aachen, Germany) was inserted into the ductus of Cuvier (25). Briefly, a small incision was made in the left cleithrum and the ductus of Cuvier was exposed using blunt dissection. A small hole was cut in the vessel and the optode was inserted pointing towards the sinus venosus. The incision was then closed around the optode using a 4-0 silk suture and the optode lead was
attached to the skin with 3-0 silk sutures. Two additional sutures were placed along the dorsal fin securing and orienting the optode tip centrally in the sinus venosus.

To record Q, a small incision was made in the left opercular cavity and the ventral aorta was dissected free without damaging the pericardium. A Transonic blood flow probe (model 2.5PSL or 2.5PSB; Transonic systems Inc., Ithaca, NY, USA) was fitted around the vessel (16). The probe lead was fixed with sutures close to the opercular cavity and with the same dorsal sutures used to attach the optode lead.

Following instrumentation, the fish were individually placed in experimental chambers maintained at their respective environmental temperature and were allowed 24-48 hours of post-surgical recovery. To avoid external visual stimuli, the experimental chambers were surrounded by black plastic drapes and the fish were monitored using a camera mounted above the experimental setup.

Experimental protocol

Thermal challenge experiments were performed in reference (n=11) and Biotest (n=11) fish under normoxic conditions, and in an additional group of reference fish (n=10) under environmental hyperoxia (200% water air saturation), achieved by bubbling the water with a regulated flow of pure O₂. Three fish were examined each day, with experimental groups randomized throughout the duration of the experimental period. All experiments began in the morning by recording resting cardiovascular variables at 19°C for 4-6 hours. A resting blood sample was then collected (approx. 0.3 ml) from the ventral aortic catheter into a heparinized syringe, which was followed by an additional hour of recordings of resting variables before starting the thermal protocol. The temperature was elevated in 1°C increments using a 6 kW water heater controlled by a digital thermostat (EW 7221, Crn Tecnopart, Barcelona, Spain). Each temperature was maintained for 20 minutes (i.e. 3°C h⁻¹) to allow the body temperature
of the fish to reach thermal equilibrium with the surrounding water (13, 59). Loss of the
righting reflex for more than 3 seconds was considered as the endpoint to determine $CT_{\text{max}}$
(3). Immediately following $CT_{\text{max}}$, a second blood sample was collected. The fish was
subsequently removed from the experimental chamber and sacrificed with a sharp blow to the
head. In the rare instances where the second (final) blood sample could not be collected from
the catheter, blood was drawn from the caudal vessels using a heparinized syringe. Following
termination of the experiments, the ventricle was dissected out and the ventricle mass was
measured.

Data acquisition

The ventral aortic catheter was connected to a pressure transducer (model DPT-6100, Pvb
Medizintechnik, Kirchseeon, Germany) calibrated against a static water column with the
water level in the experimental chamber as the zero reference. The blood pressure signal was
amplified using a Senselab 4ChAmp amplifier (Somedic sales, Hörby, Sweden). The oxygen
optode was connected to a Firesting fibreoptic $O_2$ meter (Pyroscience, Aachen, Germany),
which was calibrated by two-point calibration prior to implantation. The blood flow probe
was connected to a Transonic blood flowmeter (model T403, Transonic Systems Inc., Ithaca,
NY, USA). The water temperature in the experimental chamber was recorded continuously
using a temperature logger integrated with the water thermostat (EW 7221, Crn Tecnopart,
Barcelona, Spain). The analog outputs from the recording equipment were relayed to a
PowerLab system (ADinstruments, Sydney, Australia) connected to a computer running
LabChart Pro (version 7.03; ADInstruments, Bella Vista, Australia) for on-line data

Data analysis
Mean routine cardiovascular values (i.e. $Q$, $f_H$, $V_S$, CPO, $P_{Va}$ and $R_{tot}$) were typically taken during the last 1-2 minutes prior to an increase in temperature when variables were stable. Fish that were anemic (hematocrit < 15%) were excluded from the analysis. Cardiac output was determined from the blood flow data from the Transonic flow probes and normalized to body mass. All Transonic flow probes were factory calibrated to 10°C and flow recordings were not temperature corrected during the thermal challenge experiments. Bench calibration of similar probes according to manufacturer's instructions at different temperatures have revealed that flow is overestimated by ~20% at 19°C, and that this deviation is reduced with increasing temperature as flow is only overestimated by ~15% or less at temperatures exceeding 25°C (Ekström et. al. unpublished). While this overestimation should be taken into account when comparing the absolute flow data to previous studies, it does not affect the comparisons between experimental groups in this paper as the same probes were used across experimental groups. Ventral aortic blood pressure was measured in the ventral aortic catheter and mean pressure was calculated using the blood pressure module in LabChart Pro. Heart rate was determined from the pulsatile blood flow or pressure traces. Stroke volume was calculated according to equation [1]:

\[
V_S = \frac{Q}{f_H} \quad [1]
\]

Cardiac power output (in mW g ventricle$^{-1}$) was estimated according to equation [2] from Farrell et al. (20):

\[
CPO = \frac{(Q \times P_{Va}) \times K}{m_v} \quad [2]
\]

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 grams. Total vascular resistance was calculated according to equation [3]:

\[
R_{tot} = \frac{P_{Va}}{Q} \quad [3]
\]

The $P_{VO2}$ was recorded continuously in LabChart as % O$_2$ saturation and converted to partial pressure for O$_2$ ($P_{VO2}$) according to equation [4]:
\[ P_{VO2} = (P_a - P_w) \times 0.2095 \times (\% \text{ air saturation} / 100) \]  \[4\]

where \( P_a \) and \( P_w \) are the temperature-dependent atmospheric pressure and water vapor pressure, respectively, and 0.2095 is the fraction of available oxygen in air.

Values for the peak response and the temperature where the peak response in \( Q, f_H \) and CPO occurred during the thermal challenge were determined for each individual and averaged. Consequently, due to the slight differences in thermal reaction norms among individual fish, data presented from this analysis (i.e. Table 2) may differ numerically from the apparent peak responses in the mean curves presented in Figure 1.

The \( P_{VO2} \) thresholds for cardiac performance were taken as the \( P_{VO2} \) values where \( Q \) and \( f_H \) peaked. The magnitude of the change (i.e. the scope) in \( f_H, Q \) and CPO was assessed by calculating the difference between routine values at 19°C and peak values. Similarly, \( Q_{10} \) values were calculated between 19°C and the temperature where the peak value occurred using Van’t Hoff’s equation (53) where relevant.

All peak, threshold, scope and \( Q_{10} \) values were calculated and expressed as the mean for all individuals within a given experimental group.

Blood hemoglobin concentration (Hb) was determined in duplicate using a handheld Hb 201+ meter (HemoCue® AB, Ängelholm, Sweden) with the values corrected for fish blood according to Clark et al. (7). Hematocrit (Hct) was determined in duplicate using heparinized microhematocrit capillary tubes, spun at 10000 \( g \) for 5 minutes. Mean corpuscular hemoglobin concentration (MCHC) was determined according to equation [5].

\[ \text{MCHC} = \frac{\text{Hb}}{\text{Hct} / 100} \]  \[5\]

**Statistics**

To describe the temporal effects of the temperature ramping protocol we used a linear mixed model with individuals as subject variables and temperature as the repeated variable. In the
model we included temperature (19-32°C), experimental group (reference, reference hyperoxia and Biotest) and their interaction as fixed effects. First-Order Autoregressive (AR(1)) was the type of covariance structure that gave the best fit to the data (i.e. lowest Akaike’s Information Criterion indicated that recordings that were close in time were also more dependent than temporally-distant recordings). If significant main effects were observed, these were further explored using 1) a pair-wise comparison of all variables at each temperature (i.e. to identify differences between the three experimental groups) and 2) a comparison of all variables at all temperatures to the 19°C reference value (i.e. to identify differences caused by temperature within the three experimental groups).

For the non-repeated variables (i.e. $CT_{\text{max}}$, temperature threshold, $Q_{10}$, hematological, morphometric, cardiovascular routine-, peak- and scope-values) we used one-way ANOVAs to identify statistical differences. If significant main effects were observed, these were further explored with pair-wise comparisons between the three treatments. For within-group comparisons of changes in hematological variables between the resting state (at 19°C) and at $CT_{\text{max}}$, a paired t-test was performed.

Statistical significance was accepted at $p \leq 0.05$ and all p-values were adjusted for multiple testing using the Holm-Bonferroni method. Due to the high number of tests performed, the risk of type II error was relatively large in some of the more detailed comparisons. For this reason, comparisons of routine values between treatments are included both in the complex mixed model, as well as a non-repeated variable in a one-way ANOVA analysis. Statistical analyzes were performed in SPSS for Windows (v. 21, SPSS Inc., Chicago, IL, USA). All data are presented as means ± S.E.M.
Results

Effects of thermal acclimation on cardiovascular performance and thermal tolerance in normoxia

Biotest perch had significantly lower routine Q (F₂,₃₃= 6.1, P=0.005), fₜ (F₂,₃₃= 5.8, P=0.007) and cardiac power output (CPO, F₂,₂₅= 5.1, P=0.014) in normoxia at 19°C when compared to reference perch (Table 2). There was no differences in routine values between experimental group in regards to Pᵥᵥ, Rₜₜ and Vₛ.

During the thermal challenge, Q increased progressively until peak values were reached (F₁₃,₂₄₀=7.5, P<0.0001; Fig 1A). While the scope, peak and Q₁₀ values (Q₁₀ values of 2.2±0.2 and 2.3±0.1 for reference and Biotest fish, respectively) for Q were not significantly different between Biotest and reference perch in normoxia, the peak occurred at a significantly higher temperature in Biotest fish compared to reference fish (F₂₉= 14.0, P<0.0001, Table 2). Cardiac output then declined until the respective CTₘₚₐₓ of each group was reached (Fig. 1A). The CTₘₚₐₓ of Biotest fish was significantly higher (32.0 ± 0.3°C) than reference fish (29.8 ± 0.2°C; F₂,₃₆= 20.4, P=<0.0001, Table 2). While Pᵥₒ₂ tended to decrease throughout the thermal challenge in both reference and Biotest perch (F₁₅₃= 7.4, P<0.0001), it was significantly higher in Biotest perch across the higher test temperatures (i.e. between 28-30°C, F₂,₂₆= 11.8, P=0.002, Fig. 1G). However, the Pᵥₒ₂ thresholds (i.e. the Pᵥₒ₂ values where Q and fₜ peaked) did not differ significantly between reference and Biotest fish (Table 2).

In both groups, the increase in Q with warming was primarily due to a significant increase in fₜ (F₁₃,₂₄₄=15.2, P<0.0001; Fig 1B), which increased with a Q₁₀ of 1.8±0.1 and 2.3±0.3 in reference and Biotest fish, respectively. However, both Q and fₜ tended to remain slightly lower in Biotest perch throughout the thermal challenge, with significant differences between reference and Biotest fish occurring at 26-28°C for Q and at 30°C for fₜ (F₂,₃₆ = 9.0, P>0.001 and F₂₃,₂₆₁ = 3.2, P<0.0001, respectively; Fig. 1A, B). Similar to the response in Q,
the peak response and $Q_{10}$ for $f_H$ did not differ between Biotest and reference fish, while the
peak response occurred at a significantly higher temperature in Biotest fish ($F_{2,29}= 7.6, \ P=0.002$, Table 2). Nonetheless, due to the lower routine $f_H$ in Biotest fish, the scope for $f_H$
during warming was significantly greater ($F_{2,30}= 9.1, P<0.001$; Table 2). Although stroke
volume ($V_S$) did not change significantly during warming in the two normoxic groups (Fig.
1C), the greater change in $f_H$ in Biotest fish may have been associated with a somewhat
reduced stroke volume in this group during the thermal challenge, which was significantly
different from reference fish at 26-28°C ($F_{2,30}= 5.1, P=0.012$, Fig 1C).

Cardiac power output also increased significantly with temperature and plateaued or
decayed immediately prior to $CT_{\text{max}}$ in both Biotest and reference groups ($F_{13,204}= 6.3, \ P<0.0001$, Fig. 1D). There were no differences in the peak response or scope for CPO
between groups, but similar to Q and $f_H$, the peak occurred at a significantly higher
temperature in the Biotest fish ($F_{2,25}= 13.3, P=0.0001$; Table 2). The changes in CPO during
warming were mainly explained by the changes in Q, but possibly also by a general increase
in $P_{Va}$, which was significant in Biotest fish between 28-31°C ($F_{13,195}= 3.0, P<0.001$, Fig. 1E).
The total vascular resistance ($R_{tot}$) decreased with increasing temperatures in both groups and
was higher in Biotest fish ($F_{2,31}=4.8, P<0.0001$ Fig. 1F).

Effects of ambient hyperoxia on cardiovascular performance and thermal tolerance

The routine $P_{VO2}$ at 19°C prior to the thermal challenge was nearly doubled in the hyperoxic
fish compared with the reference fish in normoxia ($F_{2,21}= 5.1, P=0.017$), but there were no
significant differences in other routine cardiovascular variables (Table 2).

Cardiac output in the hyperoxic group initially increased during warming in a similar
fashion as observed in the normoxic group, primarily via an increase in $f_H$ ($F_{13,244}=15.2, \ P<0.0001$; Fig. 1A, B). While no significant differences in peak responses and $Q_{10}$ values for
Q and $f_H$ were detected between normoxic and hyperoxic reference fish ($Q_{10}$ for $Q=2.2 \pm 0.2$ vs. $2.7 \pm 0.2$ and $Q_{10}$ for $f_H=1.8 \pm 0.1$ vs. $2.0 \pm 0.1$, respectively), the scopes for $Q$ and $f_H$ were significantly greater in hyperoxic compared to normoxic reference fish ($F_{2,28}=5.0, P=0.032$ and $F_{30}=9.1, P=0.024$, respectively; Table 2). Furthermore, the peak response in $Q$ for hyperoxic reference fish was significantly higher than for Biotest fish in normoxia ($F_{2,31}=5.7, P=0.007$), despite occurring at a significantly lower temperature ($F_{2,29}=14, P=0.018$; Table 2).

There were no differences between hyperoxic reference and Biotest fish in regards to the scope for $f_H$ or the peak for $f_H$, nor were there differences in scope for CPO or the peak for CPO between any of the experimental groups (Table 2).

While ambient hyperoxia in reference fish significantly increased $CT_{\text{max}}$ by 1.1°C ($30.9 \pm 0.3°C; F_{2,36}=20.4, P=0.024$; Table 2), this upper thermal limit was still significantly lower than in Biotest fish ($F_{2,36}=20.4, P=0.0015$; Table 2). However, as temperature increased above approximately 22°C, the $f_H$ tended to increase more in the hyperoxic fish compared with the normoxic reference fish, with statistically significant differences between experimental groups occurring at 29-30°C ($F_{23,261}=3.2, P<0.0001$; Fig. 1B). Unlike the response observed in normoxic reference and Biotest fish, a significant compensatory increase in $V_S$ and CPO ($F_{13,240}=7.4, P<0.0001$ and $F_{13,204}=6.3, P<0.0001$; Fig. 1C) was observed in the hyperoxic reference group when $f_H$ reached its peak value and started to decline (29-30°C), resulting in maintenance of $Q$ rather than a decline prior to $CT_{\text{max}}$ (Fig. 1A).

$P_{\text{VO2}}$ remained significantly higher in the hyperoxic group than in the normoxic reference group throughout the thermal challenge, although a sharp decline in $P_{\text{VO2}}$ occurred beyond 28°C in the hyperoxic fish ($F_{18}=22.5, P<0.0001$; Fig. 1G). Consequently, the $P_{\text{VO2}}$ threshold where $f_H$ reached its peak was significantly higher in hyperoxic reference fish compared to the normoxic reference fish ($F_{2,19}=4.9, P=0.018$), but not significantly different
from the Biotest fish (Table 2). There were no differences in the $P_{\text{VO2}}$ threshold for $Q$ between the experimental groups (Table 2).

**Cardiovascular function at critical temperatures in normoxia vs. hyperoxia**

At $CT_{\text{max}}$, there were no significant differences in $P_{\text{VO2}}$ or cardiovascular variables between the two normoxic groups (Fig. 2A-E). However, the $Q$ of the hyperoxia-treated fish was significantly higher compared to the normoxic reference ($F_{2,29}=9.5, P=0.019$) and Biotest fish ($F_{2,29}=9.5, P=0.001$, Fig. 2A), which was explained by a significantly higher $V_S$ ($F_{2,26}=10.1, P=0.036$ and $F_{26}=10.1, P<0.0001$, respectively; Fig. 2C), as there were no differences in $f_{\text{H}}$ at $CT_{\text{max}}$ between experimental groups (Fig 2B). The higher $Q$ and $V_S$ coincided with a significantly higher $P_{\text{VO2}}$ in the hyperoxic fish at $CT_{\text{max}}$ compared to normoxic reference fish ($F_{2,20}=3.5, P=0.049$, Fig. 2E). However, despite the approximately twofold increase in $P_{\text{VO2}}$ at critical temperatures, the $CT_{\text{max}}$ of the hyperoxic fish was still significantly lower than in the Biotest fish ($F_{2,36}=20.4, P=0.0015$). There were no differences in CPO between hyperoxic and normoxic reference fish at $CT_{\text{max}}$, but the CPO of Biotest fish was significantly lower compared to hyperoxia-treated reference fish ($F_{2,22}=3.8, P=0.036$, Fig 2D).

**Hematology and morphometrics**

There were no significant differences in body mass, relative ventricular mass, length, condition factor or hematological variables between the experimental groups (Tables 1 and 3). There was a significant reduction in MCHC in all experimental groups at $CT_{\text{max}}$ compared to resting conditions at $19^\circ C$ ($T_{1,5}=6.0, P=0.0018$; $T_{1,4}=3.8, P=0.0189$; $T_{1,6}=3.2, P=0.0197$ for reference, Biotest and hyperoxic reference fish, respectively; Table 3).
Discussion

The upper limit of thermal tolerance in fish has been suggested to be linked to an oxygen-limited deterioration of cardiovascular performance at critically high temperatures (8, 21, 41). By experimentally manipulating myocardial oxygen availability (hyperoxia and hyperoxemia) in European perch that lack a coronary oxygen supply to the heart, we show that while luminal oxygen supply is important in maintaining V\textsubscript{S} at critically high temperatures, the observed decline in heart rate likely reflects a direct effect of temperature \textit{per se} rather than a limitation in cardiac oxygen availability. Thus, an increase in cardiac and whole animal thermal tolerance with warm acclimation is only partially explained by improved cardiac oxygen supply. The current results emphasize that thermal tolerance is not determined by a sole factor such as oxygen, but more likely by multiple limiting factors.

Routine P\textsubscript{VO2} and changes with acute warming and ambient hyperoxia

The routine P\textsubscript{VO2} of 4.8 kPa in the reference fish examined here at 19°C is similar to the range of values (~4.9-6.4 kPa) previously reported for rainbow trout (\textit{Onchorhynchus mykiss}) using implanted oxygen optodes, albeit at lower temperatures (6-15°C) (24). Our P\textsubscript{VO2} values for European perch are also within the range of previously reported values in cannulated fishes (4.1–5.5 kPa at 9-20°C; (8, 17, 40, 57)), providing further confirmation of the reliability of the P\textsubscript{VO2} measurement technique used in the present study.

The overall trend for a higher P\textsubscript{VO2} in the warm acclimated Biostest fish compared to reference fish, especially at high test temperatures (see Fig. 1), is also in agreement with the study by Farrell and Clutterham (24) who reported a higher resting P\textsubscript{VO2} in rainbow trout acclimated to 13-15°C (~6.4 kPa) than in conspecifics acclimated to 6-10°C (~4.9kPa). Such increases in P\textsubscript{VO2} with warm acclimation are likely caused by a combination of thermally-compensated metabolic rate and tissue oxygen extraction, as observed previously in the warm
acclimated Biotest perch (48), and a right-shift in the oxygen-hemoglobin dissociation curve (46).

The \( P_{\text{VO}_2} \) in the reference fish exposed to ambient hyperoxia was maintained at a significantly elevated level throughout the thermal challenge, which was most likely the result of increased oxygen diffusion at the gills, and possibly increased cutaneous oxygen diffusion (25, 33). Interestingly, there was a sharp drop in \( P_{\text{VO}_2} \) in the hyperoxic group at 28°C that occurred just prior to the onset of the decline in heart rate. Although we did not measure oxygen consumption rates in the present study, numerous other studies have reported a gradual increase in whole animal oxygen consumption rate with acute warming in normoxic fish (5, 8, 30, 34). While we cannot fully explain the mechanism behind the apparent sharp drop in \( P_{\text{VO}_2} \) at 28°C in hyperoxia, it is possible that this relates to a greatly increased tissue oxygen extraction at this point, concomitant with the observed plateau in \( Q \). Indeed, Brijs et al (5) recently demonstrated a sharp increase in aerobic metabolic rate in hyperoxic Biotest perch 2-3°C before \( C_{\text{T max}} \) was reached, a response that was not observed in normoxic perch. Again, the underlying cause for this dramatic metabolic response in hyperoxia is presently unknown. The relative importance of cutaneous oxygen uptake has been suggested to decrease with increasing temperature in salmonid fish (25), and so another fascinating possibility is that the sharp drop in \( P_{\text{VO}_2} \) could be at least partly explained by a sudden decrease in cutaneous blood perfusion at temperatures approaching \( C_{\text{T max}} \).

**Oxygen- and temperature-limitations to cardiovascular performance during acute warming**

The cardiac performance (i.e. \( f_h \) and \( Q \)) of the normoxic reference and Biotest perch subjected to an acute thermal challenge started to deteriorate at a \( P_{\text{VO}_2} \) ranging between 2.3 and 4.0 kPa. These \( P_{\text{VO}_2} \) threshold values for cardiac performance are similar to previously reported values for Atlantic cod under comparable experimental conditions (41), but are higher than \( P_{\text{VO}_2} \).
thresholds reported for various salmonid species ($P_{VO2}= 0.8-2.0$ kPa) challenged with acute warming or exercise (8, 14, 24, 57).

While these differences may partly reflect differences in thermal acclimation history affecting tissue metabolism and characteristics of the oxygen-hemoglobin dissociation curve (46), another interesting possibility is that they are caused by the important anatomical difference between species lacking coronaries (e.g. European perch and Atlantic cod) and species with a well-developed coronary supply to the heart (e.g. salmonids). Therefore, the lower $P_{VO2}$ threshold for cardiac performance in salmonids may reflect a greater contribution of coronary oxygen supply to the salmonid heart during warming. Indeed, the coronary supply has been shown to be crucial for cardiac performance during intensive exercise and environmental hypoxia in salmonid fish (28, 31). It also should be noted that any interspecies comparisons regarding the $P_{VO2}$ threshold may be confounded by potential differences in CPO, and hence cardiac oxygen demand, between species.

In the two normoxic groups in the present study, the decline in cardiac output at critically high temperatures was primarily caused by a plateau and subsequent precipitous fall in $f_H$ that started approximately 2°C prior to $CT_{max}$. Stroke volume remained largely unchanged throughout the experimental protocol in normoxic fish and, if anything, CPO only showed tendencies to decline immediately before $CT_{max}$, suggesting that cardiac contractility (i.e. the ability of the ventricle to contract and eject blood) was sufficient under routine conditions. However, since Q began to decline at a similar $P_{VO2}$ in both normoxic experimental groups it may be conceivable that a common $P_{VO2}$ threshold was reached where $f_H$ was compromised, leading to impaired Q, which is consistent with the line of reasoning in previous $in vivo$ experiments (8, 24, 37, 41). However, when $P_{VO2}$ was elevated in the hyperoxic reference fish, Q was maintained by a compensatory increase in $V_S$ at critically high temperatures, although $f_H$ still decreased drastically ~3°C prior to reaching $CT_{max}$. The
increase in $V_s$ with declining $f_{H}$ may be expected due to the inverse relationship between these variables (see 55), as well as the increase in cardiac filling pressure with increasing temperature (8). Nonetheless, it is clear that the normoxic fish did not have the ability to exploit this compensatory potential, which may suggest an aerobic limitation of cardiac contractility at critical temperatures in vivo. Indeed, reduced oxygen availability negatively affects contractility of the fish heart in in situ and in vitro experimental setups (15, 22, 44). Thus, the inability to increase $V_s$ at critically high temperatures in the normoxic fish may reflect insufficient cardiac aerobic energy production, as well as a potential accumulation of anaerobic end-products resulting in a decrease in extracellular pH and hyperkalemic conditions of the venous blood, further impairing cardiac function (8, 44, 58, 68). In contrast to the idea that cardiac oxygen availability limits $f_{H}$ in fish (8, 41), the current results show that the drastic drop in $f_{H}$ occurred in spite of significantly higher $P_{VO2}$ in the hyperoxic reference fish. This provides evidence that factors other than myocardial oxygen supply caused the declining $f_{H}$ in these fish. Indeed, myocardial signal transduction may become compromised at elevated temperatures due to changes in membrane fluidity causing disturbances in cardiomyocyte ion flux (42), and cardiac Na$^+$ channel function is impaired during acute warming (69), which could both explain the sudden decline in $f_{H}$.

Mechanisms of whole animal thermal tolerance

Friedlander et al. (29) demonstrated that the temperature for loss of equilibrium was similar when locally heating the cerebrum of goldfish and when heating the whole animal, suggesting that $CT_{max}$ is related to a temperature dependent failure of the central nervous system. While it is possible that similar neural mechanisms related to cardiac signal transduction underlie these central nervous responses, this would suggest that direct temperature effects unrelated to cardiac and systemic oxygen supply determine $CT_{max}$ in fish. Warm acclimation alters cardiac
ion channel function in fish (36, 69). Hence, it is likely that the higher CT$_{\text{max}}$ of the warm
acclimated Biotest fish compared to normoxic and hyperoxic reference fish may in part be
attributed to an improvement of electrophysiological properties at high temperatures, which
was reflected in maintenance of $f_{\text{H}}$ at higher temperatures. This would also be crucial for the
maintenance of neural signal transduction and function in the central nervous system.

The slight, yet significant increase in the thermal tolerance of hyperoxic reference perch
is consistent with previous studies in hyperoxic goldfish (67), but contrasts with a recent study
on hyperoxic (200% air saturation) Biotest perch examined at a similar acclimation
temperature (24°C), which showed no significant increase in CT$_{\text{max}}$ (5). This may suggest that
these fish had reached a physiological ceiling at which their acute thermal tolerance was
maximized (5, 48). As the CT$_{\text{max}}$ of the hyperoxia treated reference fish did not increase to the
level of the Biotest fish, despite significantly elevated $P_{\text{VO2}}$ and a general maintenance of $Q$, it
is clear that additional factors beyond cardiovascular oxygen supply play pivotal roles in
setting acute thermal limits in fish (9, 10, 19, 35, 39, 45).

**Chemoreceptor interactions and cardiovascular performance during acute warming**

While there was no statistically significant difference in the temperature where heart rate
peaked and started to decline during warming in hyperoxic and normoxic reference fish, an
interesting observation was the divergence in the $f_{\text{H}}$ response during warming between the
normoxic and hyperoxic reference fish that started to develop at approximately 22°C. This
resulted in a lower scope for $f_{\text{H}}$ during warming and significantly lower heart rates at test
temperatures preceding CT$_{\text{max}}$ in normoxic reference fish. This pattern may be indicative of an
oxygen sensitive cardio-inhibitory influence on $f_{\text{H}}$ that started to develop at 22°C in the
normoxic reference fish. Indeed, both environmental (external) and systemic (internal)
hypoxia is known to elicit reflex bradycardia in fish (see 23), which is mediated by
stimulation of external or internal oxygen sensitive chemoreceptors (47, 63, 64). Thus, the contrasting patterns in the $f_R$ response during warming in the normoxic and hyperoxic reference fish may be mediated by an increasingly greater stimulation of internal chemoreceptors in the normoxic fish as warming progressed and $P_{VO2}$ declined (63), because the external (environmental) $P_O2$ remained constant in the two experimental groups throughout the entire thermal challenge.

_Perspectives and significance_

This study thoroughly details the relationship between myocardial oxygenation, cardiac function and acute thermal tolerance in European perch. We show that the primary cause of the deterioration of cardiac performance and cardiovascular oxygen transport at critically high temperatures is caused by a temperature dependent decline in heart rate that is further exacerbated by an oxygen-limited impairment of cardiac contractility and an inability to elevate stroke volume. These findings contrast with the suggestion that the decline in heart rate is primarily attributed to a diminishing oxygen supply to cardiac tissues. Although the cause of the decline in heart rate cannot be identified in the present study, it may be the result of a temperature-induced impairment of the conductive properties of the myocardial cells. Furthermore, we demonstrate that the increase in cardiac and thermal tolerance with warm acclimation is only partially explained by improved cardiac oxygen supply. Collectively, this indicates that additional mechanisms are involved in determining the critical thermal maximum in European perch, likely involving modifications to neural tissues that affect performance on several levels of physiological organization. A future challenge lies in investigating and determining the relative contribution of these causal mechanisms to fully unravel the full mechanistic basis underlying thermal tolerance in fish and other ectothermic animals.
Acknowledgements

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Author contributions

A.E and E.S. designed the experimental protocols and performed the experiments. A.E, E.S and A.G conducted the data analysis and the statistical analyses. All authors participated in the interpretation of the data and in drafting the manuscript. All authors have given final approval for publication.

Additional information

The authors declare no financial or other conflicts of interest.
Table 1. Morphological characteristics of European perch (Perca fluviatilis) in the different experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Reference hyperoxia</th>
<th>Biotest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>14</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Environmental temperature (°C)</td>
<td>17.2±0.5</td>
<td>17.2±0.5</td>
<td>23.1±2.2</td>
</tr>
<tr>
<td>Acclimation temperature (°C)</td>
<td>17.7±0.7</td>
<td>17.7±0.7</td>
<td>23.8±1.1</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>465±36</td>
<td>428±30</td>
<td>451±32</td>
</tr>
<tr>
<td>Relative ventricle mass (%)</td>
<td>0.054±0.02</td>
<td>0.056±0.02</td>
<td>0.052±0.02</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>308±7</td>
<td>303±6</td>
<td>308±5</td>
</tr>
<tr>
<td>Condition factor</td>
<td>1.57±0.03</td>
<td>1.51±0.02</td>
<td>1.51±0.05</td>
</tr>
</tbody>
</table>

The groups are reference fish, reference fish under hyperoxic conditions (200% water air saturation) and Biotest fish. ‘Environmental temperature’ represents the temperature at the locations where the fish were caught and ‘Acclimation temperature’ is the holding temperature in the lab (see Materials and methods for details). Data are presented as means ± SEM. No significant differences were found between the experimental groups.
Table 2. Cardiovascular variables, venous oxygen tension and thermal tolerance during an acute thermal challenge in European perch (Perca fluviatilis).

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Reference hyperoxia</th>
<th>Biotest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>8-11</td>
<td>7-10</td>
<td>9-11</td>
</tr>
<tr>
<td>Acclimation temperature (°C)</td>
<td>17.7±0.7</td>
<td>17.7±0.7</td>
<td>23.8±1.1</td>
</tr>
<tr>
<td>CT&lt;sub&gt;max&lt;/sub&gt; (°C)</td>
<td>29.8±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.9±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.0±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Cardiac output (ml min<sup>-1</sup> kg<sup>-1</sup>)

- **Routine at 19°C**: Reference 32.3±2.0<sup>a</sup>, Reference hyperoxia 31.1±3.0<sup>ab</sup>, Biotest 22.5±1.9<sup>b</sup>
- **Peak**: Reference 66.0±4.1<sup>ab</sup>, Reference hyperoxia 81.9±6.7<sup>a</sup>, Biotest 57.2±4.7<sup>b</sup>
- **Temperature at peak response (°C)**: Reference 28.4±0.3<sup>a</sup>, Reference hyperoxia 29.4±0.4<sup>a</sup>, Biotest 30.8±0.3<sup>b</sup>
- **Scope**: Reference 34.5±3.8<sup>a</sup>, Reference hyperoxia 51.4±5.7<sup>b</sup>, Biotest 34.7±3.2<sup>a</sup>

Heart rate (beats min<sup>-1</sup>)

- **Routine at 19°C**: Reference 67.8±1.9<sup>a</sup>, Reference hyperoxia 66.0±4.1<sup>a</sup>, Biotest 54.0±3.2<sup>b</sup>
- **Peak**: Reference 115.9±4.6, Reference hyperoxia 132.1±5.8, Biotest 124.3±4.9
- **Temperature at peak response (°C)**: Reference 28.1±0.3<sup>a</sup>, Reference hyperoxia 29.5±0.3<sup>ab</sup>, Biotest 30.3±0.6<sup>b</sup>
- **Scope**: Reference 48.1±3.8<sup>a</sup>, Reference hyperoxia 66.0±5.0<sup>b</sup>, Biotest 73.3±4.2<sup>b</sup>

Cardiac power output (mW g<sup>-1</sup>)

- **Routine at 19°C**: Reference 5.4±0.5<sup>a</sup>, Reference hyperoxia 4.7±0.5<sup>ab</sup>, Biotest 3.5±0.3<sup>b</sup>
- **Peak**: Reference 14.0±1.5, Reference hyperoxia 17.5±1.8, Biotest 13.3±1.1
- **Temperature at peak response (°C)**: Reference 28.4±0.3<sup>a</sup>, Reference hyperoxia 29±0.5<sup>a</sup>, Biotest 31±0.3<sup>b</sup>
- **Scope**: Reference 8.5±1.2, Reference hyperoxia 12.8±1.7, Biotest 9.7±1.0

Partial pressure of venous oxygen (kPa)

- **Routine at 19°C**: Reference 4.8±0.5<sup>a</sup>, Reference hyperoxia 8.5±1.5<sup>b</sup>, Biotest 5.7±0.4<sup>ab</sup>
- **P<sub>VO2</sub> threshold at peak cardiac output**: Reference 2.3±0.6, Reference hyperoxia 4.2±0.7, Biotest 3.9±0.7
- **P<sub>VO2</sub> threshold at peak heart rate**: Reference 2.6±0.5<sup>a</sup>, Reference hyperoxia 5.2±0.7<sup>b</sup>, Biotest 4.0±0.6<sup>ab</sup>

Stroke volume (ml kg<sup>-1</sup>)

- **Routine at 19°C**: Reference 0.48±0.03, Reference hyperoxia 0.50±0.03, Biotest 0.42±0.03

Ventral aortic pressure (kPa)

- **Routine at 19°C**: Reference 5.2±0.3, Reference hyperoxia 4.8±0.1, Biotest 4.9±0.1

Total vascular resistance (kPa ml min<sup>-1</sup> kg<sup>-1</sup>)

- **Routine (at 19°C)**: Reference 0.17±0.01<sup>ab</sup>, Reference hyperoxia 0.15±0.01<sup>a</sup>, Biotest 0.22±0.02<sup>b</sup>

Values are reported for reference fish, reference fish under hyperoxic conditions (200% air saturation) and warm acclimated Biotest fish. Dissimilar letters denote statistically significant differences between experimental groups. Values are means ± SEM.
Table 3. Hematological variables at rest and at CT<sub>max</sub> in European perch (Perca fluviatilis).

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Reference hyperoxia</th>
<th>Biotest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>CT&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Rest</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>10-11</td>
<td>9-10</td>
<td>9-11</td>
</tr>
<tr>
<td>Acclimation temperature (°C)</td>
<td>17.7 ± 0.7</td>
<td>17.7 ± 0.7</td>
<td>23.8 ± 1.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>21.4±0.7</td>
<td>25.8±1.3</td>
<td>21.8±1.1</td>
</tr>
<tr>
<td>Hemoglobin concentration (g l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>53.7±1.7</td>
<td>51.1±2.0</td>
<td>57.9±2.6</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (g l&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>252±4</td>
<td>200±8*</td>
<td>267±5</td>
</tr>
</tbody>
</table>

Values are reported for reference fish, reference fish under hyperoxic conditions (200% air saturation) and warm acclimated Biotest fish. Data are presented as means ± SEM. Statistical differences between experimental groups were determined by One-way ANOVA followed by a Tukey post-hoc test and within group comparisons between values at rest and at CT<sub>max</sub> was determined by a paired t-test. * denotes statistical differences between resting conditions and at CT<sub>max</sub>. 
**Fig. 1.** Cardiovascular performance during acute warming (3°C h⁻¹) in European perch (*Perca fluviatilis*). Cardiac output (A), heart rate (B), stroke volume (C), cardiac power output (D), ventral aortic pressure (E), total vascular resistance (F) and venous oxygen tension (G). The experimental groups are reference fish acclimated to 18°C (n=7-11; white triangles), reference fish under hyperoxic conditions (200% water air saturation; n=4-10; black circles) and Biotest fish acclimated to 24°C (n= 4-11; grey squares). Horizontal lines indicate significant change from the initial value at 19°C within experimental groups. The symbols above the lines denotes statistical differences for the following comparisons: * reference vs. biotest, # reference vs. reference hyperoxia and □ biotest vs. reference hyperoxia at specific test temperatures. Values are means ± SEM. For further comparisons of routine values at 19°C, see Table 2.

**Fig. 2.** Cardiovascular variables in European perch (*Perca fluviatilis*) at the critical thermal maximum following acute thermal challenge (3°C h⁻¹). The variables are cardiac output (A), heart rate (B), stroke volume (C), cardiac power output (D) and partial pressure of venous oxygen (E). The experimental groups are reference fish acclimated to 18°C (n=11; white bars), reference fish under hyperoxic conditions (200% air saturation; n=14; black bars) and biotest fish acclimated to 24°C (n=14; grey bars). Dissimilar letters denote statistically significant differences among groups. Values are means ± SEM.


56. **Somero GN.** Comparative physiology: a "crystal ball" for predicting consequences of
global change. *American journal of physiology Regulatory, integrative and comparative physiology*
301: R1-14, 2011.

57. **Steffensen JF, and Farrell AP.** Swimming performance, venous oxygen tension and
cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to
progressive hypoxia. *Comparative biochemistry and physiology Part A, Molecular & integrative

58. **Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, and Farrell AP.** The effect of
acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye

59. **Stevens ED, and Sutterlin AM.** Heat transfer between fish and ambient water. *The

60. **Stillman JH.** Acclimation capacity underlies susceptibility to climate change. *Science*

61. **Stitt BC, Burness G, Burgomaster KA, Currie S, McDermid JL, and Wilson CC.**
Intraspecific variation in thermal tolerance and acclimation capacity in brook trout (*Salvelinus
fontinalis*): physiological implications for climate change. *Physiological and biochemical zoology :PBZ*
87: 15-29, 2014.

62. **Sunday JM, Bates AE, and Dulvy NK.** Global analysis of thermal tolerance and latitude
in ectotherms. 2010.

63. **Sundin LI, Reid SG, Kalinin AL, Rantin FT, and Milsom WK.** Cardiovascular and
respiratory reflexes: the tropical fish, traira (*Hoplias malabaricus*) O2 chemoresponses. *Respiration

64. **Taylor EW.** Control and co-ordination of gill ventilation and perfusion. *Symposia of the

65. **Tota B.** Vascular and metabolic zonation in the ventricular myocardium of mammals

66. **Wahlqvist I, and Nilsson S.** The role of sympathetic fibres and circulating
catecholamines in controlling the blood pressure and heart rate in the cod, *Gadus morhua*.

67. **Weatherley AH.** Effects of superabundant oxygen on thermal tolerance of goldfish.
*The Biological bulletin* 139: 229-238, 1970.

68. **West JL, and Driedzic WR.** Mitochondrial protein synthesis in rainbow trout
(*Oncorhynchus mykiss*) heart is enhanced in sexually mature males but impaired by low temperature.

69. **Vornanen M, Haverinen J, and Egginton S.** Acute heat tolerance of cardiac excitation
Biotest (24°C)

Reference (18°C)

Reference hyperoxia (200% O₂ sat., 18°C)

0  20  40  60  80  100

f (beats min⁻¹)

0  20  40  60  80

Q (ml min⁻¹ kg⁻¹)

0  20  40  60  80  100

fₚ (beats min⁻¹)

0  20  40  60  80

Vₔ (ml kg⁻¹)

0  0.2  0.4  0.6  0.8  1.0

CPO (mW g⁻¹)

0  2  4  6  8  10  12

PᵥO₂ (kPa)

0  1  2  3

□ Reference (18°C)
■ Reference hyperoxia (200% O₂ sat., 18°C)
□ Biotest (24°C)