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Mechanisms of cardiac collapse at high temperature in a marine teleost (*Girella nigrians*)

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ABSTRACT

Heat-induced mortality in ectotherms may be attributed to impaired cardiac performance, specifically a collapse in maximum heart rate (f_{Hmax}), although the physiological mechanisms driving this phenomenon are still unknown. Here, we tested two proposed factors which may restrict cardiac upper thermal limits: noxious venous blood conditions and oxygen limitation. We hypothesized elevated blood [K^+] (hyperkalemia) and low oxygen (hypoxia) would reduce cardiac upper thermal limits in a marine teleost (*Girella nigricans*), while high oxygen (hyperoxia) would increase thermal limits. We also hypothesized higher acclimation temperatures would exacerbate the harmful effects of an oxygen limitation. Using the Arrhenius breakpoint temperature test, we measured f_{Hmax} in acutely warmed fish under control (saline injected) and hyperkalemic conditions (elevated plasma [K^+]) while exposed to hyperoxia (200% air saturation), normoxia (100% air saturation), or hypoxia (20% air saturation). We also measured ventricle lactate content and venous blood oxygen partial pressure (PO_2) to determine if there were universal thresholds in either metric driving cardiac collapse. Elevated [K^+] was not significantly correlated with any cardiac thermal tolerance metric. Hypoxia significantly reduced cardiac upper thermal limits (Arrhenius breakpoint temperature [T_{AB}], peak f_{Hmax}, temperature of peak heart rate [T_{Peak}], and temperature at arrhythmia [T_{ARR}]). Hyperoxia did not alter cardiac thermal limits compared to normoxia. There was no evidence of a species-wide threshold in ventricular [lactate] or venous PO_2. Here, we demonstrate that oxygen limits cardiac thermal tolerance only in instances of hypoxia, but that other physiological processes are responsible for causing temperature-induced heart failure when oxygen is not limited.

1. Introduction

With rising global temperatures, increases in marine heatwaves, and more extreme thermal fluctuations (Gissi et al., 2021; Pörtner et al., 2022), understanding the factors influencing the upper thermal limits of ectotherms is of increasing importance (Lefevre et al., 2021; Oliver et al., 2021; Smith et al., 2021; Stuart-Smith, 2021). Across taxa, ectotherms exhibit cardiac collapse at high temperatures, frequently before other organ systems begin to shut down (Eliason et al., 2022; Somero, 2010). The heart is critical to support whole-organism function via its role in transporting oxygen, nutrients, hormones, and metabolic waste products throughout the body. Thus, it follows that impaired cardiac function at high temperatures impacts (and perhaps sets) organismal thermal limits (Eliason and Anttila, 2017; Frederich and Pörtner, 2000; Hall and Sun, 2021; Oellermann et al., 2020; Stenseng et al., 2005; Stillman, 2002).

As temperatures rise, cardiac output concomitantly increases to support a rising metabolic demand of the tissues. In most fishes, this is

Abbreviations: ABT, Arrhenius breakpoint temperature; CTmax, critical thermal maxima; f_H, heart rate; f_{Hmax}, maximum overall heart rate; T_{AB}, breakpoint temperature on the heart; T_{ARR}, temperature at the first cardiac arrhythmia; T_{Peak}, temperature corresponding to maximum heart rate; AP, action potential; DO, dissolved oxygen; PO_2, partial pressure of oxygen; Pv_{O2}, partial pressure of oxygen in venous blood; RVM, relative ventricular mass; ROS, reactive oxygen species.

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primarily accomplished by an increase in heart rate (f_H) (Eliason and Anttila, 2017). It is well known that maximum heart rate (f_{Hmax}) has a parabolic relationship with temperature, increasing until it reaches a peak or plateau, with subsequent declines indicative of approaching arrhythmias and complete cardiac collapse (Anttila et al., 2013; Casselman et al., 2012; Eliason and Anttila, 2017). Thus, the inability of f_{Hmax} to continue to increase indefinitely means total cardiac output may be insufficient to meet the temperature-induced increased metabolic demands of the tissues, contributing to organism mortality (Eliason et al., 2013; Farrell, 2009). We have evidence for this in vivo, where collapsing cardiac scope, mediated by a collapse in scope for heart rate, contributed to declines in aerobic scope in migrating sockeye salmon (*Oncorhynchus nerka*) (Eliason et al., 2013). While the mechanisms underlying temperature effects on cardiac function have been well studied (see Chung and Schulte, 2020; Eliason and Anttila, 2017; Iftikar and Hickey, 2013; Kalinin et al., 2009; Moyes and Ballantyne, 2011; Vornanen, 2016), no single component has fully explained heat-induced cardiac failure. Mechanistic assessments of what might be driving the plateau and eventual collapse in f_{Hmax} specifically are still lacking. This represents a critical gap in our knowledge of what constrains ectotherm thermal tolerance.

One hypothesis regarding f_{Hmax} collapse is that the noxious venous environment associated with high temperature (i.e., a combination of low oxygen, low pH, and hyperkalemia) impairs cardiac excitability, with stressors acting concomitantly and synergistically (Hanson et al., 2009; Schwieterman et al., 2021). At high temperatures, insufficient oxygen delivery to the tissues (whether through insufficient blood oxygen levels or impaired blood flow due to inadequate cardiac output) causes an upregulation of anaerobic metabolism, resulting in high blood lactate and low blood pH levels (metabolic acidosis). Acidosis may disrupt cardiac function directly through changes in contractility or through decreases in f_H (Farrell and Milligan, 1986; Gesser and Jorgensen, 1982), although some species can mitigate this response with increased intracellular Ca^{2+} activity (Gesser and Poupa, 1979). Acidosis also disrupts hemoglobin-oxygen (Hb-O_2) binding affinity, exacerbating oxygen delivery impairment. High inter-specific variability has, however, limited our understanding of whether there exists a critical plasma pH or lactate level for cardiac function disruption. Similarly, increasing temperatures increase K^+ leakage from skeletal muscle, causing hyperkalemia (high potassium concentration, [K^+]), which is known to impair cardiac function (Hanson et al., 2006; Schwieterman et al., 2021). In isolated teleost myocardium, hyperkalemia decreases transcellular sodium-potassium (Na^+-K^+) gradients and alters calcium-troponin binding. These, in turn, result in depolarized membrane potentials, increased action potential (AP) length, reduced influx of sarcolemmal calcium (Ca^{2+}), and decreased contractile force (Badr et al., 2018; El-Sayed and Gesser, 1989; Kalinin and Gesser, 2002; Nielsen and Gesser, 2001). It is unknown, however, how hyperkalemia affects f_{Hmax} specifically, or how this may interact with other components of a noxious blood environment in vivo.

Another hypothesis explaining f_{Hmax} collapse at high temperatures is that oxygen limitation alone drives cardiac collapse (Pörtner, 2010). As fish have a single circulatory system, the spongy myocardium is the last tissue in the body to receive oxygen from the venous blood, though fish with a coronary circulation do receive a more reliable source of oxygenated blood to the outer compact myocardium. At warm temperatures, a cardiac oxygen limitation could occur either through low oxygen availability at the gills due to the decrease in oxygen solubility in warm water, or through temperature-induced elevated oxygen consumption by the tissues which draws down the venous reserve. Particularly in maximally exercising animals, the partial pressure of oxygen in venous blood (Pv_{O2}) decreases dramatically such that there may be insufficient partial pressure of oxygen (PO_2) remaining to meet the demand of the spongy myocardium, capping aerobic ATP production in the cardiomyocytes and leading to disruptions in contraction/relaxation cycling (Ekström et al., 2016; Eliason et al., 2013; Farrell, 2009; Lannig et al., 2004; Steinhausen et al., 2008). It has been suggested that there may be a critical Pv_{O2} threshold for cardiac performance, though the proposed thresholds vary (7.5 Torr, Clark et al., 2008; 6–16 Torr, Davie and Farrell, 1991; 7.8–9.9 Torr, Farrell and Clutterham, 2003; Steffensen and Farrell, 1998).

In support of the oxygen limitation hypothesis, studies using isolated myocardium have found that hypoxia limits cardiac performance at high temperatures (Driedzic and Gesser, 1994; Gesser, 1985; Paajanen and Vornanen, 2003; Rodnick and Gesser, 2017). Further, hyperoxia improved the performance of isolated ventricular myocardial strips (Gesser and Rodnick, 2019) and in vivo cardiac contractility (McArley et al., 2022) in rainbow trout (*Oncorhynchus mykiss*), suggesting that the heart may always be limited by the oxygen it receives. In contrast, European perch (*Perca fluviatilis*) exposed to normoxic or hyperoxic (200% saturation) conditions during a critical thermal maxima (CTmax) test where *resting* heart rate was simultaneously recorded, showed no impact of environmental oxygen on cardiac thermal limits (Ekström et al., 2016). More work is needed to assess how oxygen may impact the relationship between f_{Hmax} and temperature.

Many studies on cardiac thermal tolerance have focused on processes occurring over time scales of days to years. While this information is critical to our understanding of species plasticity and vulnerability to climatic change, it does not account for the physiological processes that can occur on much shorter time scales (i.e., seconds to hours), which also have important impacts on physiology (Gamperl and Shiels, 2014). For example, digestion, and exercise can increase circulating levels of glucose, decrease blood PO_2, increase [K^+], and alter pH; all of which are known to negatively impact cardiac function in vitro (Brill and Lai, 2016; Eliason and Anttila, 2017; Gamperl and Shiels, 2014). Further, for ectotherms, environmental challenges, such as marine heat waves, storm-induced mixing (which distribute hypoxic water throughout the water column), or upwelling events (which cause simultaneous temperature changes and dissolved oxygen concentrations) can occur over mere hours (Bakun et al., 2015; Bianchi et al., 2010; Oliver et al., 2021). These abrupt environmental shifts also can have negative impacts on the ability of the heart to function properly (Lannig et al., 2004; Leeuwis et al., 2021; Petersen and Gamperl, 2010; van der Walt et al., 2021). Investigating how these rapid processes can constrain f_{Hmax}, and how they may differ between groups with different thermal histories, may therefore elucidate a critical understanding of the mechanisms that limit cardiac and organismal thermal tolerance. Fish living near their upper thermal limits, for example, may have limited abilities to respond to acute stressors and may thus be more susceptible to cardiac collapse.

The objective of this study was to evaluate two potential drivers of cardiac upper thermal limits for their role in f_{Hmax} collapse at high temperatures. We specifically sought to test the following hypotheses: *(1)* Hyperkalemia will reduce the upper thermal limits of the heart, and *(1b)* this will be exacerbated when co-occurring with other stressors (i.e., oxygen limitation), and remediated by hyperoxia (i.e., removal of synergistic stressors). *(2)* hypoxia will reduce the upper thermal limits of the heart and hyperoxia will increase the upper thermal limits of the heart, and *(2b)* thermally stressful warm acclimation temperatures will exacerbate any observed differences among the aforementioned treatments. *(3)* Hearts will fail at a common PO_2 or a common lactate level. We tested these hypotheses on a coastal teleost (opaleye, *Girella nigricans*) using cardiac Arrhenius Breakpoint Temperature (ABT) tests (also referred to as the rapid screening protocol) on maximum heart rate (f_{Hmax}) (Casselman et al., 2012; Schwieterman et al., 2022). This temperate species inhabits intertidal zones where they are subject to seasonal and daily temperature changes (Hardison et al., 2021). This method allowed us to continuously monitor f_{Hmax} (achieved through pharmacological stimulation) during acute warming until arrhythmia was reached, while simultaneously inducing hypoxia, hyperoxia, and/or hyperkalemia to directly test the proposed mechanisms setting upper thermal limits in the fish.

2. Methods

2.1. Collections and holding

All work was approved by the University of California, Santa Barbara Institutional Animal Care and Use Committee. Juvenile opaleye (body mass = 89.3 ± 2.74 g; average length = 16.83 ± 0.15 cm; mean ± SE; total $n = 98$) were collected via hook and line from the Santa Barbara Harbor, CA, USA during the Spring and Summer of 2021 and transported back to the University of California, Santa Barbara within 3 h of capture. Harbor water temperature at the time of capture ranged from 16.5 to 21.2 °C. Tanks were heated or cooled at a rate of 1 °C h^{-1} until the target acclimation temperature (14 °C or 22 °C) was achieved. These temperatures represent a near-optimal temperature and a thermally stressful, climate change scenario temperature for this population. Individuals were acclimated for a minimum of 2 weeks in 25-gal flow-through seawater tanks (10–12 fish per tank, 1–2 tanks per treatment) at their treatment temperature. Fish acclimated to 14 °C were tested under all oxygen levels (hypoxic, normoxic, and hyperoxic), and potassium levels (control and high K^+) in a fully factorial design. Fish acclimated to 22 °C were tested under two oxygen levels (hypoxic and normoxic conditions only, no hyperoxia) and no potassium levels (i.e., they were not tested with hyperkalemia) due to resource limitations. Temperature was continuously monitored via iButton temperature loggers programmed to take a measurement every 30 min (Maximum Integrated, San Jose, CA), and oxygen levels were maintained above 90% air saturation determined through daily measurements (OxyGuard Handy Polaris 2; OxyGuard International A/S, Farum, Denmark). Fish were fed a diet of adult brine shrimp ad libitum, and were held under a 14:10 light:dark cycle (reflecting a natural light:dark cycle in the region).

2.2. Treatments and f_{Hmax} tests

Arrhenius breakpoint temperature tests (ABT tests) have been described elsewhere (Hardison et al., 2021; Schwieterman et al., 2022). In brief, fish were anesthetized in seawater from their acclimation tank (80 mg L^{-1} MS-222 buffered with 1 g L^{-1} $NaHCO_3^-$). Individuals were then weighed and placed ventral side up in the test tank, where oxygenated seawater with a maintenance dose of anesthetic (65 mg L^{-1} MS-222 buffered with 1 g L^{-1} $NaHCO_3^-$) was circulated past the gills. Needle tip electrodes (AD Instruments INC, Colorado Springs, CO, USA) were placed just under the skin, and the resulting ECG signal was amplified and filtered (60hz Notch filter; Mains filter; Low-Pass: 2Kz; High Pass: 10hz; Range: 2 mV) using a Dual Bio Amp (AD Instruments INC, Colorado Springs, CO, USA). Following a 30-min equilibration period at the acclimation temperature (Ferreira et al., 2014; Hansen et al., 2017), atropine sulfate (1.2 mg kg^{-1} in 0.9% NaCl) was injected intraperitoneally to block vagal tone. Fifteen min later, isoproterenol (4 μg kg^{-1} in 0.9% NaCl) was also injected intraperitoneally to maximally stimulate β-adrenoreceptors.

Fifteen min after isoproterenol injection, fish were injected intraperitoneally with either saline (5 ml kg^{-1} 0.9% NaCl) or a high potassium solution (5 mg kg^{-1} of 6 mmol KCl in 0.75% NaCl) to induce hyperkalemia. At the same time as the K^+ injection, dissolved oxygen (DO) manipulation began, by bubbling of pure O_2 into the test tank until a saturation of 200% was achieved for the hyperoxic treatments (~15 min), and bubbling N_2 until a saturation of 20% was achieved for the hypoxic treatments. In the normoxia treatments, DO levels were maintained at 90–100% air saturation. DO was continuously monitored during manipulation and throughout the trial using a Firesting oxygen meter (Pyroscience, Germany).

Once the desired oxygen saturation level was reached, water temperature was heated at 1 °C every 6 min using a Polystat recirculating heater/chiller (Cole-Palmer, Vernon Hills, IL, USA). At each 1 °C interval, f_{Hmax} and temperature were allowed to stabilize to record f_{Hmax}. Temperature was increased until the onset of cardiac arrhythmia (T_{ARR}) evidenced by a missing QRS wave, irregular spacing of QRS waves (>10 bpm), or two tightly grouped QRS waves (Casselman et al., 2012; Hardison et al., 2023). Immediately upon the onset of arrhythmias, fish were removed from the test chambers and an incision was made to expose the heart. A blood sample was taken directly from the sinus venosus. The needle tip was plugged with putty to limit air exposure and the syringe was set on ice for later measurement of PO_2. A second blood sample was taken for [K^+] measurements, then the ventricle was dissected out, frozen in liquid nitrogen, and stored at −80 °C. All fish were euthanized at the end of the test, and both total length and ventricle mass were recorded.

2.3. [K^+] Measurements

Blood from the second blood draw was immediately spun down at 10,000 rpm for 3 min to separate the RBCs from the plasma and the plasma was stored on ice until analysis (<30 min later). The plasma [K^+] was measured using the Diazyme liquid stable potassium enzymatic assay (DZ113C-K01, Diazyme, Hannover, Germany) for fluorescence on a FLOUstar Omega Microplate reader (BMG Labtech, Germany), with a calibration curve of [K^+] ranging from 0.0 to 10 mmol.

2.4. PO_2 Measurements

PO_2 was measured using a water jacketed glass chamber held at the same temperature of T_{ARR}. Blood was injected into the chamber where oxygen was measured using a Firesting oxygen meter and robust oxygen probe (Pyroscience) and was allowed to equilibrate until the rate of change of measured PO_2 was <0.1 Torr/s. Following each measurement, the chamber was flushed with heparinized saline (150 IU/ml in 0.9% NaCl) to ensure no lingering effects between samples. All blood measurements were made within 1 h of collection. Samples that were suspected of being air exposed during collection due to presence of bubbles in the sample during the blood draw were excluded ($n = 17$, 17% of total samples).

2.5. Lactate measurements

Frozen ventricles were ground in liquid nitrogen using a mortar and pestle and weighed (10 mg – 28 mg; 4 samples <10 mg were excluded), before being treated with ice-cold 8% $HClO_4$, and sonicated on ice (3 × 5 s). The homogenate was then centrifuged at 10000 rpm at 4 °C for 10 min. A 600 μl aliquot of the supernatant was neutralized with 140 μl 3 M K_2CO_3. Samples were centrifuged once more at 10000 rpm at 4 °C for 10 min, and supernatants were frozen at −80 °C until assayed. Lactate assays were carried out using previously described protocols (Bergmeyer, 1983; Richards et al., 2002). Absorbance was read at 340 nm at 37 °C on a FLUOstar Omega Microplate reader (BMG Labtech, Germany), with all samples assayed in triplicate.

2.6. Data analysis for Arrhenius breakpoint test

All ECG analyses were performed in LabChart software (AD Instruments, Dunedin, New Zealand). f_{Hmax} was calculated for each temperature increment from 15 continuous s of measurements using automated ECG analysis software (Gradil et al., 2016). Arrhenius breakpoint tests were performed on plots of log(f_{Hmax}) against the Arrhenius temperature using the "segmented" package in R (version 1.1–0 Muggeo, 2008), and the temperature corresponding to the breakpoint in f_{Hmax} was defined as T_{AB}. This represents when the heart first starts showing signs of impairment due to temperature (Hardison et al., 2023). The overall maximum heart rate (peak f_{Hmax}) was defined as the highest f_{Hmax} recorded during the 15 s measurement periods. Peak temperature (T_{Peak}) was the temperature corresponding to peak f_{Hmax}.

2.7. Statistical analysis

All data were statistically analyzed using R (version 3.5.1). Linear models for all measures of cardiac thermal tolerance (T_{AB}, T_{Peak}, T_{ARR}, peak f_{Hmax}) as well as other physiological metrics (lactate, PO_2) were tested for significance with body mass, total length, and relative ventricle mass (RVM = [heart mass/body mass]100). Polynomial curves were fit to f_{Hmax} data and compared using Bayesian information criterion (BIC). We also included a random effect for individual fish to account for repeated measures of f_{Hmax} on the same individual. Metrics of upper thermal limits, ventricle lactate, and venous PO_2 were each analyzed using an analysis of variance (ANOVA; car package version 3.0–2) with post-hoc Tukey HSD when there were significant main effects. To calculate percent change in mean measures of cardiac thermal tolerance between treatments, a bootstrap analysis was conducted using 1000 replicates and subsequent *t*-tests were conducted between resulting values for fish acclimated to 14° and 22 °C. Bootstrap analysis was required to account for variance associated with the mean values used in the percent change calculation. Because our potassium injections did not produce consistent plasma [K^+], concentrations were treated as continuous variable and were analyzed with general linear models (GLMs). Model selection was done with BIC to determine if interactions should be included in the final model.

3. Results

A summary of fish used is included in Supplemental Table 1. We found no significant relationships between mass, total length, or relative ventricle mass (RVM) and any response variables, so these were not included in subsequent analyses.

3.1. Impacts of hyperkalemia

Preliminary data from opaleye (n = 4, data not shown) that were exhaustively chased and air exposed for 1 min showed plasma [K^+] ranging from 8 to 10 mmol, and high potassium injected fish showed similar [K^+] 30 min post-injection (n = 2; data not shown). The K^+ injections did not, however, have the intended effect of consistently raising blood K+ levels in injected groups compared to control. The K^+ injections resulted in plasma [K^+] levels varying between 4.67 and 12.17 mmol l^{-1} in the hyperkalemia groups at the time of T_{ARR}, compared to 2.64 to 13.22 mmol l^{-1} in the control groups (saline injected) at 14 °C, and from 0.69 to 4.57 mmol l^{-1} in the control groups at 22 °C (Supplemental Table 1). Oxygen treatment had no significant

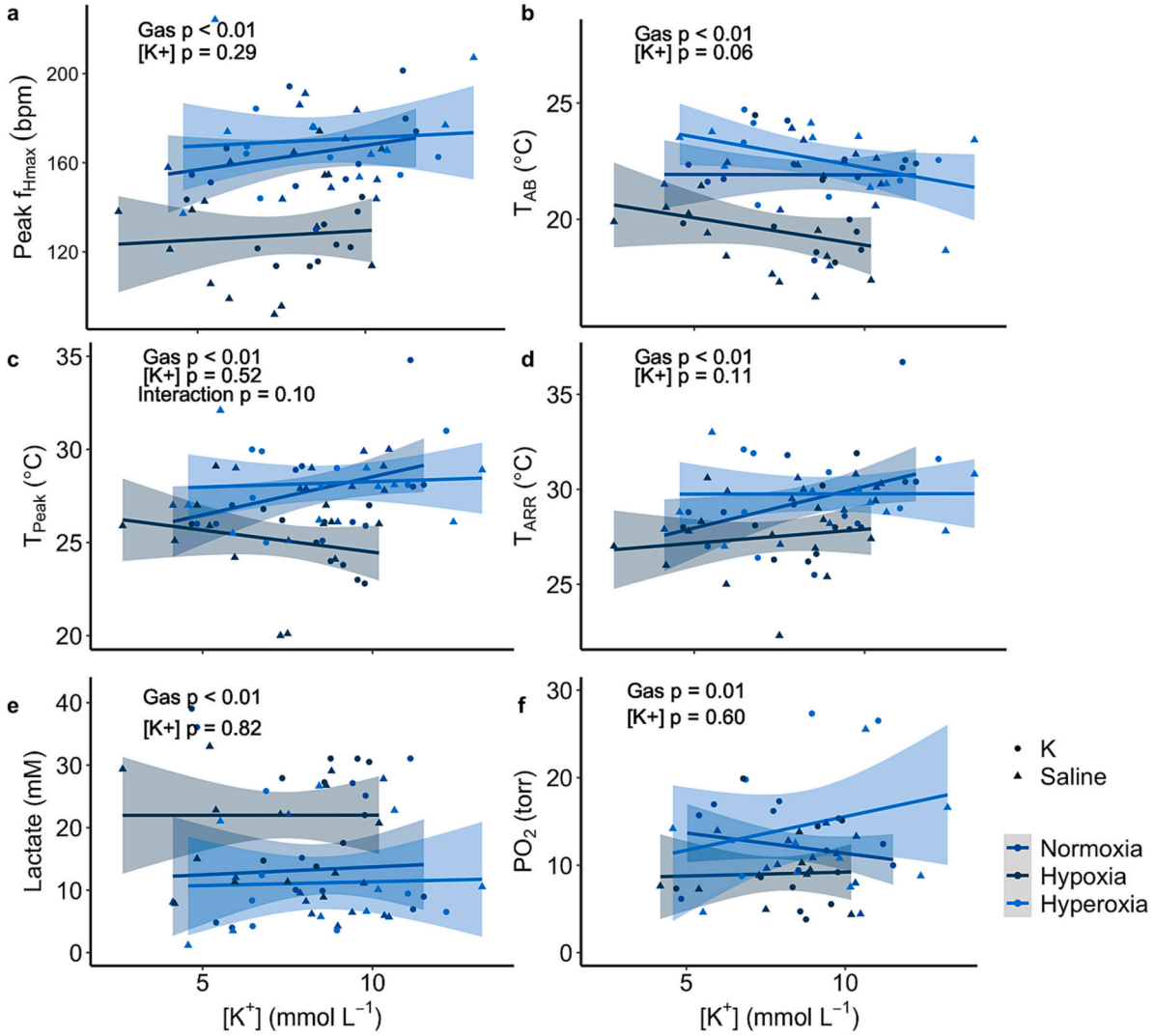

Fig. 1. Correlations between blood potassium concentration ([K^+]) at T_{ARR} and cardiac thermal limits (a-d) ventricle lactate concentration (e), or venous PO_2 (f) in 14 °C acclimated opaleye (n = 24 per treatment) tested under hypoxia (20% saturation), normoxia (100% saturation) and hyperoxia (200% saturation). BIC was used to determine whether to include an interaction in the model. Main effects from the general linear model are shown in the top left of each panel.

impact on mean [K^+] ($p > 0.05$). There was still a considerable range of blood K+ levels across individuals, which permitted an evaluation of [K^+] level impacts on response metrics. Using the GLMs, we found no significant impacts of hyperkalemia level on any of our three metrics of thermal tolerance, or f_{Hmax} ($p > 0.05$; Fig. 1). We also found no significant impacts of hyperkalemia on ventricle lactate or venous blood PO_2 ($p > 0.05$; Fig. 1). Given the lack of significant findings, subsequent analyses pooled data from control and hyperkalemia groups.

3.2. Impacts of oxygen saturation

For both acclimation groups (14 °C and 22 °C) and all oxygen treatments (hypoxia, normoxia, hyperoxia), the best fit model was a third order polynomial curve with an interaction between acute test temperature and oxygen treatment (Fig. 2). Other models and BIC values are presented in Supplemental Table 2.

Cardiac thermal limits and peak f_{Hmax} did not differ between fish acclimated to 14 °C and tested under normoxic and hyperoxic conditions ($p > 0.05$). We did, however, see significant reductions in T_{AB}, T_{Peak}, T_{ARR}, and peak f_{Hmax} between fish tested under hypoxia and those tested

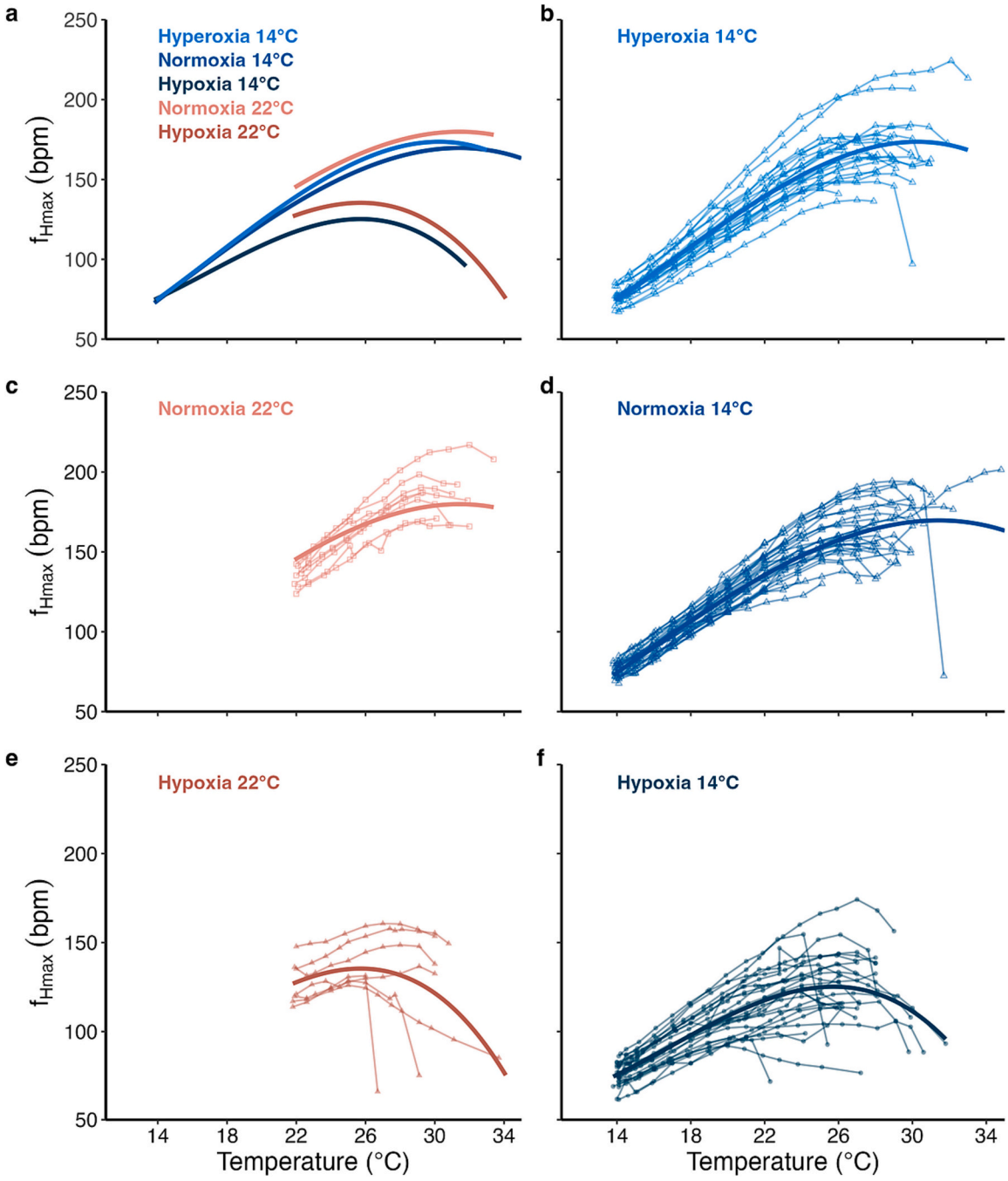

Fig. 2. Acute thermal performance curves on maximum heart rate (f_{Hmax}) from 14 °C (n = 24 fish per treatment; combined data from saline and high [K^+] injections) and 22 °C ($n = 7$ fish per treatment) acclimated fish under different gas treatments. Panel a shows the model-predicted curves for fish tested under hypoxia (20% saturation), normoxia (100% saturation), or hyperoxia (200% saturation) and the associated acclimation temperatures. The best-fitting model as selected by BIC was a third-order polynomial that included an interaction between test temperature and oxygen treatment, as well as individual fish as a random effect. Panels b-f show cardiac thermal performance curves for each treatment with individual fish and the best-fitting model.

at either hyperoxia or normoxia ($p < 0.01$ in all pairwise comparisons conducted with Tukey's post-hoc test, Fig. 3). For fish acclimated to 22 °C and tested under either hypoxia or normoxia, there was a trend for lower thermal tolerance under hypoxia but this was only significant for peak f_{Hmax} (Fig. 3).

Acclimation to warm temperature significantly increased T_{AB} and peak f_{Hmax} (Fig. 3, Supplemental Table 1). However, the negative impact of hypoxia was not equivalent across the two acclimation temperatures or across cardiac performance metrics. When comparing the magnitude of the detriments caused by hypoxia for fish acclimated to 22 °C compared to those acclimated to 14 °C, we found significantly smaller detriments in T_{AB}, T_{Peak}, and T_{ARR} in fish acclimated to 22 °C ($t = -227$, $t = -400$, and $t = -362$, respectively; $p < 0.01$ in all cases; Fig. 4). For example, hypoxia reduced T_{ARR} by 3% at 22 °C but by 7% at 14 °C. There was, however, a larger detriment in f_{Hmax} in fish acclimated to the warmer temperature, with reductions due to hypoxia of 24% at 22 °C but by 21% at 14 °C ($t = 133.2$, $p < 0.01$; Fig. 4).

3.3. Thresholds for the induction of cardiac failure

We did not find any universal threshold values for cardiac failure in either ventricle lactate or venous blood PO_2 (Fig. 5), which would have been indicated by a lack of any significant effects in our two-way ANOVA. Only the oxygen treatment significantly impacted ventricle lactate ($p = 0.05$), with hypoxia leading to increased ventricle lactate levels. In general, lactate varied widely (from 1.2 to 47.8 mmol kg^{-1}) at the time of arrhythmia (Fig. 5b). There was a significant effect of gas and acclimation temperature on the mean PO_2 values collected at the time of arrhythmia ($p = 0.01$ and $p < 0.01$, respectively), with hypoxic treatments exhibiting lower PO_2 at the time of arrhythmia than the normoxic and hyperoxic treatments (Supplemental Table 1). The combined PO_2 data shows a single peak in frequency between 8 and 11 Torr, representing a total of 10.4% of the total data (Fig. 4d).

4. Discussion

Studies on cardiac thermal limits have often assessed how they change across populations (Chen et al., 2018), acclimation temperatures (Anttila et al., 2014; Gilbert and Farrell, 2021; Schwieterman et al., 2022), diets (Hardison et al., 2021), or life stages (Drost et al., 2016).

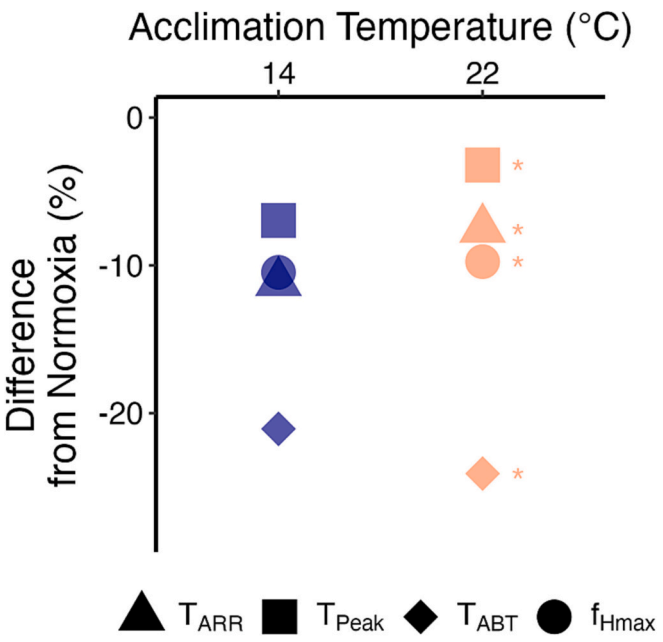

Fig. 4. Percent difference in cardiac thermal limits and peak maximum heart rate (peak f_{Hmax}) between fish tested under hypoxia (20% saturation) and normoxia (100% saturation) acclimated at 14° and 22 °C. All differences within metric and between temperatures are significant following bootstrap analysis with 1000 replicate calculations of percent change under hypoxia compared to normoxia. For each metric, *t*-tests were calculated on bootstrap analysis results. For all metrics, there was a significant difference between the mean values from fish acclimated to 14 °C and 22 °C.

Here, we assessed two of the leading hypotheses regarding the mechanism of f_{Hmax} collapse at high temperatures. Specifically, we tested the impacts of oxygen availability and simulated hyperkalemia on the relationship between temperature and f_{Hmax}. We found that there was no significant impact of hyperoxia or simulated plasma hyperkalemia on cardiac thermal limits. Hypoxia, however, did significantly decrease cardiac thermal limits, suggesting that oxygen can act as a limiting factor. The lack of consistency across measures of venous PO_2 levels and

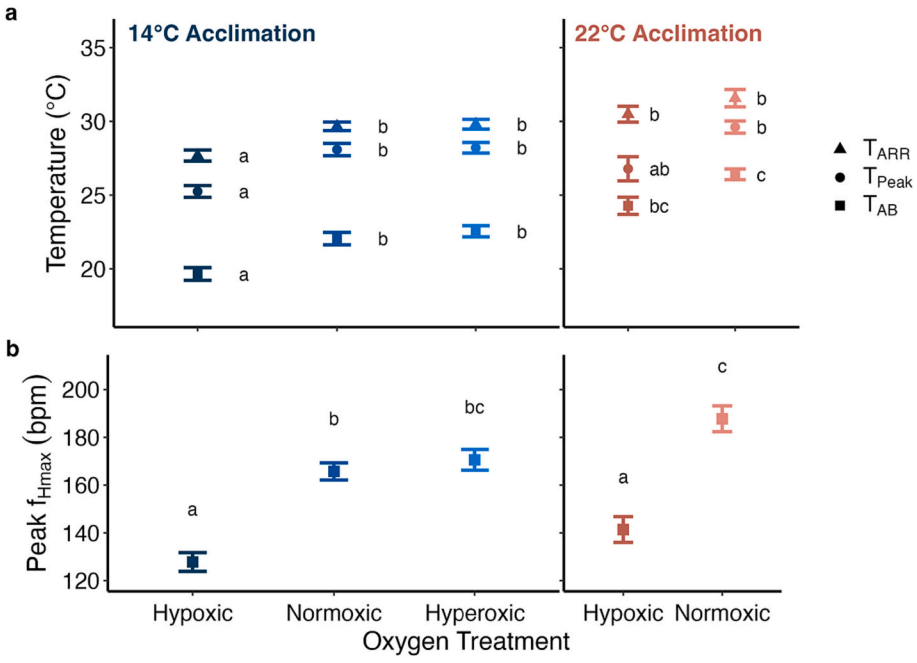

Fig. 3. The mean ± SE for thermal limits and peak maximum heart rate (peak f_{Hmax}) for fish acclimated to 14 °C (n = 24 per treatment) or 22 °C (n = 7 per treatment) and tested under hypoxia (20% saturation), normoxia (100% saturation), or hyperoxia (200% saturation). Lowercase letters indicate statistical differences within a given metric as analyzed by a two-way ANOVA including acclimation temperature and test oxygen condition as factors and Tukey's post-hoc test for pairwise comparisons. For all metrics the main effects of oxygen treatment and acclimation temperature were significant ($p < 0.05$).

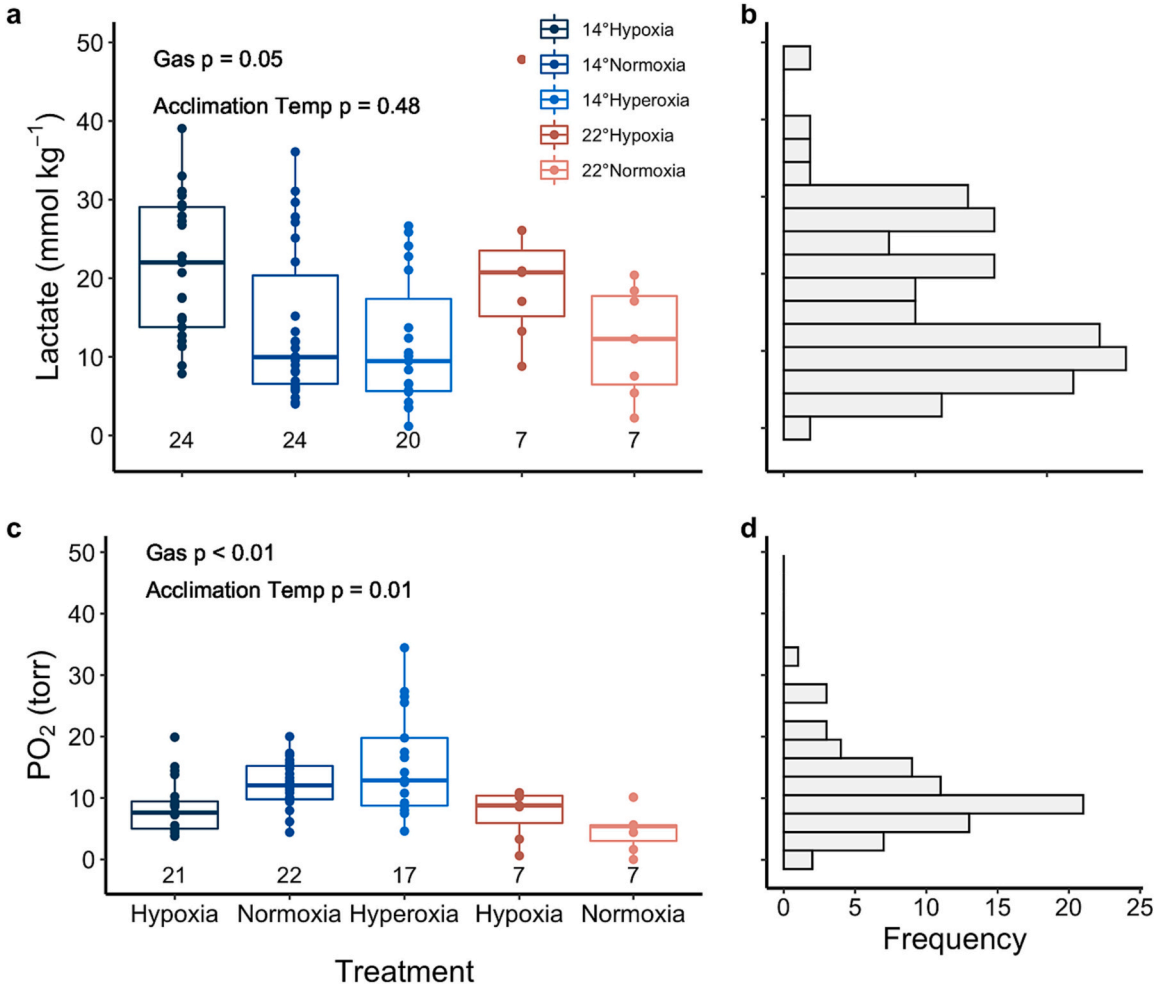

Fig. 5. The ventricle lactate concentration by treatment (a), lactate distribution (b), the venous PO_2 by treatment (c) and PO_2 distribution (d) at the time of arrhythmia in all fish included in this experiment (n values shown along the x axis). Hearts that were too small (<10 mg) were excluded from lactate analysis ($n = 4$), and blood that may have been air exposed during collection was also excluded ($n = 12$). Boxplots represent the median, and upper and lower quartile, with data from individual fish in overlayed points and the total sample size reported along the bottom of the plot. Determination of model selection regarding interactions was done with BIC scores, and the best fitting models did not include an interaction between oxygen level and acclimation temperature. The main effects are shown in the top left corner of each panel. Histograms used a bin size of 3.

ventricular lactate concentrations, however, suggests that additional mechanisms besides oxygen limitation cause cardiac failure at high temperatures.

4.1. Impacts of hyperkalemia

Noxious blood environments have been proposed to cause hearts failure in ectotherms, especially at high temperatures. Studies examining hyperkalemia and cardiac function in vitro have looked at the effects of $[K^+]$ values ranging from biologically relevant levels (e.g., 5 mmol l^{-1}, Hanson et al., 2006) to those far exceeding realistic in vivo concentrations (i.e., 300 mgm percent, Hiatt, 1943). Although the level which is biologically relevant is likely highly species-specific (Marshall et al., 2012), attempting to extrapolate the findings of studies using levels of potassium several orders of magnitude greater than measured values is difficult. It is thought that hyperkalemia occurs from K^+ leakage from skeletal muscle and is widely observed following enforced exercise (Schlenker et al., 2016; Wells et al., 1986). Hyperkalemia can lengthen action potential plateaus through elevated extracellular potassium, thus, decreasing heart rate (Badr et al., 2018; Hanson et al., 2006; Schwieterman et al., 2021)

We did not find any significant correlations between cardiac thermal limits or peak f_{Hmax} and level of hyperkalemia in fish injected with a high potassium solution. Notably, our K^+ injections did not induce a consistent increase in $[K^+]$ values relative to the saline injections, perhaps because the fish were able to rapidly metabolize the excess K^+ and excrete it through the gills, kidneys, and intestine (Presas-Basalo, 2022). As such, plasma $[K^+]$ values were relatively low, though some fish did exceed values we observed from forced exercise (e.g., 8–10 mmol l^{-1} $[K^+]$) and those observed in other fish species (e.g., *Oncorhynchus mykiss* 3.4–4.7 mmol l^{-1} Hanson et al., 2009; *Mustelus antarcticus* 2.7–5.2 mmol l^{-1}, Frick et al., 2010). Regardless, had we been able to achieve a more consistent elevated state of high $[K^+]$, we may have observed significant effects on cardiac performance. This method of assessing cardiac upper thermal limits does use isoproterenol to maximally stimulate the heart via stimulation of the β-adrenergic receptors. Isoproterenol has been demonstrated to improve function of isolated cardiac strips from elasmobranch fishes following exposure to hyperkalemia (Schwieterman et al., 2021), and 500 nmol L^{-1} adrenaline has been shown to ameliorate detrimental impacts of hyperkalemia in rainbow trout (Hanson et al., 2006). This method may therefore have obscured detriments of hyperkalemia alone, but may still be useful for interpreting in situ cardiac responses as maximum heart would only occur with adrenergic stimulation. Alternatively, this species may have high inter-individual variability in the capacity to excrete excess potassium. Interestingly, the noxious venous blood environment is often

considered a combination of low pH, low PO_2, and high [K^+] which all have demonstrated a reduction in contractility (Hanson et al., 2006). High [K^+] would rarely (if ever) occur in isolation, but we did not see these stressors act synergistically (e.g., we did not see increased detriments to cardiac function in the low oxygen hyperkalemia fish, Fig. 1).

4.2. Impacts of oxygen saturation

We found that low oxygen saturation reduced cardiac thermal tolerance in opaleye as evidenced by decreases in the fish's T_{AB}, T_{Peak}, T_{ARR} and peak f_{Hmax}. This supports previous work on isolated myocardium which has demonstrated reductions in oxygen can cause detriments in cardiac function such as decreases in contractile force, rate of contraction and relaxation, and maximum contraction frequency (Gesser, 1985; Joyce et al., 2016; Shiels et al., 2002). In vivo work has shown that hypoxia induces bradycardia in water-breathing fishes (Mendonca and Gamperl, 2010), which aligns with our observations of lower peak f_{Hmax}.

In fishes, the spongy myocardium of the heart is the last organ to receive oxygen in the body, and it is entirely supplied by the venous oxygen reserve. Accordingly, decreases in the PO_2 of the venous blood is likely to be detrimental to the aerobic production of ATP in the cardiomyocytes themselves. Indeed, according to the oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT), detriments to oxygen delivery mechanisms drive organismal thermal tolerance (Pörtner, 2010; Pörtner et al., 2017), although this hypothesis is still under debate (Jutfelt et al., 2018). While this hypothesis can be applied to any step in the oxygen cascade (e.g., gill permeability, Hb-O_2 binding affinity, mitochondrial efficiency), many of these proposed detriments will impact the oxygen available to the cardiac tissue itself, and thus limit cardiac performance under rising temperatures. These hypotheses also suggest that hyperoxia should benefit performance, as it would remove constraints due to oxygen limitation.

We did not, however, find any benefits to thermal tolerance when fish were tested under hyperoxic conditions. Work on isolated myocardium has demonstrated that the heart is functionally oxygen limited, with increases in contractility under hyperoxic conditions (Gesser and Rodnick, 2019; Syme et al., 2013). Our results, however, are consistent with work on European perch (*Perca fluviatilis*) which found that hyperoxia increased venous PO_2, but did not increase cardiac thermal limits (Ekström et al., 2016). Similarly, in giant goby (*Gobius cobitis*), hyperoxia (175%–285% saturation) had no impact on f_H at 12.5 °C, nor did it affect f_H following acute warming to 25 °C (Berschick et al., 1987). Rainbow trout (*Oncorhynchus mykiss*) undergoing modified CTmax tests in hyperoxia (200% saturation) also showed no difference in f_H at peak MO_2 (~25 °C), although they did have higher venous PO_2 and lower plasma lactate compared to fish tested in normoxia (McArley et al., 2022).

Although there is debate on its ecological relevance, critical thermal maxima (CTmax) may help us compare physiological processes underpinning mechanisms conferring thermal tolerance across species, particularly as many studies have examined the impact of hyperoxia on this metric (Desforges et al., 2023; Ern et al., 2023). For example, hyperoxia increases CTmax in goldfish (*Cassarius arratus*) (Weatherley, 1970), rainbow trout (McArley et al., 2022), silversides (*Atherinomorous* sp.), damselfish (*Dascyllus* sp.) (Giomi et al., 2019), brycon (*Brycon amazonicus*), marbeled hatchetfish (*Carnegiella strigata*), Schwartz's catfish (*Corydoras schwartzi*), cardinal tetras (*Paracheirodon axelrodi*) (Jung et al., 2020), and in common triplefin (*Forsterygion lapillum*), although the last was not biologically significant (McArley et al., 2018). There is, however, variability in this response across taxa, with no significant change in CTmax under hyperoxic conditions in European perch (Brijs et al., 2015), twister (*Bellapiscis medius*) (McArley et al., 2018), brook charr (*Salvelinus fontinalis*) (Ellis et al., 2013), zebrafish (*Danio rerio*) (Reiersen, 2021), blackstripe topminnow (*Fundulus notatus*), red shiner (*Notropis lutrensis*), bullhead minnow (*Pimephales vigilax*) (Rutledge and Beitinger, 1989), tambaqui (*Colossoma macropomum*), and pretty corycatfish (*Corydoras pulcher*) (Jung et al., 2020). Nevertheless, the disparate responses across species suggests that the physiological processes governing cardiac thermal tolerance and CTmax may not always be the same (Desforges et al., 2023; Ern et al., 2023; McArley et al., 2022; McArley et al., 2021).

The lack of benefit of hyperoxia observed here could be due to limitations of the oxygen transport cascade, or due to actual physiological detriments directly related to excess oxygen. Hyperoxia can cause decreased respiration leading to respiratory acidosis, and can also increase levels of oxidative stress (McArley et al., 2021). To better understand these mechanisms and how they relate to thermal tolerance in fish, future work should assess the impact of hyperoxia on cardiac thermal tolerance, specifically in species where increases in CTmax have been observed, and with simultaneous measures of reactive oxygen species (ROS) and antioxidant responses.

When fish were acclimated to 22 °C rather than 14 °C, we saw significant increases in thermal limits. This is consistent with previous work in this species and many others showing warm acclimation can increase acute thermal tolerance (Beitinger et al., 2000; Gilbert and Farrell, 2021; Hardison et al., 2023; Safi et al., 2019; Schulte et al., 2011; Stenseng et al., 2005; Stillman, 2003). Interestingly, warm acclimation *increased* the detriment caused by hypoxia for peak f_{Hmax}, but *decreased* the magnitude of the effect of hypoxia on cardiac thermal limits (i.e., T_{AB}, T_{Peak}, T_{ARR}). This suggests there are different mechanisms governing peak f_{Hmax} and cardiac thermal limits. While peak f_{Hmax} may be limited by the ability of the cardiomyocytes to move ions across the cell membrane rapidly, T_{ARR} may be driven by a lack of Ca^{2+} movement either between intra- and extra-cellular environments, or between the sarcoplasmic reticulum and the intracellular environment (reviewed by Shiels et al., 2002).

4.3. Thresholds for the induction of cardiac failure

If oxygen limits cardiac thermal tolerance, we would expect to find a threshold level of venous PO_2, below which, cardiac failure occurs (Clark et al., 2008; Davie and Farrell, 1991; Farrell and Clutterham, 2003; Lannig et al., 2004; Syme et al., 2013). While this may hold true in some species (e.g., European perch, Ekström et al., 2016; rainbow trout, Farrell and Clutterham, 2003; Atlantic cod, Lannig et al., 2004), for opaleye in this experiment, we found significantly variable levels of venous PO_2 at the time of cardiac arrhythmia. Interestingly, the values of PO_2 at the time of arrhythmia in opaleye (10.8 ± 0.78 Torr, mean ± SE) which lack coronary circulation, are less similar to values from other species lacking coronary circulation (European perch, 17.3–30 Torr), and more in line with the threshold found in salmonids (6–15 Torr) which do possess coronary circulation and thus are less dependent upon the venous reserve (Clark et al., 2008; Ekström et al., 2016; Eliason et al., 2013; Farrell and Clutterham, 2003). The coronary circulation supplies the outer compact myocardium with a reliable source of oxygen-rich blood, so fish lacking this oxygen supply are more dependent upon the venous reserve to fuel aerobic metabolism of the cardiomyocytes. While the importance of coronary circulation is well known (Davie and Farrell, 1991; Ekström et al., 2018), these results suggest that other factors may influence the level of oxygen necessary to maintain cardiac function in spongy myocardium. Interspecific differences in PO_2 thresholds could also be explained, for example, by differences in the cardiac power needed to perfuse important tissues, with some species requiring higher oxygen levels at the heart due to an active lifestyle or residence in a warm habitat. It may be that under normoxic and hyperoxic conditions, other factors besides venous PO_2 cause arrhythmia, thus driving the observed difference between the PO_2 values from 14 °C acclimated fish tested under hypoxia (9.61 ± 1.64 Torr, mean ± SE) and those tested under hyperoxia (21.61 ± 3.95 Torr). Importantly, when oxygen is limiting, either via environmental hypoxia or via internal hypoxemia, venous PO_2 levels may reach a threshold

value (e.g., ~ 8–11 Torr) that limits cardiac function before other mechanisms essential for regular excitation/relaxation coupling processes can fail.

We observed variable levels of ventricle lactate, with the highest lactate levels in fish tested under hypoxia at 14 °C. This is supported by hypoxic fish having low PO_2, suggesting these fish were relying more heavily on anaerobic metabolism. Acclimation temperature had no significant impact on lactate. This may be species-specific, as other work has shown up to a three-fold increase in resting lactate levels in sockeye salmon with increased temperatures (Eliason et al., 2013) and red seabream (*Pagrus major)* show a positive relationship with lactate and acclimation temperature (Jeong et al., 2021). In contrast, studies on rainbow trout showed no difference across a range of 8 °C of acclimation temperatures (Connors et al., 1978). These species-specific differences may be indicative of differences in oxygen utilization by other tissues (drawing down of the venous reserve) under thermal stress.

4.4. Remaining questions

Other potential mechanisms that may cause the onset of cardiac arrhythmia include mismatches in the temperature sensitivity of inward Na^+ movement and rectifier K^+ during the membrane depolarization and repolarization cycle (Vornanen, 2020), or a buildup of ROS that causes disruptions to contractility (Iftikar and Hickey, 2013). Changes in the fluidity or excitability of the membrane may also be implicated in the cause of arrhythmias at high temperatures (Haverinen and Vornanen, 2020; Lennard and Huddart, 1991; Moyes and Ballantyne, 2011; Vornanen, 2020, 2021). Disruptions to brain function due to overheating could also cause cardiac arrhythmias (Ern et al., 2023; Jutfelt et al., 2019), however in our experimental design we removed sympathetic and parasympathetic control of the heart by injecting the fish with atropine and isoproterenol. Work on Atlantic cod and spotty (*Notolabrus celidotus*) have found that oxidative phosphorylation in isolated cardiac mitochondria fails at high temperatures despite high oxygen availability (Iftikar and Hickey, 2013; Rodnick et al., 2014), suggesting this subcellular mechanism may be implicated in the driver of cardiac collapse at high temperatures. More work linking these mechanisms specifically to f_{Hmax} is needed to fully understand the potential drivers of f_{Hmax} collapse at high temperatures (Eliason and Anttila, 2017 (Ern et al., 2023)).

Here, we assessed the impacts of acute physiological stress on the relationship between temperature and f_{Hmax} to investigate drivers of f_{Hmax} collapse, and thus ectothermic thermal limits. We successfully ruled out oxygen limitation as the sole metric determining cardiac upper thermal limits. Although we found that hypoxia reduces cardiac thermal tolerance, we did not find any benefit to cardiac thermal tolerance under hyperoxia. This was further corroborated by the lack of a species-wide threshold for either blood PO_2 at the onset of arrhythmia or cardiac lactate levels. Although hyperkalemia is known to have detrimental impacts on isolated myocardium function, it did not significantly affect cardiac thermal limits in this study. We posit there is likely a suite of factors that can cause cardiac failure at high temperatures, any one of which may become limiting in different species or under different physiological conditions.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made publicly available through Dryad data repository.

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Appendix A. Supplementary data

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References

Anttila, K., Casselman, M.T., Schulte, P.M., Farrell, A.P., 2013. Optimum temperature in juvenile salmonids: connecting subcellular indicators to tissue function and whole-organism thermal optimum. Physiol. Biochem. Zool. 86, 245–256.
Anttila, K., Couturier, C.S., Overli, O., Johnsen, A., Marthinsen, G., Nilsson, G.E., Farrell, A.P., 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. Nat. Commun. 5, 4252.
Badr, A., Abu-Amra, E.S., El-Sayed, M.F., Vornanen, M., 2018. Electrical excitability of roach (Rutilus rutilus) ventricular myocytes: effects of extracellular K(+), temperature, and pacing frequency. Am. J. Phys. Regul. Integr. Comp. Phys. 315, R303–R311.
Bakun, A., Black, B.A., Bograd, S.J., García-Reyes, M., Miller, A.J., Rykaczewski, R.R., Sydeman, W.J., 2015. Anticipated effects of climate change on coastal upwelling ecosystems. Curr. Clim. Change Rep. 1, 85–93.
Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ. Biol. Fish 58, 237–275.
Bergmeyer, H.U., 1983. Methods of Enzymatic Analysis. Academic Press, New York.
Berschick, P., Bridges, C.R., Grieshaber, M.K., 1987. The influence of hyperoxia, hypoxia and temperature on the respiratory physiology of ther intertidal rockpool fish *Gobius cobitis* Pallas. J. Exp. Biol. 130, 369–387.
Bianchi, T.S., DiMarco, S.F., Cowan Jr., J., Hetland, R.D., Chapman, P., Day, J., Allison, M., 2010. The science of hypoxia in the Northern Gulf of Mexico: a review. Sci. Total Environ. 408, 1471–1484.
Brijs, J., Jutfelt, F., Clark, T.D., Grans, A., Ekstrom, A., Sandblom, E., 2015. Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. J. Exp. Biol. 218, 2448–2454.
Brill, R.W., Lai, N.C., 2016. Elasmobranch cardiovascular system. Physiol. Elasmobranch Fishes: Intern. Process. 34.
Casselman, M.T., Anttila, K., Farrell, A.P., 2012. Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon Oncorhynchus spp. J. Fish Biol. 80, 358–377.
Chen, Z., Farrell, A.P., Matala, A., Hoffman, N., Narum, S.R., 2018. Physiological and genomic signatures of evolutionary thermal adaptation in redband trout from extreme climates. Evol. Appl. 11, 1686–1699.
Chung, D.J., Schulte, P.M., 2020. Mitochondria and the thermal limits of ectotherms. J. Exp. Biol. 223.
Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., Farrell, A.P., 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (Oncorhynchus tshawytscha). Am. J. Phys. Regul. Integr. Comp. Phys. 295, R1631–R1639.
Connors, T.J., Schneider, M.J., Genoway, R.G., Barraclough, S.A., 1978. Effect of acclimation temperature on plasma levels of glucose and lactate in rainbow trout, Salmo gairdneri. J. Exp. Zool. 206, 443–449.
Davie, P.S., Farrell, A.P., 1991. The coronary and luminal circulations of the myocardium of fishes. Can. J. Zool. 69, 1993–2001.
Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L., McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fangue, N., Cooke, S.J., 2023. The ecological relevance of critical thermal maxima methodology for fishes. J. Fish Biol. 102 (5), 1000–1015.
Driedzic, W.R., Gesser, H., 1994. Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis, and low temperature. Physiol. Rev. 74, 221–258.
Drost, H.E., Fisher, J., Randall, F., Kent, D., Carmack, E.C., Farrell, A.P., 2016. Upper thermal limits of the hearts of Arctic cod *Boreogadus saida*: adults compared with larvae. J. Fish Biol. 88, 718–726.
Ekström, A., Brijs, J., Clark, T.D., Grans, A., Jutfelt, F., Sandblom, E., 2016. Cardiac oxygen limitation during an acute thermal challenge in the European perch: effects

of chronic environmental warming and experimental hyperoxia. Am. J. Phys. Regul. Integr. Comp. Phys. 311, R440–R449.
Ekström, A., Axelsson, M., Grans, A., Brijs, J., Sandblom, E., 2018. Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (Oncorhynchus mykiss). Biol. Lett. 14.
Eliason, E.J., Anttila, K., 2017. Temperature and the cardiovascular system. Fish Physiol. 235–297. Elsevier.
Eliason, E.J., Clark, T.D., Hinch, S.G., Farrell, A.P., 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv. Physiol. 1, cot008.
Eliason, E.J., Van Wert, J.C., Schwieterman, G.D., 2022. Applied aspects of the cardiorespiratory system. In: Cooke, S.J., Fangue, N.A., Farrell, A.P., Brauner, C.J., Eliason, E.J. (Eds.), Fish Physiology. Elsevier, pp. 189–252.
Ellis, L.E., Sacobie, C.F., Kieffer, J.D., Benfey, T.J., 2013. The effects of dissolved oxygen and triploidy on critical thermal maximum in brook charr (Salvelinus fontinalis). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166, 426–433.
El-Sayed, M.F., Gesser, H., 1989. Sarcoplasmic reticulum, potassium, and cardiac force in rainbow trout and plaice. Am. J. Phys. Regul. Integr. Comp. Phys. 257, R599–R604.
Ern, R., Andreassen, A.H., Jutfelt, F., 2023. Physiological mechanisms of acute upper thermal tolerance in fish. Physiology (Bethesda) 38, 141–158.
Farrell, A.P., 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J. Exp. Biol. 212, 3771–3780.
Farrell, A., Clutterham, S., 2003. On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J. Exp. Biol. 206, 487–496.
Farrell, A., Milligan, C., 1986. Myocardial intracellular pH in a perfused rainbow trout heart during extracellular acidosis in the presence and absence of adrenaline. J. Exp. Biol. 125, 347–359.
Ferreira, E.O., Anttila, K., Farrell, A.P., 2014. Thermal optima and tolerance in the eurythermic goldfish (*carassius auratus*): Relationships between whole-animal aerobic capacity and maximum heart rate. Physiol. Biochem. Zool. 87, 599–611.
Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance definded by cardiac and ventilatory performance in spider crab, Maja squinado. Am. J. Phys. Regul. Integr. Comp. Phys. 279, R1531–R1538.
Frick, L.H., Reina, R.D., Walker, T.I., 2010. Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. J. Exp. Mar. Biol. Ecol. 29–37.
Gamperl, A.K., Shiels, H.A., 2014. Cardiovascular System. In: Evans, D.H., Claiborne, J. B., Currie, S. (Eds.), The Physiology of Fishes, 4th ed. CRC Press, Boca Raton, FL, pp. 34–69.
Gesser, H., 1985. Effects of hypoxia and acidosis on fish heart performance, Circulation, Respiration, and Metabolism. Springer 402–411.
Gesser, H., Jorgensen, E., 1982. pHi, contractility and Ca-balance under hypercapnic acidosis in the myocardium of different vertebrate species. J. Exp. Biol. 96, 405–412.
Gesser, H., Poupa, O., 1979. Effects of Different Types of Acidosis and Ca2+ on Cardiac Contractility in the Flounder (Pleuronectes flesus). J. Comp. Physiol. A. 131, 293–296.
Gesser, H., Rodnick, K.J., 2019. Is the teleost heart oxygen limited? – Insights using "hyperoxic" incubations of contracting cardiac tissue from rainbow trout. Comp. Biochem. Physiol. A Mol. Integr. 231, 124–130.
Gilbert, M.J.H., Farrell, A.P., 2021. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (Salvelinus alpinus), a northern cold-water specialist. J. Therm. Biol. 95, 102816.
Giomi, F., Barausse, A., Duarte, C.M., Booth, J., Agusti, S., Saderne, V., Anton, A., Daffonchio, D., Fusi, M., 2019. Oxygen supersaturation protects coastal marine fauna from ocean warming. Science. Advances 5 eaax1814.
Gissi, E., Manea, E., Mazaris, A.D., Fraschetti, S., Almpanidou, V., Bevilacqua, S., Coll, M., Guarnieri, G., Lloret-Lloret, E., Pascual, M., Petza, D., Rilov, G., Schonwald, M., Stelzenmuller, V., Katsanevakis, S., 2021. A review of the combined effects of climate change and other local human stressors on the marine environment. Sci. Total Environ. 755, 142564.
Gradil, K.J., Garner, S.R., Wilson, C.C., Farrell, A.P., Neff, B.D., 2016. Relationship between cardiac performance and environment across populations of atlantic salmon (*salmo salar*): A common garden experiment implicates local adaptation. Evol. Ecol. 30, 877–886.
Hall, J.M., Sun, B.J., 2021. Heat tolerance of reptile embryos: Current knowledge, methodological considerations, and future directions. J. Exp. Zool. A Ecol. Integr. Physiol. 335, 45–58.
Hanson, L.M., Obradovich, S., Mouniargi, J., Farrell, A.P., 2006. The role of adrenergic stimulation in maintaining maximum cardiac performance in rainbow trout (*Oncorhynchus mykiss*) during hypoxia, hyperkalemia and acidosis at 10° C. J. Exp. Biol. 209, 2442–2451.
Hansen, A.K., Byriel, D.B., Jensen, M.R., Steffensen, J.F., Svendsen, M.B.S., 2017. Optimum temperature of a northern population of arctic charr (*salvelinus alpinus*) using heart rate arrhenius breakpoint analysis. Polar Biol 40, 1063–1070.
Hanson, L.M., Baker, D.W., Kuchel, L.J., Farrell, A.P., Val, A.L., Brauner, C.J., 2009. Intrinsic mechanical properties of the perfused armoured catfish heart with special reference to the effects of hypercapnic acidosis on maximum cardiac performance. J. Exp. Biol. 212, 1270–1276.
Hardison, E.A., Kraskura, K., Van Wert, J., Nguyen, T., Eliason, E.J., 2021. Diet mediates thermal performance traits: implications for marine ectotherms. J. Exp. 224 (1), jeb242846.
Hardison, E.A., Schwieterman, G.D., Eliason, E.J., 2023. Diet changes thermal acclimation capacity, but not acclimation rate, in a marine ectotherm (Girella nigricans) during warming. Proc. Biol. Sci. 290, 20222505.
Haverinen, J., Vornanen, M., 2020. Reduced ventricular excitability causes atrioventricular block and depression of heart rate in fish at critically high temperatures. J. Exp. Biol. 223.
Hiatt, E.P., 1943. The action of adrenaline, acetylcholine and potassium in relation to the innervation of the isolated auricle of the spiny dogfish (*Squalus acanthias*). Am. J. Physiol.-Legal Content 139, 45–48.
Iftikar, F.I., Hickey, A.J., 2013. Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in Notolabrus celidotus. PLoS One 8, e64120.
Jeong, H., Kang, M., Cha, S.-Y., Byun, J., Kim, J., Baek, J.W., Park, J.J., Shin, S.R., Kim, H.J., Lee, J.S., 2021. Usefulness of clustering blood biochemical markers to assess thermal stress and acclimation in red seabream, Pagrus major. Aquaculture 545, 737197.
Joyce, W., Ozolina, K., Mauduit, F., Ollivier, H., Claireaux, G., Shiels, H.A., 2016. Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. Biol. Lett. 12, 20150708.
Jung, E.H., Brix, K.V., Richards, J.G., Val, A.L., Brauner, C.J., 2020. Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world. Sci. Total Environ. 748, 141349.
Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D.J., Lefevre, S., Nilsson, G.E., Metcalfe, N.B., Hickey, A.J.R., Brijs, J., Speers-Roesch, B., Roche, D.G., Gamperl, A.K., Raby, G.D., Morgan, R., Esbaugh, A.J., Grans, A., Axelsson, M., Ekstrom, A., Sandblom, E., Binning, S.A., Hicks, J.W., Seebacher, F., Jorgensen, C., Killen, S.S., Schulte, P.M., Clark, T.D., 2018. Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. J. Exp. Biol. 221 jeb169615.
Jutfelt, F., Roche, D.G., Clark, T.D., Norin, T., Binning, S.A., Speers-Roesch, B., Amcoff, M., Morgan, R., Andreassen, A.H., Sundin, J., 2019. Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. J. Exp. Biol. 222.
Kalinin, A., Gesser, H., 2002. Oxygen consumption and force development in turtle and trout cardiac muscle during acidosis and high extracellular potassium. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 172, 145–151.
Kalinin, A., Costa, M., Rantin, F., Glass, M., 2009. Effects of temperature on cardiac function in teleost fish. Cardio-Respir. Control Vertebr. 121–160.
Lannig, G., Bock, C., Sartoris, F.J., Portner, H.O., 2004. Oxygen limitation of thermal tolerance in cod, Gadus morhua L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287, R902–R910.
Leeuwis, R.H.J., Zanuzzo, F.S., Peroni, E.F.C., Gamperl, A.K., 2021. Research on sablefish (Anoplopoma fimbria) suggests that limited capacity to increase heart function leaves hypoxic fish susceptible to heat waves. Proc. Biol. Sci. 288, 20202340.
Lefevre, S., Wang, T., McKenzie, D.J., 2021. The role of mechanistic physiology in investigating impacts of global warming on fishes. J. Exp. Biol. 224.
Lennard, R., Huddart, H., 1991. The effect of thermal stress on electrical and mechanical responses and associated calcium movements of flounder heart and gut. Comp. Biochem. Physiol. 98A, 221–229.
Marshall, H., Field, L., Afiadata, A., Sepulveda, C., Skomal, G., Bernal, D., 2012. Hematological indicators of stress in longline-captured sharks. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 162, 121–129.
McArley, T.J., Hickey, A.J.R., Herbert, N.A., 2018. Hyperoxia increases maximum oxygen consumption and aerobic scope of intertidal fish facing acutely high temperatures. J. Exp. Biol. 221.
McArley, T.J., Sandblom, E., Herbert, N.A., 2021. Fish and hyperoxia—From cardiorespiratory and biochemical adjustments to aquaculture and ecophysiology implications. Fish Fish. 22, 324–355.
McArley, T.J., Morgenroth, D., Zena, L.A., Ekstrom, A.T., Sandblom, E., 2022. Prevalence and mechanisms of environmental hyperoxia-induced thermal tolerance in fishes. Proc. Biol. Sci. 289, 20220840.
Mendonca, P.C., Gamperl, A.K., 2010. The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (Pseudopleuronectes americanus). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 155, 245–252.
Moyes, C., Ballantyne, J., 2011. TEMPERATURE Membranes and Temperature: Homeoviscous Adaptation.
Muggeo, V.M., 2008. Segmented: an R package to fit regression models with broken-line relationships. R News 8, 20–25.
Nielsen, J.S., Gesser, H., 2001. Effects of high extracellular [K^+] and adrenaline on force development, relaxation and membrane potential in cardiac muscle from freshwater turtle and rainbow trout. J. Exp. Biol. 204, 261–268.
Oellermann, M., Hickey, A.J.R., Fitzgibbon, Q.P., Smith, G., 2020. Thermal sensitivity links to cellular cardiac decline in three spiny lobsters. Sci. Rep. 10, 202.
Oliver, E.C.J., Benthuysen, J.A., Darmaraki, S., Donat, M.G., Hobday, A.J., Holbrook, N. J., Schlegel, R.W., Sen Gupta, A., 2021. Marine Heatwaves. Annu. Rev. Mar. Sci. 13, 313–342.
Paajanen, V., Vornanen, M., 2003. Effects of Chronic Hypoxia on Inward Rectifier K^+ Current (I_{K1}) in Ventricular Myocytes of Crucian Carp (*Carassius carassius*) Heart. J. Membr. Biol. 194, 119–127.
Petersen, L.H., Gamperl, A.K., 2010. Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (Gadus morhua). J. Exp. Biol. 213, 808–819.
Pörtner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–893.
Pörtner, H.O., Bock, C., Mark, F.C., 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J. Exp. Biol. 220, 2685–2696.
Pörtner, H.-O., Roberts, D.C., Adams, H., Adler, C., Aldunce, P., Ali, E., Begum, R.A., Betts, R., Kerr, R.B., Biesbroek, R., 2022. Climate change 2022: Impacts, adaptation and vulnerability. IPCC Geneva, Switzerland.

Presas-Basalo, F., 2022. Potassium homeostasis and fish welfare in coupled aquaponic systems. Fish. Aquac. J. 13.
Reiersen, M., 2021. The effects of oxygen availability and acclimation temperature on upper thermal tolerance in zebrafish (*Danio rerio*). Department of Biology. Norwegian University of Science and Technology.
Richards, J.G., Heigenhauser, G.J.F., Wood, C.M., 2002. Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. Am. J. Physiol.- Regul. Integr. Comp. Physiol. 282, R89–R99.
Rodnick, K.J., Gesser, H., 2017. Cardiac energy metabolism. Fish Physiol. Elsevier 317–367.
Rodnick, K.J., Gamperl, A.K., Nash, G.W., Syme, D.A., 2014. Temperature and sex dependent effects on cardiac mitochondrial metabolism in Atlantic cod (*Gadus morhua* L.). J. Therm. Biol. 44, 110–118.
Rutledge, C.J., Beitinger, T.L., 1989. The effects of dissolved oxygen and aquatic surfact respiration on the critical thermal mexima of three intermittent-stream fishes. Environ. Biol. Fish 24, 137–143.
Safi, H., Zhang, Y., Schulte, P.M., Farrell, A.P., 2019. The effect of acute warming and thermal acclimation on maximum heart rate of the common killifish Fundulus heteroclitus. J. Fish Biol. 95, 1441–1446.
Schlenker, L.S., Latour, R.J., Brill, R.W., Graves, J.E., 2016. Physiological stress and post-release mortality of white marlin (*Kajikia albida*) caught in the United States recreational fishery. Conserv. Physiol. 4 cov066.
Schulte, P.M., Healy, T.M., Fangue, N.A., 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr. Comp. Biol. 51, 691–702.
Schwieterman, G.D., Winchester, M.M., Shiels, H.A., Bushnell, P.G., Bernal, D., Marshall, H.M., Brill, R.W., 2021. The effects of elevated potassium, acidosis, reduced oxygen levels, and temperature on the functional properties of isolated myocardium from three elasmobranch fishes: clearnose skate (*Rostroraja eglanteria*), smooth dogfish (*Mustelus canis*), and sandbar shark (*Carcharhinus plumbeus*). J. Comp. Physiol. B. 127–141.
Schwieterman, G.D., Hardison, E.A., Eliason, E.J., 2022. Effect of thermal variation on the cardiac thermal limits of a eurythermal marine teleost (*Girella nigricans*). Curr. Res. Physiol. 5, 109–117.
Shiels, H.A., Vornanen, M., Farrell, A.P., 2002. The force-frequency relationship in fish hearts- a review. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 132, 811–826.
Smith, K.E., Burrows, M.T., Hobday, A.J., Sen Gupta, A., Moore, P.J., Thomsen, M., Wernberg, T., Smale, D.A., 2021. Socioeconomic impacts of marine heatwaves: Global issues and opportunities. Science 374 eabj3593.
Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. 213, 912–920.
Steffensen, J.F., Farrell, A.P., 1998. Swimming Performance, Venous Oxygen Tension and Cardiac Performance of Coronary-Ligated Rainbow Trout, *Oncorhynchus mykiss*, Exposed to Progressive Hypoxia.
Steinhausen, M.F., Sandblom, E., Eliason, E.J., Verhille, C., Farrell, A.P., 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (Oncorhynchus nerka). J. Exp. Biol. 211, 3915–3926.
Stenseng, E., Braby, C.E., Somero, G.N., 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus *Tegula*): Implications for vertical zonation. Biol. Bull. 208, 138–144.
Stillman, J.H., 2002. Causes and consequences of thermal tolerance limits in Rocky Intertidal Porcelain Crabs, Genus *Petrolisthes*. Integr. Comp. Biol. 42, 790–796.
Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.
Stuart-Smith, R.D., 2021. Climate change: large-scale abundance shifts in fishes. Curr. Biol. 31, R1445–R1447.
Syme, D.A., Gamperl, A.K., Nash, G.W., Rodnick, K.J., 2013. Increased ventricular stiffness and decreased cardiac function in Atlantic cod (Gadus morhua) at high temperatures. Am. J. Phys. Regul. Integr. Comp. Phys. 305, R864–R876.
van der Walt, K.-A., Potts, W.M., Porri, F., Winkler, A.C., Duncan, M.I., Skeeles, M.R., James, N.C., 2021. Marine heatwaves exceed cardiac thermal limits of adult Sparid Fish (Diplodus capensis, Smith 1884). Front. Mar. Sci. 8.
Vornanen, M., 2016. The temperature dependence of electrical excitability in fish hearts. J. Exp. Biol. 219, 1941–1952.
Vornanen, M., 2020. Feeling the heat: source-sink mismatch as a mechanism underlying the failure of thermal tolerance. J. Exp. Biol. 223.
Vornanen, M., 2021. Effects of acute warming on cardiac and myotomal sarco(endo) plasmic reticulum ATPase (SERCA) of thermally acclimated brown trout (Salmo trutta). J. Comp. Physiol. B. 191, 43–53.
Weatherley, A.H., 1970. Effects of Superabundant Oxygen on Thermal Tolerance of Goldfish. Biol. Bull. 139, 229–238.
Wells, R.M.G., McIntyre, R.H., Morgan, A.K., Davie, P.S., 1986. Physiological stress responses in big gamefish after capture observations on plasma chemistry and blood factors. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 84A, 565–571.