

1 Large differences in thermal tolerance between isolated organs in rainbow trout

2

3

4 Pfeufer, L.¹, Olsson, C¹ and Jutfelt, F.¹

5 Leon Pfeufer: 0009-0008-6924-2602, leon.pfeufer@bioenv.gu.se

6 Catharina Olsson: 0009-0000-4916-8929, Catharina.olsson@bioenv.gu.se

7 Fredrik Jutfelt: 0000-0001-9838-3991, fredrik.jutfelt@bioenv.gu.se

8 ¹ Department of Biological and Environmental Sciences, Faculty of Science, University of
9 Gothenburg, Gothenburg, 413 90, Sweden

10

11 * Corresponding author: Leon Pfeufer at leon.pfeufer@bioenv.gu.se

12

Abstract

Due to climate change, research on thermal performance limits in ectotherms has received much attention in the last two decades. However, the underlying physiological mechanisms and most vulnerable organs remain largely unknown. Here, we examined contractile performance in different organs from rainbow trout during an acute warming challenge, including the heart (cardiac muscle), caudal peduncle (white skeletal muscle) and the gastrointestinal tract (smooth muscle). The preparations were electrically stimulated at three voltages *in vitro* to record contraction force, while temperature was progressively increased. To relate tissue-level responses to organismal thermal limits, whole-animal thermal tolerance (CT_{max}) was recorded. White skeletal muscle was the most vulnerable, retaining less than 25% of baseline contraction force at the whole-animal CT_{max} temperature. This suggests that white skeletal muscle may be a previously overlooked contributor to acute upper thermal limits in fish. Notably, skeletal muscle contraction force had already fallen to 50% of baseline several degrees below CT_{max} , indicating that heat waves may impair swim performance well below lethal thermal limits. Additionally, only 50% of the atrial preparations still produced spontaneous contractions at CT_{max} temperature, suggesting severe impairment of excitation-contraction coupling at high temperatures. We observed a voltage-dependent decline in ventricular contractility, consistent with predictions of the temperature-dependent deterioration of electrical excitability (TDEE) hypothesis, whereas no comparable voltage-dependent pattern was observed in white skeletal muscle. This suggests that reduced electrical excitability at elevated temperatures is not universal across electrically excitable tissues. Together, our results identify white skeletal muscle and cardiac function as potential contributors to acute upper thermal tolerance limits.

Introduction

The past decade has been the warmest on record, with 2024 ranking as the hottest year to date. (IPCC, 2023; WMO, 2025; NASA, 2025). Amongst the consequence of a warming climate are the rising average surface water temperatures and increasing frequency, duration and severity of extreme thermal events such as heat waves (Calvin et al., 2023; Smith et al., 2023). Ectotherms like fish, may be particularly vulnerable to such thermal challenges, because their body temperature is directly linked to the environmental temperature, and thus any changes are likely to affect various physiological processes. Although ectotherms can often tolerate a range of temperatures, there is typically an optimal temperature for their overall performance. Outside of the thermal tolerance range, survival becomes time-limited and is ultimately no longer possible at the acute upper thermal limit (Jørgensen et al., 2019; Jutfelt et al., 2024).

A common way to determine the acute upper thermal tolerance limit of a fish is by the thermal maximum method (CT_{max}), where the temperature is gradually increased until control of locomotor function is lost and the fish is unable to maintain an upright position in the water column. This is most commonly defined as the loss of equilibrium (LOE) (Cox, 1974; Lutterschmidt & Hutchison, 1997). While research on thermal tolerance of fish, particularly using CT_{max} , has found increasing attention in the last decade (G. Raby et al., 2025b), the underlying physiological mechanism that determines acute upper thermal tolerance limits remains debated (Ern et al., 2023; Jutfelt et al., 2018; Pörtner et al., 2017; Vornanen, 2020).

The most prominent hypothesis with regard to setting thermal tolerance limits in fish is the oxygen- and capacity limited thermal tolerance (OCLTT) hypothesis (Pörtner & Knust, 2007; Pörtner, 2010). With higher temperatures, tissue oxygen demand increases due to higher routine metabolic rates (Gillooly et al., 2001; Robinson et al., 1983; Somero, 1978). According to the OCLTT, upper thermal tolerance in aquatic ectotherms is determined by the capacity of the cardiorespiratory system to increase the oxygen supply and thereby compensate for the elevated tissue oxygen demand at rising temperatures (Pörtner & Knust, 2007; Pörtner, 2010). However, experiments investigating the validity of the OCLTT have yielded contradicting results. While some have found a connection between thermal tolerance and oxygen supply capacity (Andreassen et al., 2022; Fry & Hart, 1948; Pörtner & Knust, 2007; Verberk et al., 2016) others have not (Andreassen et al., 2025; Ern et al., 2015; Gräns et al., 2014; G. D. Raby et al., 2025a; Silva-Garay et al., 2025; Wang et al., 2014), questioning the universality of the OCLTT hypothesis (Jutfelt et al., 2018). Evidence for the OCLTT hypothesis remains weak across species, and the research focus is therefore shifting towards mechanisms not directly linked to tissue oxygen supply.

The role of the heart and cardiac performance in setting upper thermal tolerance limits is empirically supported in some contexts (Ern et al., 2023; Farrell et al., 2009). Cardiac output - the volume of blood pumped by the heart within a certain time, determined by heart rate and stroke volume - increases with

temperature, but plateaus close to critical temperatures (i.e., approaching CT_{max}) and ultimately collapses when surpassed (Eliason & Anttila, 2017; Mendonça & Gamperl, 2010; Stevens et al., 1972). This collapse of cardiac output at high temperatures is attributed to cardiac arrhythmia (Clark et al., 2008; Gollock et al., 2006) whereas stroke volume remains stable even at the upper thermal limits (Ekström et al., 2019; Haverinen & Vornanen, 2020; Sandblom & Axelsson, 2007).

It has been suggested that this malfunction of the heart at higher temperatures occurs because of a mismatch between sodium and potassium currents, that affects the excitability of cardiac myocytes. This temperature-dependent deterioration of electrical excitability (TDEE) thus offers an alternative to the OCLTT hypothesis by proposing an oxygen-independent cellular mechanism that may set thermal tolerance limits in fish (Vornanen, 2016, 2020). The TDEE is based on observations in cardiac myocytes of brown trout *Salmo trutta*, showing that the voltage-gated sodium (Na^+) current responsible for rapid membrane depolarisation and the outward K^+ -current involved in stabilising the resting membrane potential differ in their thermal sensitivity (Vornanen et al., 2013). In atrial and ventricular myocytes, the negative resting membrane potential is largely maintained by a small outward K^+ -current through inward rectifier K^+ -channels (Vornanen et al., 2002). During acute warming, this efflux increases causing the resting membrane potential to become more negative. At the same time, warming initially increases Na^+ -currents, supporting electrical excitability (Vornanen et al., 2013). However, the breakpoint temperature, the temperature at which an ion current does not increase with warming anymore, of the Na^+ -current in brown trout is at approximately 21°C whereas the outwards K^+ -current continues to increase beyond 32°C (Vornanen et al., 2013). Thus, rapid warming beyond the Na^+ -current breakpoint causes a more negative resting membrane potential, increasing the magnitude of depolarization needed to trigger an action potential, while reducing the Na^+ -currents during depolarisation. Eventually, the imbalance between the two ion currents becomes large enough that incoming signals may no longer sufficiently depolarize the membrane, resulting in failure to trigger action potentials (Vornanen, 2016, 2020).

The TDEE hypothesis is presumably not limited to cardiac muscle, but may apply to all cells whose electrical excitability is connected to voltage-gated ion channels, like neural or skeletal muscle cells (Koester & Siegelbaum, 2000; Vornanen, 2020). However, this has not yet been tested experimentally. While a few studies have investigated whether neural function is linked to upper thermal tolerance in fish (Andreassen et al., 2022; Friedlander et al., 1976; Jutfelt et al., 2019), we found no previous experiments examining skeletal muscle function during acute thermal challenges. This is relevant, because skeletal muscle is essential for functions such as ventilation and movement and is directly involved in loss of equilibrium. Consequently, this represents a major knowledge gap in our understanding of the mechanisms and organs involved in limiting acute thermal tolerance.

We therefore investigated the vulnerability to warming of different contractile tissues during an acute slow-ramping temperature challenge to assess whether the upper thermal tolerance limits differ between tissues. In addition, we tested whether the temperature-dependent decline in electrical excitability predicted by the TDEE hypothesis can be observed in *in vitro* preparations of cardiac and white skeletal muscle. For that, the contractile performance of cardiac muscles (ventricle and atrium), white skeletal muscle, and smooth muscles (stomach and intestine) of rainbow trout (*Oncorhynchus mykiss*) was recorded *in vitro*, while temperature was ramped stepwise until the loss of contractile function.

2. Methods

Ethics statement

The experimental procedures were conducted in line with and approved by the Swedish Board of Agriculture's ethical committee and the University of Gothenburg (Ethics Permit Dnr 5.8.18-07417/2024). It followed the regulations set by the Animal Welfare Body at the University of Gothenburg.

Holding

Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial fish farm (Vänneåns fiskodling AB, Knäred, Sweden) and randomly distributed in three 750 L freshwater tanks (11.5°C – 13.2°C) at the animal facility of the University of Gothenburg. They were fed three times a week and fasted for 24 h prior to the experiments. Water was constantly aerated and filtered and exchanged via a central recirculating aquaculture system. The fish were kept in the facilities for up to nine months (experimental period: 23.02.2025 – 30.05.2025) and had by the time of the experiments an average weight of 413 g (standard error (SE): ± 17 g) by the time of the experiments. In total, 41 fish were used, 21 for the *in vitro* study (n = 16 for temperature ramping; n = 5 for constant temperature controls) and 20 for the whole-animal CT_{max}.

Tissue preparations for the in vitro study

One fish was randomly caught out of one of the three holding tanks on each day of the experiments. They were euthanized by a sharp blow to the head and subsequently dissected. Atrium, ventricle, stomach and intestine were taken out of the fish and immediately transferred into ice-cooled Ringer's solution (in mmol/l: NaCl, 140; KCl, 2.5; CaCl₂ x 2H₂O, 1.5; MgSO₄ x 7H₂O, 0.8; NaHCO₃, 15; KH₂PO₄, 1; Hepes-buffer, 5; pH = 7.8) and kept on ice before further preparation.

To obtain skeletal muscle strips, the skin above the caudal peduncle was removed to access the area where caudal fin rays and skeletal muscles are connected. To identify fibre orientation, two neighbouring tendinous attachments (the continuations of the myosepta connecting to the fin rays) were carefully lifted and fixated with forceps. The two myosepta, together with the white muscle tissue in between them were cut out of the fish with a scalpel until a strip of white skeletal muscle fibres (mean $\pm$ SE; length: 17.7 ± 0.09 mm, breadth: 4.1 ± 2.4 mm, weight: 0.32 ± 0.05 g) were obtained and cleaned by carefully removing loose white muscle tissue and red muscle fibres lying on top. Only after all tissues were dissected from the fish (within 5 min), was further preparation started.

For the ventricle, circular preparations (breadth: 1.47 ± 0.05 mm; weight: 0.1 ± 0.01 g) were excised by two parallel cuts along its transverse axis, which resulted in the highest consistency in the quality of preparations (Aho & Vornanen, 1999). The atrium was not further processed but was mounted in the tissue bath as a whole (weight: 0.07 ± 0.01 g). For smooth muscle preparations, the cardiac stomach

and the proximal-section of the distal intestine were cut open, gently stretched and pinned to a Sylgard coated Petri dish with needles. A single strip was then cut either in the circular (stomach, length: 11.8 ± 0.4 mm, breadth: 3.47 ± 0.15 mm, weight: 0.16 ± 0.01 g) or longitudinal (intestine, length: 9.2 ± 0.6 mm, breadth: 2.29 ± 0.13 mm, weight: 0.05 ± 0.01 g) direction. To account for size differences in tissue preparations the tissues were blotted dry after the experiment and size, length and weight were measured for a normalised developed force per gram of muscle.

All preparations (for n, see Table 1) were placed in double-walled organ baths (Fig. 1), filled with Ringer's solution bubbled with 0.3% CO₂ in air at 12°C (matching acclimation temperature during holding). The preparations were mounted with regular cotton sewing threads tied to both sides of each preparation. One end of the thread was fixated in the organ bath between two platinum electrodes, and the other end of the thread was connected to a force transducer (SENSELab 10-700-0003; Somedic Sales AB, Hörby, Sweden) to measure developed contraction force by the preparations (Figure 1). The signal was amplified with 4Champs (SENSELab, 4Champ amplifier) connected to a Powerlab (ADInstruments, Powerlab/16SP). Data was recorded and analysed using LabChart (LabChart 7 pro v7.3.8) software.

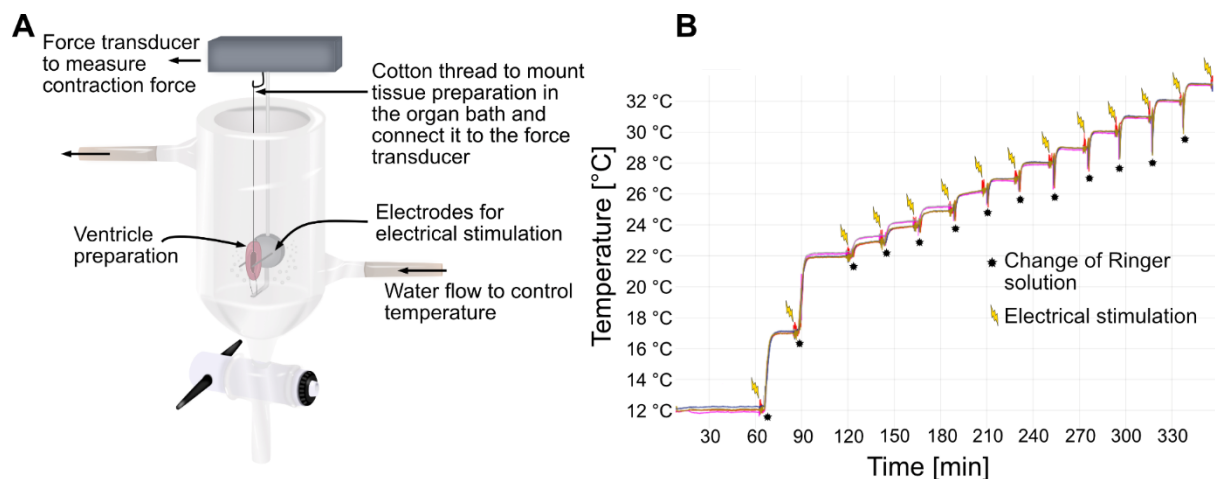


Figure 1. Organ bath set-up and ramping protocol. *A)* Schematic drawing of an organ bath with a mounted circular ventricle preparation. *B)* Temperature ramping steps within each organ bath for one thermal ramping sample (recorded with thermocouples). The black stars show when the Ringer solution was exchanged and the yellow flashes mark electrical stimulations.

Measuring the effect of temperature on contractility requires a consistent length of the muscle fibres (Shiels & White, 2008). To accomplish this, all tissues were stretched in the beginning of the experiment. The smooth muscle strips were stretched by a force of 10 mN (isometric force equivalent to 1 g; following (Gräns et al., 2013). All other tissue preparations were stretched stepwise whilst stimulated to the length where developed force was maximal and further stretching of the tissue would

not lead to an increase in contractility anymore (Aho & Vornanen, 1999). The force applied varied between and within tissues, depending on size (isometric force equivalent; atrium: 1.12 ± 0.14 g; ventricle: 1.53 ± 0.22 g; skeletal muscle: 2.12 ± 0.28 g). After stretching, tissues were allowed to equilibrate until a stable baseline was reached (50-75 min).

All preparations were stimulated using a Grass stimulator (Astro-med, Grass stimulator, Model SD9 J). For cardiac and smooth muscle preparations, electrical field stimulation was used (no contact with the electrodes; distance between tissue and electrodes was *ca.* 1mm) while, due to the bigger size of the strips, skeletal muscles were in direct contact with the platinum electrodes.

The stimulation settings depended on the tissue type. Cardiac and skeletal muscle preparations were stimulated with 16 ms pulses at a frequency of 0.2 Hz and three different voltages (in that order: 10 V, 40 V and 80 V). Ten volts represent the minimum voltage needed to elicit contractions in both the skeletal muscle (8-10 V) and ventricle (4-7 V with field stimulations). For each voltage and temperature the stimulations were repeated ten times with 15 s between the three different voltages were. Of those ten stimulations the first and last were discarded. While the eight remaining peaks were analysed using the “Peak analysis” module (Peak Analysis v1.3.2) in Labchart 7. All atrial preparations showed spontaneous contractions, which often interfered with the contractions induced by stimulation. Thus, contraction force and frequency of the spontaneous contractions were extracted by analysing all peaks two minutes before the start of electrical stimulations at every temperature. When arrhythmia occurred within those two minutes, the time span of the analysis was reduced to get a minimum of 30s of consecutive peaks without arrhythmia interference.

Smooth muscles were stimulated with 70 V and a frequency of 10 Hz (10 ms per pulse) (adapted from Olsson et al., 2008). Since smooth muscles showed a graded response to stimulations, tissues were stimulated until contraction force did not increase anymore. Contraction force was analysed, by marking the start of each contraction onset and the end of contraction defined as the point where maximum force was reached. Contraction force was derived by subtracting the baseline force of each strip right before stimulation from the maximum force developed after stimulation.

Table 1. Number of preparations included in the analysis for each tissue and stimulation voltage. Exclusion criteria are listed on the right.

Tissue	Treatment	Stimulation	n	Reason of exclusion
Ventricle	Control	All voltages	4	One preparation did not react to electrical stimulation
		10 V	9	
	Thermal ramping	40 V	12	Two preparations did not react to electrical stimulation. Five of the remaining 14 exhibited spontaneous contractions that interfered with electrically evoked contractions, particularly at 10V
		80 V	13	

Atrium	Control	All voltages	4	One preparation did not react to electrical stimulation
	Thermal ramping	All voltages	15	One preparation did not react to electrical stimulation
Skeletal muscle	Control	10 V	4	One strip did not respond to stimulation at 10 V
		40 & 80 V	5	
	Thermal ramping	10 V	13	Two strips did not react to stimulations at all, and one strip did not respond to stimulation at 10 V
		40 & 80 V	14	
Stomach	Control	70 V	5	Two strips did not react to electrical stimulations and one strip ripped during the experiment
	Thermal ramping	70 V	13	
Intestine	Control	70 V	5	Two strips did not react to electrical stimulations
	Thermal ramping	70 V	14	

198

199 Heating protocol

200 Temperature in the organ baths was controlled via a thermostat (LAUDA immersion thermostat Alpha
201 A) connected to a circulating water bath. Temperature in each bath was monitored continuously with
202 type K thermocouples (Pico Technology) connected to TC08 data loggers and recorded every second
203 using the PicoLog 6 software (6.2.13). Following the equilibration period, the first stimulation series
204 started at 12°C (=t(0)). Subsequently, temperature was increased stepwise, first in two 5°C increments
205 (17°C and 22°C) with 30 min between stimulation rounds to allow tissue re-equilibration. Thereafter,
206 temperature was increased in 1°C steps every 20 min, with stimulations at the end of each period.
207 Temperature ramping and contractility recordings continued until tissue contractions ceased. After each
208 stimulation round, Ringer's solution was replaced with fresh solution preheated to the corresponding
209 bath temperature to ensure consistency in concentrations and pH.

210 For control preparations, temperature was maintained at 12°C throughout the experiment while
211 stimulation frequency and solution exchange matched that of temperature-ramped tissues in time.
212 Contractility of control preparations was recorded for at least 6 h, exceeding the duration over which
213 any temperature-ramped preparation remained contractile. Two control preparations for each organ
214 were kept in the organ baths for 24 h to assess long-term tissue stability. The first 7h, they were
215 stimulated every 20 min; from hour 8 to 12, hourly; and then once more after 24 h.

216 Normalising data and correcting for natural decline over time

217 All data were normalised by setting the mean amplitude of the first contraction series after the
218 equilibration period (t(0)) for each tissue to 100% with subsequent values presented as percentages
219 relative to that.

To isolate the effect of temperature on tissue contractility, we calculated an estimated natural decline in contractility over time for each tissue based on control measurements. This decay factor was defined as the difference between baseline contraction force at $t(0)$ and force at a given time point $t(x)$. For example, if the contraction force of a preparation maintained at constant temperature declined to 95% after 30 min, the corresponding decay factor would be 0.05. This decay factor was then used to correct contractility values of temperature-treated samples for their estimated natural, time-dependent decline according to the following equation:

$$\text{Corrected Force} = \frac{\text{Observed retained contraction force at temperature } X \text{ (which took } Z \text{ minutes to reach)}}{1 - \text{estimated decay factor for time point } Z}$$

Figures without correction for the natural decline over time (Figure S1-S4) and figures with absolute force values per gram (Figure S6-S8) can be found in the supplementary material.

Whole-animal $CT_{\max}$

To determine the acute upper thermal tolerance for the whole fish, a tank (104 x 104 x 62cm) containing 184 l of 12°C water was used. The tank was divided into two sections, comprising one-third and two-thirds of the total volume, separated by a plastic partition. The smaller section contained an air stone and two water pumps (Eheim Universal 10W, Deizisau, Germany). One pump circulated water through a thermostat controlled electrical heater (Pahlén Titanium, Aqua Compact AC60T, 6500W), while the other transferred water from the smaller to the larger section of the arena to ensure a homogenous temperature throughout the entire arena. The larger section was the actual arena for the fish. Temperature was measured with a high precision digital thermometer throughout the experiment (Testo-112 Digital Thermometer, Lenzkirch, Germany).

For each run of experiments (four in total), five rainbow trout were randomly chosen from one of the three holding tanks and transferred to the heating tank. The same stepwise ramping protocol that was used to measure tissue contractility was implemented to measure $CT_{\max}$ for the whole fish. As the $CT_{\max}$ endpoint, loss of equilibrium (LOE) was used, defined as disorganised locomotory activity for three consecutive seconds (Lutterschmidt & Hutchison, 1997; Morgan et al., 2018). To avoid observer bias, one observer blind to temperature and time determined all LOE events, while another person recorded time and temperature of all endpoints (Raby et al., 2025b). As soon as LOE was observed, the individual was transferred into a recovery container (12°C). After successful recovery within 30 min, fish were anaesthetized with MS-222 to take weight and length and subsequently euthanised by a sharp blow to the head.

Statistical analyses

Data was processed and analysed in Rstudio, version 2025.05.0 (RStudio Team, 2025). Bayesian Regression Models (brms) were fitted using “Stan” via the package brms (Bürkner, 2021). To estimate

the time-dependent natural decline in contractility for each tissue, normalised contraction force was modelled as a smooth function of time with separate curves for each stimulation voltage (applicable for ventricle and white skeletal muscle; $\text{percentage} \sim \text{s}(\text{time}, \text{by} = \text{volts}, k = 10) + \text{volts} + (1 \mid \text{fish_id})$). After correcting contraction force values for natural decline in the 16 thermal ramping preparations (as described under “*Normalising data and correcting for natural decline over time*”), models were fitted to the normalised and corrected sample data, modelling contraction force as a smooth function of temperature with voltage-specific curves ($\text{corrected_force} \sim \text{s}(\text{temperature}, \text{by} = \text{volts}, k = 10) + \text{volts} + (1 \mid \text{fish_id})$).

Since we wanted to investigate if there were differences in the temperature–contractility relationships between stimulation voltages, we always fitted separate curves for each voltage in white skeletal muscle and ventricle preparation models to visualise and estimate potential differences. To test whether the added complexity of voltage-specific curves was supported by the data, we used expected log predictive densities (elpd) derived from leave-one-out cross validation comparisons (LOO-CV). We compared models fitting voltage-specific temperature responses with models assuming a shared temperature response across voltages ($\text{corrected_force} \sim \text{s}(\text{temperature}, k = 10) + \text{volts} + (1 \mid \text{fish_id})$). However, model comparison was used to assess the evidence for voltage-dependent temperature effects, rather than to select a single final model. In this context, preference for the simpler model was interpreted as evidence against meaningful differences between voltages. Default brm priors were used for all models. Models were fitted using four Markov chains with 4,000 iterations per chain, including 2,000 warm-up iterations. The target acceptance probability (adapt_delta) was set to 0.99.

Lastly, we calculated posterior density distributions from the sample models of a) contractility per tissue and voltage at 27.46°C (i.e. CT_{max} temperature of the whole fish) and b) the temperature at which each tissue is estimated to only retain 50% of their baseline contraction force, defined as T_{50} . In addition, we conducted a pairwise comparison between tissues and conditions to generate a distribution of the differences. We extracted posterior predictions from the fitted models and calculated differences draw-by-draw, summarizing it using means, medians and 95% credible intervals (Supplementary material Table S1 and S2).

3. Results

Whole-animal CT_{max}

Fish started to lose equilibrium when temperature reached 26°C, with a mean CT_{max} of 27.46°C (SE: ± 0.15). Due to stepwise ramping of the water, CT_{max} values are clustered around whole temperature steps (~28°C, n = 9; ~27°C, n = 7; ~26°C, n = 1; Also, see figure S12 in the supplementary material). LOE usually occurred after the fish spent several minutes at the respective CT_{max} temperature. For the analysis, 17 out of 20 fish were used, since two managed to jump out of the tub before losing equilibrium and one fish lost equilibrium at 23.06°C (~3.5°C below average) and did not manage to recover afterwards.

In vitro experiment

Ventricle

Credible intervals (CrI) of all three voltages overlap in the control preparations, suggesting that the effect of natural decline of contractility over time was independent of voltage. Consistent with this, LOO-CV showed no support for fitting separate curves for each voltage (elpd ± SE= -14.2 ± 3.9). The final contraction observed in a ventricle preparation during thermal ramping occurred after 5h 39 min, a time point that lay well within the duration of the control experiments. At this time, control preparations estimated an average reduction in contraction force to 69.8% (95% CrI: 58.3-81.1%; 10 V); 76.3% (95% CrI 64.7-87.6%; 40 V) and 80.1% (95% CrI: 68.5-91.5%; 80 V) relative to their respective baseline values.

LOO-CV comparison provided moderate evidence (elpd: -29.9 ± 10) that separate smooths per voltage provided the better fit, indicating a temperature-dependent interaction between stimulation voltage and ventricle contractility. This interaction appears to be driven primarily by differences in the temperature range at which contraction force declines. At temperatures up to 24°C, estimated means and credible intervals overlapped across voltages, whereas at higher temperatures clear differences emerged between 10 V and the two higher voltages (Figure 2). These differences reflect both an earlier onset and a steeper decline in contraction force at 10 V (start of decline: 22–23°C) compared with 40 V and 80 V (24–25°C). Accordingly, during 10 V stimulation, contractions first failed at 24°C, and at 31°C, no preparations responded to stimulation anymore. In contrast, stronger stimulation voltage narrowed the temperature window over which contractions ceased and shifted this window to higher temperatures (30–35°C at 40 V and 33–35°C at 80 V; Figure 2).

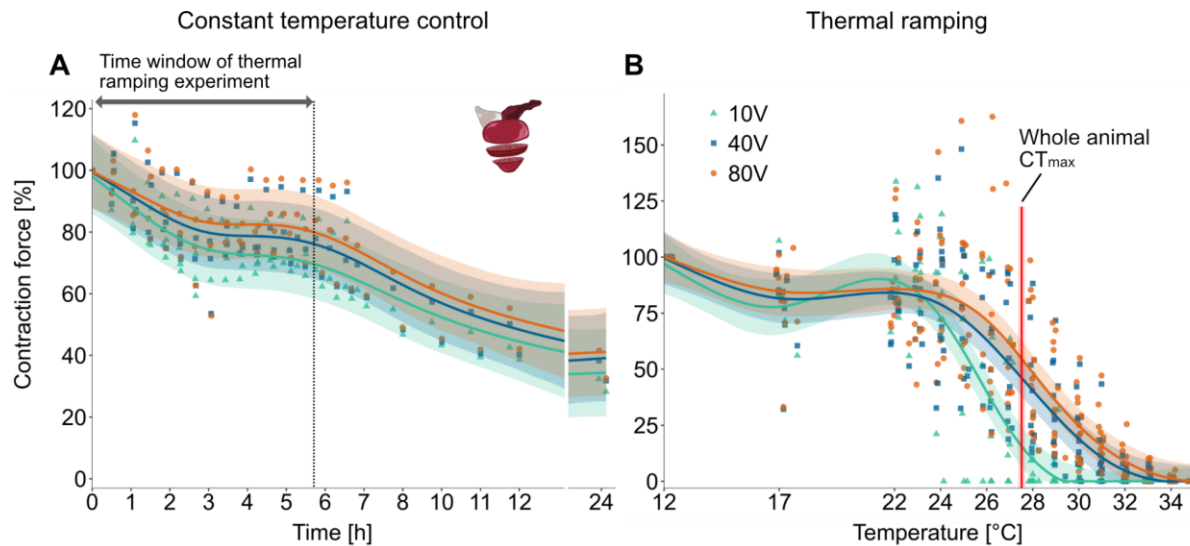


Figure 2. Relative contraction force of isolated ventricle preparations in organ baths. A) Natural decline in contraction force over time at constant temperature (12°C) and **B)** temperature dependent decline of contraction force (corrected for the natural decline, based on model predictions of the control samples) in circular ventricle preparations from rainbow trout hearts in an in vitro organ bath set-up. Contraction force was normalized by setting the first measurement at time point 0 (A) or 12°C (B) to 100%, with all subsequent values expressed as relative percentages. Tissues were stimulated at three different voltages (10 V, 40 V, 80 V). Points represent the relative force measured for individual ventricle preparations, smooth curves depict the estimated mean contraction force derived from the brms models, and ribbons indicate the 95% credible interval. The red vertical line shows the mean (SE as transparent) CT_{max} value of the whole fish experiment.

Atrium

Most of the atrial preparations (15/16 and 4/5 for thermal ramping and controls respectively) exhibited spontaneous contractions that interfered with electrical stimulations. Consequently, no control data were available and the sample size at lower temperatures is thus low (no preparations responded to stimulation at 12°C and only two responded up to 22°C). This prevented relative comparisons due to the lack of a baseline contraction force at 12°C. We therefore focused on contraction force generated by the spontaneous contractions. Raw force values (N g⁻¹) of electrically stimulated contractions during thermal ramping are shown in figure S9 of the supplementary material without correction for natural decline over time.

A potential pitfall with analysing the spontaneous contractions of the atrium was that the contraction frequency was not constant, in contrast to the other tissues. In this context, lower contraction force does not necessarily indicate a reduced performance, as increased contraction frequency may partially compensate for reduced force. However, the relationship between frequency and force was non-linear (see Supplementary Figure S13). We therefore present contraction force values only and did not attempt to integrate frequency into a combined metric. Accordingly, subsequent interpretations are done with caution and remain within the limits of what can be inferred from contraction force alone and direct comparisons with other tissues will only be done with the ventricle and not the atrium results.

At constant temperature, atrial contraction force increased from the first to second contraction and subsequently declined linearly over time, although it remained above 100% after 6 h, covering the duration of the ramping experiment. During thermal ramping, atrial contractility stayed stable up to approximately 25°C before declining from 26°C onwards. Spontaneous contractions ceased between 26 and 31°C, with only six out of fifteen preparations still contracting at 28°C, half a degree above whole-animal CT_{max} (Figure 3).

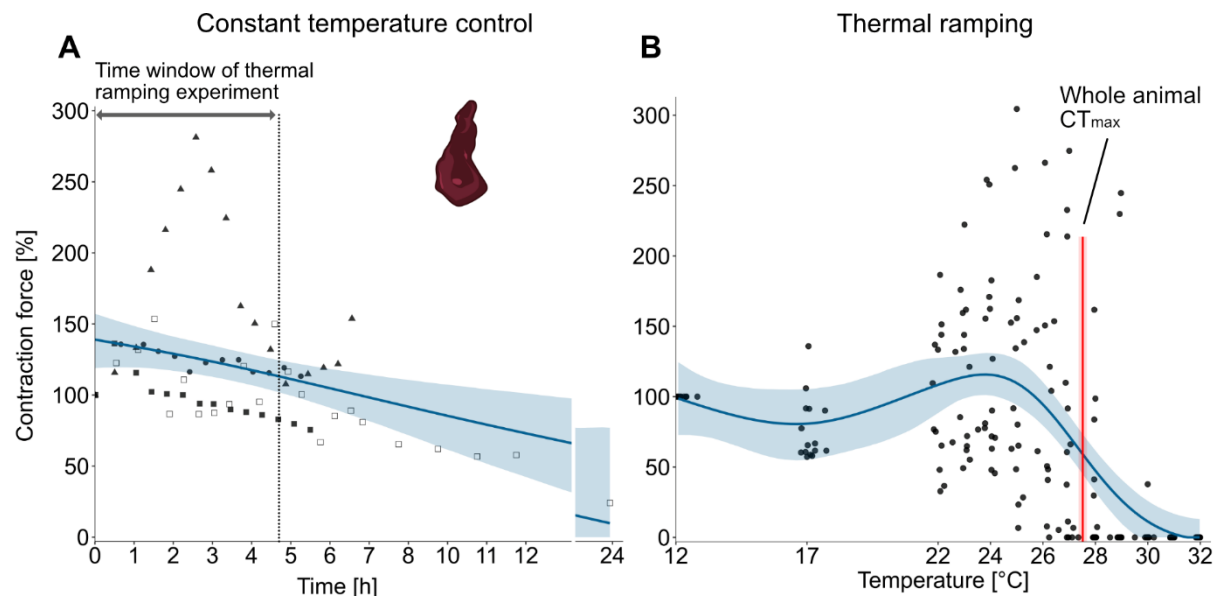


Figure 3. Relative spontaneous contraction force of isolated atrium preparations in organ baths. **A)** Natural decline in contraction force over time at constant temperature (12°C) and **B)** temperature dependent decline of contraction force (corrected for the natural decline, based on model predictions of the control samples) in atrium preparations from rainbow trout hearts in an in vitro organ bath set-up. Contraction force was normalized by setting the first measurement at time point 0 (**A**) or 12°C (**B**) to 100%, with all subsequent values expressed as relative percentages. Contraction force of spontaneous contractions was measured over two minutes at each temperature. Points represent the relative force measured for individual atrium preparations, smooth curves depict the estimated mean contraction force derived from the brms models, and ribbons indicate the 95% credible interval. The red vertical line shows the mean (SE as transparent) CT_{max} value of the whole fish experiment. In **A**) different symbols represent individual fish.

Spontaneous vs forced contractions

To assess which stimulation voltage may provide the most relevant estimate of cardiac contractile failure under in vivo conditions, we compared the proportion of preparations, generating spontaneous contractions and the proportions responding to 10 V, 40 V, and 80 V stimulation across temperatures

(Figure 4). In both atrial and ventricular tissue, the proportion of preparations producing spontaneous contractions closely followed those responding to 10 V stimulation. The close match between spontaneous activity and 10 V responses suggests that lower-voltage stimulation provides a better estimate of the temperature at which cardiac tissue loses spontaneous contractile activity than higher-voltage stimulation. However, it cannot be assumed to fully represent excitation in vivo. Because spontaneous atrial contractions interfered with electrical stimulations, comparisons were restricted to higher temperatures. In contrast, stimulations at 40 V and 80 V likely overestimate the ability of cardiac tissue to contract at high temperatures. At 31°C and 32°C respectively, no spontaneous contractions of the atrium and ventricle were observed anymore, yet 87% and 60% of those preparations still contracted when stimulated with 40 V.

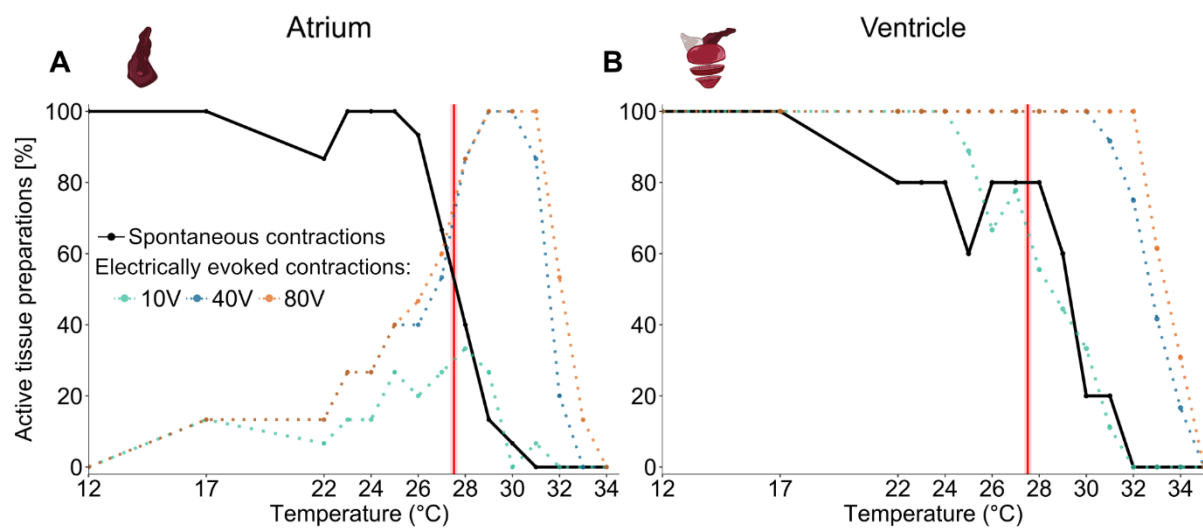


Figure 4. Number of preparations still able to produce a contraction (= active) over the range of tested temperatures for atrium (A) and ventricle (B). The black line depicts the percentage of tissue preparations still able to contract spontaneously (without external stimulation). The coloured lines show the percentage of tissues that are still reacting to external stimulation, when stimulated with 10 V (turquoise), 40 V (blue) and 80 V (orange). The red vertical line shows the mean (SE as transparent) CT_{max} value of the whole fish experiment.

Skeletal muscle

When stimulated at 40 V or 80 V, white skeletal muscle showed no decline in contractility over time and was the most stable tissue regarding natural degeneration in the tissue baths (Figure 5A). In contrast, stimulation with 10 V resulted in a large drop in contraction force between the first stimulation (t_0) and the second stimulation (t_1 , 30 min later). Because contraction force values were normalised to t_0 , this abrupt initial decrease, could not be adequately captured by a smooth model fit, likely leading to an underestimation of the magnitude of natural decline at 10 V. To avoid this bias toward higher force

values, t_0 was therefore excluded from the control model at 10 V. Inclusion of t_0 would artificially constrain the fitted curve toward the normalised baseline and thereby underestimate the extent of the early force loss (See Figure S10, for the model including t_0 for 10 V and the corresponding corrected sample values).

The final contraction observed in a white skeletal muscle preparation during thermal ramping occurred after 4 h 42 min, a time point that lay well within the duration of the control experiments (Figure 5A). At this time, control preparations retained an estimated contraction force of 72% (95% CrI: 61-83.4%; 10 V); 98.1% (95% CrI: 87.2-109.2%; 40 V) and 94.7% (95% CrI: 83.7-105.8%; 80 V) relative to their respective baseline values (Figure 5B). Despite these clear differences between 10 V and 40/80 V, the LOO-CV did not indicate clear support for fitting separate curves for each voltage (elpd difference = -4.1 ± 6.3), which can likely be attributed to the low sample size per voltage.

When stimulated at 40 V and 80 V, white skeletal muscle maintained near-maximal contractility up to approximately 20°C, after which contraction force declined steadily with further warming. At 10 V, the decline in contraction force began at temperatures slightly above acclimation temperature. However, this reduction was small, and credible intervals overlapped extensively with those of the higher voltages. Apart from the earlier onset of decline at 10 V, contraction force trajectories ran largely in parallel across stimulation voltages. Furthermore, credible intervals for 40 V overlapped with those for both 10 V and 80 V across most of the temperature range, with non-overlapping intervals observed only between 10 V and 80 V at temperatures above 20°C. Accordingly, LOO-CV comparison provided no evidence that fitting separate smooths for each voltage improved model performance (elpd difference = -11.6 ± 7.9), indicating that the effect of temperature on skeletal muscle contractility was largely independent of stimulation voltage.

Consistent with these patterns, the temperature windows over which contraction failure occurred lay closer to each other in white skeletal muscle than in ventricle preparations. Loss of contractile activity occurred at 24–30°C at 10 V, 29–32°C at 40 V, and 30–32°C at 80 V (Figure 5).

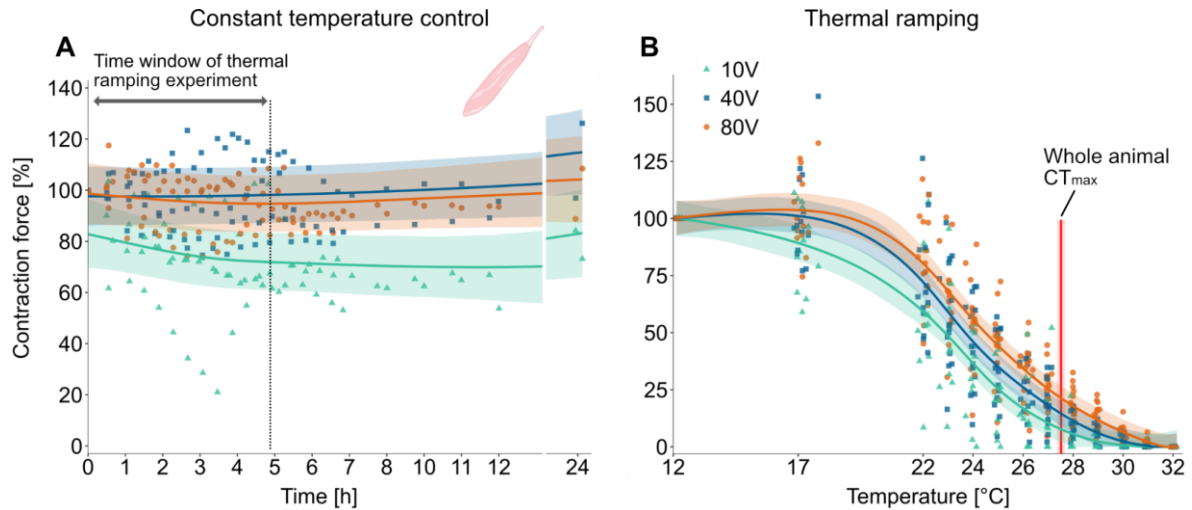


Figure 5. Relative contraction force of isolated skeletal muscle preparations in organ baths. *A)* Natural decline in contraction force over time at constant temperature (12°C) and *B)* temperature dependent decline of contraction force (corrected for the natural decline, based on model predictions of the control samples) in skeletal muscle strip preparations from rainbow trout in an in vitro organ bath set-up. Contraction force was normalized by setting the first measurement at time point 0 (*A*) or 12°C (*B*) to 100%, with all subsequent values expressed as relative percentages. Tissues were stimulated at three different voltages (10 V, 40 V, 80 V). Points represent the relative force measured for individual white muscle preparations, smooth curves depict the estimated mean contraction force derived from the brms models, and ribbons indicate the 95% credible interval. The red vertical line shows the mean (SE as transparent) CT_{max} value of the whole fish experiment.

Smooth muscle

Both stomach and intestine showed a strong natural decline in contraction force over time in our set-up. Contractility of the intestine preparations decreased steadily over time to 59.1% (95% CrI: 32.5-84.3%) after 5h 14min, when contractions ceased in the tissues undergoing thermal ramping (at 32°C). For the stomach controls, mean contraction force dropped to 44.8% (95% CI: 15.1-73%) from the first to second contraction. After this drop they stayed relatively stable and averaged a contraction force of 38.2% (95% CI: 10.6 - 65.6%) after 5h 49min - the time corresponding to the final contraction observed in temperature-treated stomach tissues (at 34°C). Just like in the white skeletal muscle at 10 V, this abrupt initial decrease from the first (t_0) to the second (t_1 , 30 min later) contraction cannot be adequately captured by a smooth model fit. Once again, to avoid an underestimation of this decline in stomach preparations, t_0 was excluded from the control model (see Appendix, Figure S11, for the model including t_0 and the corresponding corrected sample values).

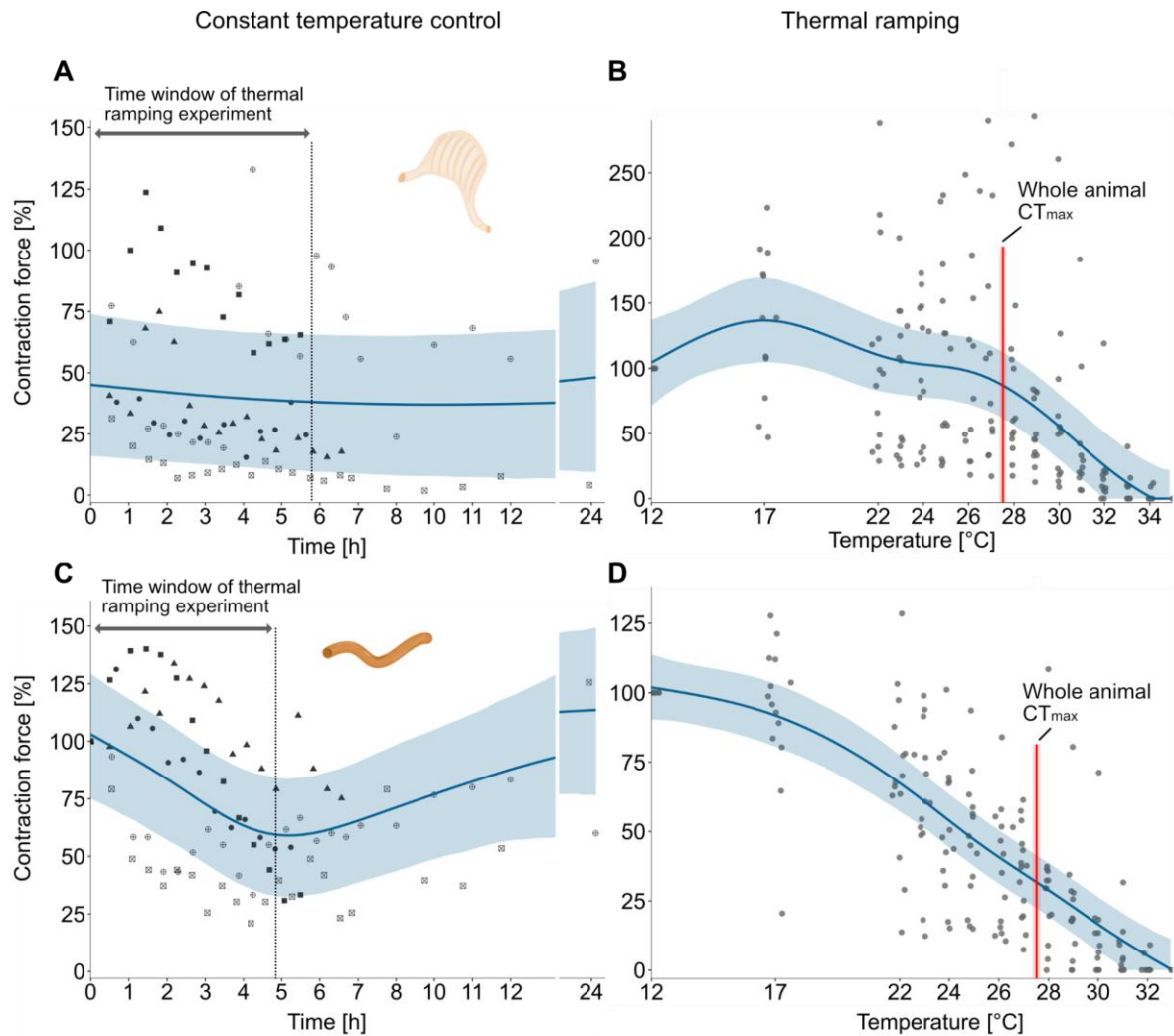


Figure 6. Relative contraction force of isolated circular stomach and longitudinal intestine smooth muscle preparations in an organ bath. Natural decline in contraction force over time, at constant temperature (12°C) for (A) stomach and (C) intestine preparations and temperature dependent decline of contraction force (corrected for the natural decline, based on model predictions of the respective control samples shown in A) and C)) in (B) stomach and (D) intestine preparations of rainbow trout in an in vitro organ bath set-up. Contraction force was normalized by setting the first measurement at time point 0 (A and C) or 12°C (B and D) to 100%, with all subsequent values expressed as percentages. Contractions were induced using electrical stimulations (70 V). Points represent the relative force measured for all stomach preparations, smooth curves depict the estimated mean contraction force derived from the brms models, and ribbons indicate the 95% credible interval. The red vertical lines shows the mean (SE as transparent) CT_{max} value of the whole fish experiment.

Amongst all tested tissues, stomach preparations were the most robust towards thermal ramping. Contractility reached its maximum with 125% at 17°C and only dropped below baseline (100%) at temperatures above 24°C. The decline in contractility from 17°C to 27°C was slow but picked up at temperatures above 27°C until preparations lost their ability to contract between 32 and 34°C. Contrary to this, the intestine was quite sensitive towards warming. Intestine preparations retained their original contraction force up until 17°C, after which further warming decreased contractility steadily, with preparations ceasing to contract between 28 and 32°C.

Posterior distributions

To compare differences between the tissues in their ability to maintain relative contraction force over a range of temperatures, two critical points were chosen. First, the retained relative contraction force of the organs at the CT_{max} temperature of the whole fish (mean = 27.46°C) and second, the temperature at which each tissue reached 50% of their baseline contraction force (T_{50}).

In the in vitro setting, white skeletal muscle strips were the most temperature sensitive tissue. At CT_{max} , they were estimated to retain only 8% (95% CrI: 2.3-13.6%), of their initial contraction force measured at 12°C when stimulated with 10 V. The corresponding values at 40 V and 80 V were 15% (95% CrI: 9.6-20.4%) and 21.7% (95% CrI: 16.3-27.1%) respectively. Skeletal muscle also showed the lowest T_{50} with posterior means of 22.9°C (10V; 95% CrI: 22.3-23.4°C), 23.7°C (40V; 95% CrI: 23.3-24.1°C) and 24.5°C (80V; 95% CrI: 24-25°C). Similar to this, intestine strips reached T_{50} at 24.6°C (95% CrI: 23.6 - 26°C) and on average kept 31.9% (95% CrI: 22.4-41.4%) of baseline force at CT_{max} . Likewise, when ventricle preparations were stimulated with 10 V the T_{50} (25.45°C (95% CrI: 24.95–25.93°C)) and relative force at CT_{max} 16.41% (95% CrI: 7.05–25.21%) values were similar to those estimated for the skeletal muscle. However when stimulated with 40 V and 80 V, contractility of ventricle preparations was robust towards changes in temperature with estimated T_{50} 's of 27.2°C (40 V; 95% CrI: 26.5-27.9°C) and 27.9°C (80V; 95% CrI: 27.3-28.5°C) respectively and a retained contraction capacity of 46.8% (95% CrI: 38.4-54.8%) and 55.5% (95% CrI: 47.4-63.4%) at CT_{max} . Finally, stomach preparations were the most robust towards rising temperatures, estimated to maintain 86.9% (95% CrI: 60.7-113.5%) relative contraction force at CT_{max} with a T_{50} of 30.3°C (95% CrI: 28.4-32.1°C), almost 6°C higher than that of the intestine.

Posterior distributions of ventricle and skeletal muscle preparations showed little overlap between voltages within each tissue and limited overlap between tissues (only the 10V ventricle distribution overlapped with the 40V and 80V skeletal muscle distributions), suggesting a clear difference in retained relative contraction force across both voltage and tissue types (Figure 7). Despite the broad variation in posterior draws for the stomach, its posterior distribution remained distinct from that of the intestine, indicating a meaningful difference in retained relative contraction force between the two smooth muscle tissues.

For a more detailed comparison we computed the posterior distribution of the difference between two conditions to estimate a posterior probability that one treatment is actually higher than the other. Tables with comparisons of every temperature : tissue : voltage treatment can be found in the supplements (Table S1 and S2).

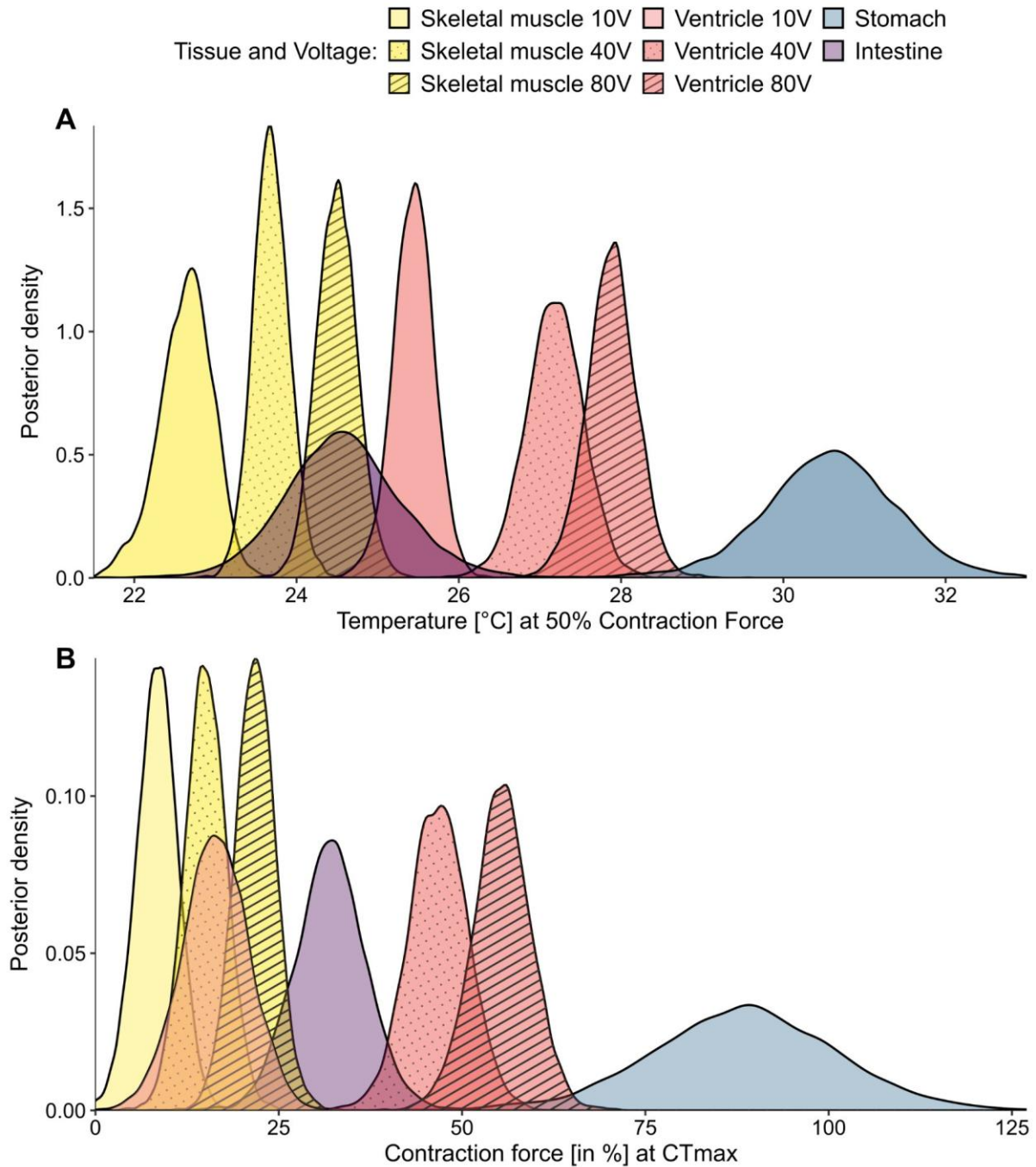


Figure 7. Posterior density distributions from the Bayesian models. A) Predicted relative contraction force capacity of each tissue, separate for stimulation voltages, at the mean CT_{max} temperature of the whole fish (27.46°C). **B)** Temperature at which 50% contraction force is reached for each tissue and voltage. Skeletal muscle are depicted in yellow, ventricle in red, stomach in blue and the intestine in purple. Different stimulation voltages are shown by the black lines (80 V) or black dots (40 V) within each peak. No hatching represents the 10 V treatments for skeletal muscle and ventricle preparations or the 70 V stimulation for the two smooth muscle preparations.

4. Discussion

By investigating thermal tolerance at the organ level in rainbow trout, we 1) discovered that there are surprisingly large differences in vulnerability to warming between tissues, 2) showed that skeletal muscle contractility is the most sensitive to temperature of all tested tissues and 3) found support for the TDEE hypothesis in cardiac myocytes, but not in white skeletal muscle, suggesting that reduced electrical excitability at elevated temperatures is not universal across electrically excitable tissues.

Big differences in vulnerability to warming between muscle tissue types

Vulnerability to warming varied substantially between muscle tissue types. Skeletal muscle was the most sensitive, followed by the cardiac muscles and intestine, with the stomach being the most heat tolerant of the tested organs. The magnitude of the difference was large, for example up to 8 °C higher in stomach than in skeletal muscle. Such a large difference is surprising from an evolutionary point of view where the thermal windows of the various organs have all been shaped by the same thermal environment. It has previously been suggested that the thermal windows of various organ systems should be similar because “*a weak link mechanism may constitute a point where selection may act, while redundancy in a system may constitute a fitness cost*” (Ern et al. 2023). This prediction appears less likely in the light of the detected differences in thermal tolerance, and it is therefore possible that a “weak link” organ system may exist, with skeletal muscle being one candidate. The differences in tolerance between organs also indicate that a single unifying molecular mechanism is unlikely to determine performance and tolerance for all organs but instead points to tissue-specific thermal limits that may stem from distinct mechanisms.

Organs are specialised for different functional roles and contain different cell types and may therefore not share the same thermal limits or optima. This aligns with the conceptual framework of “multiple performances – multiple optima”, proposed as an alternative to the idea that thermal performance curves of physiological functions generally line up with that for aerobic scope, as suggested by the OCLTT hypothesis (Clark et al., 2013). The MPMO framework predicts that different physiological functions can have distinct thermal optima and do not universally follow the thermal performance curve of aerobic scope. While we did not test for optimal temperatures and focused on a single performance trait (contractility), we showed that temperature dependence and thermal limits of contractility can differ among organs. Because the physiological functions of the tested organs are closely linked to their ability to contract, it is likely that their respective performance traits may differ in thermal optima and tolerance accordingly.

We can only speculate which underlying processes may be responsible for the observed differences in vulnerability to warming between tissues. These differences may arise from the accumulation of small, tissue-specific variation in thermal sensitivity of various physiological systems involved in excitation,

contraction or their coupling. Force generation depends strongly on intracellular Ca^{2+} concentration, yet Ca^{2+} handling differs among tissue types. Cardiac and gastrointestinal myocytes rely on extracellular Ca^{2+} influx to trigger further Ca^{2+} release from intracellular stores, whereas in teleost white skeletal muscle, Ca^{2+} release is derived almost exclusively from the sarcoplasmic reticulum (Lennard & Huddart, 1991; Lodge & Huddart, 1984; Rios & Brum, 1987; Schneider & Chandler, 1973; Vornanen, 1997). In addition, tissues express different isoforms of proteins involved in excitation–contraction coupling, such as SERCA (Sarcoplasmic reticulum calcium ATPase) or troponin complex subunits, which may differ in their thermal sensitivity (Alderman et al., 2012; Morrisette et al., 2000; Schredelseker et al., 2010; Vornanen, 2021). Thus, similar physiological processes may become thermally limiting at different temperatures across tissues, although direct evidence for tissue-specific thermal sensitivity at the level of individual protein isoforms remains scarce.

Tissue-specific differences also occur in other physiological systems, including mitochondrial function and enzyme stability or kinetics (Feng et al., 2019; Lau et al., 2020). Together, this highlights that cellular systems differ between tissues within the same individual. As a result, temperature is likely to influence performance differently in each tissue type, rather than through a single controlling mechanism. It is important to note, however, that our *in vitro* approach isolates tissues from systemic processes, and whole-animal performance or tolerance may instead be limited by other mechanisms, such as neural failure, before these tissue-specific contraction mechanisms become critical.

Implications of tissue-specific differences for thermal tolerance

The heart has been suggested as an important contributor in setting upper thermal tolerance limits in fish (Eliason & Anttila, 2017; Farrell et al., 2009; Sandblom et al., 2016). However, skeletal muscle dropped to 50% of their maximal contraction force at temperatures 3–4°C lower than ventricular myocytes. In addition, at the average whole-animal CT_{max} (27.46°C) temperature, skeletal muscle only retained 8–22% of their initial contractility compared to 16–55% for the ventricle. This implies that white skeletal muscle function may fail earlier than cardiac function during acute warming and that locomotor performance near the whole-animal upper thermal limit is likely to be strongly compromised. It is difficult to determine a percentage of contractility below which a tissue can no longer fulfil its function. However, such a pronounced loss contraction force is unlikely to support proper skeletal muscle function and indicates that skeletal muscle contractility in fish may play an important role in setting thermal tolerance limits in fish during acute thermal challenges. While not direct evidence of causation, we propose that skeletal muscle failure represents a plausible mechanism for the loss of equilibrium observed during acute thermal challenges.

Although white skeletal muscle was more temperature sensitive than ventricular tissue, our results also indicate that cardiac function is impaired near whole-animal CT_{max} . Around CT_{max} , only 50% of atrium

preparations were still able to elicit spontaneous contractions, showing that spontaneous activity of cardiac tissue declines close to the temperature at which the whole-animal loses equilibrium. These findings align with observations of cardiac arrhythmias in fish near their upper critical temperatures (Clark et al., 2008; Gollock et al., 2006) and support the suggestion of the heart as a potential determinant of upper thermal tolerance limits in fish (Pörtner et al., 2017; Vornanen, 2020).

Furthermore, white skeletal muscle contractility declined to 50% of its baseline value at temperatures around 3.5°C below CT_{max}, indicating that heat waves may substantially impair fish locomotion well before CT_{max} is reached. In fish, white muscle fibres make up most of the myotomal mass and power burst swimming, rapid acceleration and escape responses (Domenici & Blake, 1997; Hudson, 1973; James & Johnston, 1998; Walker, 1971). In the wild, such reduced muscle performance during heat waves could increase vulnerability to predation and reduce foraging success. As marine and freshwater heat waves become more frequent and prolonged under climate change, fish may therefore experience repeated, sublethal excursions into this compromised performance range without approaching the upper thermal limit. The reduction in contractility during warming could therefore have dramatic fitness consequences well below CT_{max} temperatures.

Although our findings highlight the thermal vulnerability of white skeletal muscle, it does not necessarily mean that the failure of these fast glycolytic muscle fibres during warming is directly responsible for setting upper thermal tolerance limits in fish. It is still unclear which muscle type is primarily responsible for maintaining an upright position in the water column, although recent studies attributed a central role to red, oxidative muscle fibres during static postural control in zebrafish (Nagaoka et al., 2025; Sugioka et al., 2023). Thus, loss of equilibrium – the most used proxy for upper thermal tolerance limits – could also be connected to an impairment of red skeletal muscle function rather than or in addition to the failure of white skeletal muscle. Red and white skeletal muscle fibres differ in their electrophysiology. While contractions of white skeletal muscle are always triggered by all-or-none Na⁺-induced action potentials, red skeletal muscle contractions appear to result from tonic, graded membrane depolarisation (Hidaka & Toida, 1969; Kamata & Washio, 2003; Stanfield, 1972). In zebrafish larvae an absence of depolarising Na⁺ currents in red skeletal muscle was shown (Buckingham & Ali, 2004; Coutts et al., 2006) and in adults two different isoforms of the L-type Ca²⁺ channel - responsible for mediating the influx of sarcoplasmic Ca²⁺ into the cell - are expressed in red and white muscle fibres (Morrissette et al., 2000; Schredelseker et al., 2010). Given these physiological differences - and our observation that temperature vulnerability varies substantially among tissues within the same species - the thermal tolerance of red skeletal muscle cannot be directly derived from measurements in white skeletal muscle. Unfortunately, red skeletal muscle fibres are too fragile to be reliably mounted and measured in our *in vitro* set-up, preventing a direct assessment of their

vulnerability to warming. Further method development is therefore needed before red skeletal muscle thermal tolerance can be assessed in *in vitro* chambers.

Voltage dependence in ventricle but not in skeletal muscle

The TDEE hypothesis proposes that elevated temperatures reduce cardiac myocyte excitability by creating a mismatch between depolarizing inward Na^+ - and outward K^+ -currents (Vornanen, 2016, 2020). This mechanism has been suggested to apply to all electrically excitable cells and not only to cardiac myocytes (Haverinen & Vornanen, 2020). While we found that the temperature-associated decline in contractility of ventricle preparations was clearly dependent on stimulation voltage, as predicted by the TDEE, this pattern was less pronounced in white skeletal muscle.

When stimulated with 10 V, ventricle contractility began to decline at approximately 23°C. At 40 V and 80 V, the onset of decline was shifted to temperatures roughly 2°C higher, and the decline in contraction force was less steep. No preparations responded to 10 V stimulations above 31°C, yet contractions were observed up to 34°C when preparations were stimulated with 40 V or 80 V. This suggests that the capacity for excitation–contraction coupling and force generation is not immediately lost at elevated temperatures. Instead, the complete recovery of force by higher stimulation voltages between 23–25°C shows that stronger stimulation can initially overcome the temperature-dependent impairment. This pattern is consistent with reduced electrical excitability or tissue recruitment, as predicted by the TDEE hypothesis. At higher temperatures, however, force was only partially recovered, indicating that additional mechanisms downstream of excitation may also contribute to the decline in contractility.

While the retained relative contraction force differed between stimulation voltages in skeletal muscle, this voltage effect did not change across the temperature range. Thus, we found no evidence for a temperature-dependent voltage effect in white skeletal muscle comparable to that observed in ventricle preparations. One potential explanation lies in differences in excitation contraction coupling between skeletal and cardiac muscles in fish. In cardiac myocytes, membrane depolarisation opens voltage-gated L-type Ca^{2+} channels in the sarcolemma, allowing extracellular Ca^{2+} influx, which can trigger further Ca^{2+} release from the sarcoplasmic reticulum (Lennard & Huddart, 1991; Lodge & Huddart, 1984; Vornanen, 1997). In teleost skeletal muscle, by contrast, L-type Ca^{2+} currents are not detectable during excitation, and Ca^{2+} release is derived almost exclusively from intracellular stores in the sarcoplasmic reticulum (Rios & Brum, 1987; Schneider & Chandler, 1973; Schredelseker et al., 2010). Here, Ca^{2+} channels function as voltage sensors, opening sarcoplasmic Ca^{2+} release channels (RyR1) through voltage-dependent conformational changes and direct protein-protein interaction (Schredelseker et al., 2010). This difference does not mean that white skeletal muscle contraction is independent of membrane depolarisation. However, because the depolarisation-sensitive steps of excitation–contraction coupling differ between skeletal and cardiac muscle, partial membrane depolarisation may affect Ca^{2+} release

and force generation differently in the two tissues. Thus, tissue-specific differences in excitation-contraction coupling may help explain the contrasting voltage dependencies observed between ventricle and white skeletal muscle.

Gradual decline in contraction force with warming

The vulnerability of the tested organs to warming was expressed as a gradual decline in contraction force, indicating progressive impairment rather than an abrupt threshold-dependent collapse. One potential explanation is reduced electrical excitability during warming. Because we used field stimulation, electrical pulses likely did not depolarise all cells equally; as excitability decreases, some cells may no longer reach the depolarisation threshold or action potential propagation may become impaired. This interpretation is supported by the observation that increasing the stimulation voltage recovered the lost contraction force in cardiac and skeletal muscle preparations completely for 2–3 °C and partially at higher temperatures. However, once increased stimulation voltage no longer fully recovered force, mechanisms downstream of excitation may also have contributed, including reduced myofilament Ca²⁺ sensitivity, structural damage to the contractile apparatus, impaired cross-bridge function, or metabolic limitations such as poor oxygen diffusion and/or impaired mitochondrial function leading to reduced ATP supply, (De Vos et al., 2018; Johnston & Altringham, 1985; Johnston & Brill, 1984; Michaelsen et al., 2021). While the underlying mechanisms cannot be identified with certainty, the gradual and tissue-specific decline in contraction force still allows ecophysiological consequences of warming to be examined.

Conclusion

By investigating thermal tolerance at the organ level in rainbow trout, we demonstrate a surprisingly large difference in vulnerability to warming between muscle tissue types. This indicates that temperature affects function differently across tissues, likely through partly distinct physiological pathways, rather than through a single shared mechanism. Among the tissues examined, white skeletal muscle exhibited the greatest thermal vulnerability, highlighting that skeletal muscle function may represent a previously overlooked contributor to acute upper thermal tolerance limits in fish. Cardiac myocytes showed a temperature-induced, voltage-dependent decline in contractility consistent with predictions of the TDEE hypothesis, whereas the white skeletal muscle contractility declined independently of voltage. This suggests that the TDEE hypothesis may not apply to all electrically excitable cells, including those in white skeletal muscle. In addition to the implications for acute thermal tolerance, the early reduction in skeletal muscle contraction force suggests that poor swim performance could lead to ecologically relevant impacts well below the acute thermal limits.

654 **Author contributions**

655 L.P. led the writing, data analysis and the collection of data. L.P. and F.J. designed the methodology.
656 All authors gave critical feedback on the draft and gave final approval for publication.

657 **Acknowledgements**

658 We thank Patrice Pottier at the University of Gothenburg for guidance and advice about statistical
659 analysis.

660 **Use of AI tools**

661 We used Codex to assist with the generation of code for data processing and analysis and ChatGPT
662 (version 5.5) to conduct grammar and spelling checks. All content has been reviewed and revised by all
663 authors, who take full responsibility for the accuracy and originality of the work.

664 **Data Accessibility**

665 The data and analysis script are available from the github repository: XYZ -> not ready yet

666 **Funding**

667 This work was supported by the European Research Council (ERC Consolidator Grant 101003026,
668 CLIMEVOLVE, to F. Jutfelt). L.P. was additionally supported by the *Helge Ax:son Johnsons stiftelse*.

669 **Data availability**

670 The data and analysis script are available from the GitHub repository:
671 https://github.com/LeonPfeufer/Organ_Bath_Experiment

672

References

- Aho, E., & Vornanen, M. (1999). Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout *Oncorhynchus mykiss*: Effects of thermal acclimation. *Journal of Experimental Biology*, 202(19), 2663–2677. <https://doi.org/10.1242/jeb.202.19.2663>
- Andreassen, A. H., Clements, J. C., Morgan, R., Spatafora, D., Metz, M., Åsheim, E. R., Pélabon, C., & Jutfelt, F. (2025). Evolution of warming tolerance alters physiology and life history traits in zebrafish. *Nature Climate Change*, 15(6), 665–672. <https://doi.org/10.1038/s41558-025-02332-y>
- Andreassen, A. H., Hall, P., Khatibzadeh, P., Jutfelt, F., & Kermen, F. (2022). Brain dysfunction during warming is linked to oxygen limitation in larval zebrafish. *Proceedings of the National Academy of Sciences*, 119(39), e2207052119. <https://doi.org/10.1073/pnas.2207052119>
- Buckingham, S. D., & Ali, D. W. (2004). Sodium and potassium currents of larval zebrafish muscle fibres. *Journal of Experimental Biology*, 207(5), 841–852. <https://doi.org/10.1242/jeb.00839>
- Bürkner, P.-C. (2021). Bayesian Item Response Modeling in R with **brms** and Stan. *Journal of Statistical Software*, 100(5). <https://doi.org/10.18637/jss.v100.i05>
- Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P. W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W. W. L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., ... Ha, M. (with Lee, H.). (2023). *IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland*. (P. Arias, M. Bustamante, I. Elgizouli, G. Flato, M. Howden, C. Méndez-Vallejo, J. J. Pereira, R. Pichs-Madruga, S. K. Rose, Y. Saheb, R. Sánchez Rodríguez, D. Úrge-Vorsatz, C. Xiao, N. Yassaa, J. Romero, J. Kim, E. F. Haites, Y. Jung, R. Stavins, ... C. Péan,

699 Eds.; First). Intergovernmental Panel on Climate Change (IPCC).
700 <https://doi.org/10.59327/IPCC/AR6-9789291691647>

701 Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G., & Farrell, A. P. (2008). Circulatory limits to
702 oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *American Journal of Physiology-Regulatory, Integrative and*
703 *Comparative Physiology*, 295(5), R1631–R1639.
704 <https://doi.org/10.1152/ajpregu.90461.2008>

705 Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of
706 climate change: Respirometry, relevance and recommendations. *Journal of Experimental*
707 *Biology*, 216(15), 2771–2782. <https://doi.org/10.1242/jeb.084251>

708 Coutts, C. A., Patten, S. A., Balt, L. N., & Ali, D. W. (2006). Development of ionic currents of
709 zebrafish slow and fast skeletal muscle fibers. *Journal of Neurobiology*, 66(3), 220–235.
710 <https://doi.org/10.1002/neu.20214>

711 Cox, D.K. (1974). Effects of three heating rates on the critical thermal maximum of bluegill.
712 *Thermal Ecology*, 150–163.

713 De Vos, O., Van Hecke, T., & Ghysels, A. (2018). Effect of Chain Unsaturation and Temperature on
714 Oxygen Diffusion Through Lipid Membranes from Simulations. In O. Thews, J. C.
715 LaManna, & D. K. Harrison (Eds.), *Oxygen Transport to Tissue XL* (Vol. 1072, pp. 399–
716 404). Springer International Publishing. [https://doi.org/10.1007/978-3-319-91287-](https://doi.org/10.1007/978-3-319-91287-5_64)
717 [5_64](https://doi.org/10.1007/978-3-319-91287-5_64)

718 Domenici, P., & Blake, R. W. (1997). The Kinematics and Performance of Fish Fast-Start
719 Swimming. *Journal of Experimental Biology*, 200(8), 1165–1178.
720 <https://doi.org/10.1242/jeb.200.8.1165>

722 Ekström, A., Gräns, A., & Sandblom, E. (2019). Can't beat the heat? Importance of cardiac control
 723 and coronary perfusion for heat tolerance in rainbow trout. *Journal of Comparative*
 724 *Physiology B*, 189(6), 757–769. <https://doi.org/10.1007/s00360-019-01243-7>

725 Eliason, E. J., & Anttila, K. (2017). Temperature and the Cardiovascular System. In *Fish*
 726 *Physiology* (Vol. 36, pp. 235–297). Elsevier. <https://doi.org/10.1016/bs.fp.2017.09.003>

727 Ern, R., Andreassen, A. H., & Jutfelt, F. (2023). Physiological Mechanisms of Acute Upper Thermal
 728 Tolerance in Fish. *Physiology*, 38(3), 141–158.
 729 <https://doi.org/10.1152/physiol.00027.2022>

730 Ern, R., Huong, D. T. T., Phuong, N. T., Madsen, P. T., Wang, T., & Bayley, M. (2015). Some like it
 731 hot: Thermal tolerance and oxygen supply capacity in two eurythermal crustaceans.
 732 *Scientific Reports*, 5(1), 10743. <https://doi.org/10.1038/srep10743>

733 Farrell, A. P., Eliason, E. J., Sandblom, E., & Clark, T. D. (2009). Fish cardiorespiratory physiology
 734 in an era of climate change The present review is one of a series of occasional review
 735 articles that have been invited by the Editors and will feature the broad range of
 736 disciplines and expertise represented in our Editorial Advisory Board. *Canadian Journal*
 737 *of Zoology*, 87(10), 835–851. <https://doi.org/10.1139/Z09-092>

738 Friedlander, M. J., Kotchabhakdi, N., & Prosser, C. L. (1976). Effects of cold and heat on behavior
 739 and cerebellar function in goldfish. *Journal of Comparative Physiology ? A*, 112(1), 19–45.
 740 <https://doi.org/10.1007/BF00612674>

741 Fry, F. E. J., & Hart, J. S. (1948). THE RELATION OF TEMPERATURE TO OXYGEN CONSUMPTION
 742 IN THE GOLDFISH. *The Biological Bulletin*, 94(1), 66–77.
 743 <https://doi.org/10.2307/1538211>

744 Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of Size and
 745 Temperature on Metabolic Rate. *Science*, 293(5538), 2248–2251.
 746 <https://doi.org/10.1126/science.1061967>

747 Gollock, M. J., Currie, S., Petersen, L. H., & Gamperl, A. K. (2006). Cardiovascular and
 748 haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature
 749 increase. *Journal of Experimental Biology*, 209(15), 2961–2970.
 750 <https://doi.org/10.1242/jeb.02319>

751 Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S.,
 752 Ortega-Martinez, O., Einarsdottir, I., Björnsson, B. T., Sundell, K., & Axelsson, M. (2014).
 753 Aerobic scope fails to explain the detrimental effects on growth resulting from warming
 754 and elevated CO₂ in Atlantic halibut. *Journal of Experimental Biology*, 217(5), 711–717.
 755 <https://doi.org/10.1242/jeb.096743>

756 Gräns, A., Seth, H., Axelsson, M., Sandblom, E., Albertsson, F., Wiklander, K., & Olsson, C. (2013).
 757 Effects of acute temperature changes on gut physiology in two species of sculpin from
 758 the west coast of Greenland. *Polar Biology*, 36(6), 775–785.
 759 <https://doi.org/10.1007/s00300-013-1301-0>

760 Haverinen, J., & Vornanen, M. (2020). Atrioventricular block, due to reduced ventricular
 761 excitability, causes the depression of fish heart rate in fish at critically high
 762 temperatures. *Journal of Experimental Biology*, jeb.225227.
 763 <https://doi.org/10.1242/jeb.225227>

764 Hidaka, T., & Toida, N. (1969). Biophysical and mechanical properties of red and white muscle
 765 fibres in fish. *The Journal of Physiology*, 201(1), 49–59.
 766 <https://doi.org/10.1113/jphysiol.1969.sp008741>

767 Hudson, R. C. L. (1973). On The Function of the White Muscles in Teleosts at Intermediate
 768 Swimming Speeds. *Journal of Experimental Biology*, 58(2), 509–522.
 769 <https://doi.org/10.1242/jeb.58.2.509>

James, R. S., & Johnston, I. A. (1998). Scaling of Muscle Performance During Escape Responses in the Fish *Myoxocephalus Scorpius* L. *Journal of Experimental Biology*, 201(7), 913–923. <https://doi.org/10.1242/jeb.201.7.913>

Johnston, I. A., & Altringham, J. D. (1985). Evolutionary adaptation of muscle power output to environmental temperature: Force-velocity characteristics of skinned fibres isolated from antarctic, temperate and tropical marine fish. *Pflügers Archiv European Journal of Physiology*, 405(2), 136–140. <https://doi.org/10.1007/BF00584534>

Johnston, I. A., & Brill, R. (1984). Thermal dependence of contractile properties of single skinned muscle fibres from Antarctic and various warm water marine fishes including Skipjack Tuna (*Katsuwonus pelamis*) and Kawakawa (*Euthynnus affinis*). *Journal of Comparative Physiology B*, 155(1), 63–70. <https://doi.org/10.1007/BF00688792>

Jørgensen, L. B., Malte, H., & Overgaard, J. (2019). How to assess *Drosophila* heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits. *Functional Ecology*, 33(4), 629–642. <https://doi.org/10.1111/1365-2435.13279>

Jutfelt, F., Ern, R., Leeuwis, R. H. J., & Clark, T. D. (2024). Effects of climate warming. In *Encyclopedia of Fish Physiology* (pp. 14–31). Elsevier. <https://doi.org/10.1016/B978-0-323-90801-6.00183-X>

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., Gräns, A., Axelsson, M., ... Clark, T. D. (2018). Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. *Journal of Experimental Biology*, 221(1), jeb169615. <https://doi.org/10.1242/jeb.169615>

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

795 thermal tolerance in Atlantic cod. *Journal of Experimental Biology*, jeb.208249.
796 <https://doi.org/10.1242/jeb.208249>

797 Kamata, R., & Washio, H. (2003). Neuromuscular transmission of pectoral fin muscles of the
798 goldfish *Carassius auratus*. *Comparative Biochemistry and Physiology Part A: Molecular &*
799 *Integrative Physiology*, 135(3), 435–442. <https://doi.org/10.1016/S1095->
800 6433(03)00110-7

801 Koester, J., & Siegelbaum, S. A. (2000). *Propagated Signaling: The Action Potential*.

802 Lennard, R., & Huddart, H. (1991). The effect of thermal stress on electrical and mechanical
803 responses and associated calcium movements of flounder heart and gut. *Comparative*
804 *Biochemistry and Physiology Part A: Physiology*, 98(2), 221–228.
805 [https://doi.org/10.1016/0300-9629\(91\)90524-G](https://doi.org/10.1016/0300-9629(91)90524-G)

806 Lodge, N. J., & Huddart, H. (1984). Calcium pools mobilized during excitation-contraction
807 coupling in flounder intestinal smooth muscle. *Comparative Biochemistry and Physiology*
808 *Part C: Comparative Pharmacology*, 77(1), 157–160. <https://doi.org/10.1016/0742->
809 8413(84)90145-2

810 Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: Data to support
811 the onset of spasms as the definitive end point. *Canadian Journal of Zoology*, 75(10),
812 1553–1560. <https://doi.org/10.1139/z97-782>

813 Mendonça, P. C., & Gamperl, A. K. (2010). The effects of acute changes in temperature and
814 oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes*
815 *americanus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*
816 *Physiology*, 155(2), 245–252. <https://doi.org/10.1016/j.cbpa.2009.11.006>

817 Michaelsen, J., Fago, A., & Bundgaard, A. (2021). High temperature impairs mitochondrial
818 function in rainbow trout cardiac mitochondria. *Journal of Experimental Biology*, 224(9),
819 jeb242382. <https://doi.org/10.1242/jeb.242382>

820 Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CTmax is repeatable and doesn't reduce growth
821 in zebrafish. *Scientific Reports*, 8(1), 7099. [https://doi.org/10.1038/s41598-018-25593-](https://doi.org/10.1038/s41598-018-25593-4)
822 4

823 Morrisette, J., Xu, L., Nelson, A., Meissner, G., & Block, B. A. (2000). Characterization of RyR1-
824 slow, a ryanodine receptor specific to slow-twitch skeletal muscle. *American Journal of*
825 *Physiology-Regulatory, Integrative and Comparative Physiology*, 279(5), R1889–R1898.
826 <https://doi.org/10.1152/ajpregu.2000.279.5.R1889>

827 Nagaoka, R., Katayama, T., Higashijima, S., & Tanimoto, M. (2025). Biomechanics of static roll
828 posture control by body flexion in adult zebrafish. *iScience*, 28(9), 113416.
829 <https://doi.org/10.1016/j.isci.2025.113416>

830 Olsson, C., Holbrook, J. D., Bompadre, G., Jönsson, E., Hoyle, C. H. V., Sanger, G. J., Holmgren, S., &
831 Andrews, P. L. R. (2008). Identification of genes for the ghrelin and motilin receptors and
832 a novel related gene in fish, and stimulation of intestinal motility in zebrafish (Danio
833 rerio) by ghrelin and motilin. *General and Comparative Endocrinology*, 155(1), 217–226.
834 <https://doi.org/10.1016/j.ygcen.2007.05.016>

835 Pörtner, H. O., & Knust, R. (2007). Climate Change Affects Marine Fishes Through the Oxygen
836 Limitation of Thermal Tolerance. *Science*, 315(5808), 95–97.
837 <https://doi.org/10.1126/science.1135471>

838 Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for
839 integrating climate-related stressor effects in marine ecosystems. *Journal of*
840 *Experimental Biology*, 213(6), 881–893. <https://doi.org/10.1242/jeb.037523>

841 Pörtner, H.-O., Bock, C., & Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance:
842 Bridging ecology and physiology. *Journal of Experimental Biology*, 220(15), 2685–2696.
843 <https://doi.org/10.1242/jeb.134585>

844 Raby, G. D., De Bonville, J., Reynolds, L., Storm, Z., Cowan, Z.-L., Metz, M., Andreassen, A. H.,
845 Pfeufer, L., Lechner, E. R., Stewart, E. M. C., Leeuwis, R. H. J., Ern, R., Silva-Garay, L.,
846 Skeeles, M. R., Roche, D. G., Morgan, R., Green, L., Speers-Roesch, B., Mills, S. C., ... Jutfelt,
847 F. (2025a). Oxygen supersaturation has negligible effects on warming tolerance across
848 diverse aquatic ectotherms. *PLOS Biology*, 23(11), e3003413.
849 <https://doi.org/10.1371/journal.pbio.3003413>

850 Raby, G., Morgan, R., Andreassen, A., Stewart, E., De Bonville, J., Hoots, E., Kuchenmüller, L., Metz,
851 M., Rowsey, L., Green, L., Griffin, R., Martin, S., Reid, H., Ern, R., Asheim, E., Cowan, Z.-L.,
852 Leeuwis, R., Blewett, T., Speers-Roesch, B., ... Jutfelt, F. (2025b). *Measuring critical*
853 *thermal maximum in aquatic ectotherms: A practical guide*. Life Sciences.
854 <https://doi.org/10.32942/X28S69>

855 Rios, E., & Brum, G. (1987). Involvement of dihydropyridine receptors in excitation–contraction
856 coupling in skeletal muscle. *Nature*, 325(6106), 717–720.
857 <https://doi.org/10.1038/325717a0>

858 Robinson, W. R., Peters, R. H., & Zimmermann, J. (1983). The effects of body size and
859 temperature on metabolic rate of organisms. *Canadian Journal of Zoology*, 61(2), 281–
860 288. <https://doi.org/10.1139/z83-037>

861 Sandblom, E., & Axelsson, M. (2007). Venous hemodynamic responses to acute temperature
862 increase in the rainbow trout (*Oncorhynchus mykiss*). *American Journal of Physiology-*
863 *Regulatory, Integrative and Comparative Physiology*, 292(6), R2292–R2298.
864 <https://doi.org/10.1152/ajpregu.00884.2006>

865 Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., Odelström, A., Adill, A.,
866 Aho, T., & Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow
867 principles of plastic floors and concrete ceilings. *Nature Communications*, 7(1), 11447.
868 <https://doi.org/10.1038/ncomms11447>

869 Schneider, M. F., & Chandler, W. K. (1973). Voltage Dependent Charge Movement in Skeletal
870 Muscle: A Possible Step in Excitation–Contraction Coupling. *Nature*, 242(5395), 244–
871 246. <https://doi.org/10.1038/242244a0>

872 Schredelseker, J., Shrivastav, M., Dayal, A., & Grabner, M. (2010). Non- Ca^{2+} -conducting Ca^{2+}
873 channels in fish skeletal muscle excitation-contraction coupling. *Proceedings of the*
874 *National Academy of Sciences*, 107(12), 5658–5663.
875 <https://doi.org/10.1073/pnas.0912153107>

876 Shiels, H. A., & White, E. (2008). The Frank–Starling mechanism in vertebrate cardiac myocytes.
877 *Journal of Experimental Biology*, 211(13), 2005–2013.
878 <https://doi.org/10.1242/jeb.003145>

879 Silva-Garay, L., Ern, R., Andreassen, A. H., Reiersen, M., & Jutfelt, F. (2025). No oxygen limitation
880 of upper thermal tolerance in zebrafish regardless of acclimation temperature. *Journal of*
881 *Thermal Biology*, 131, 104157. <https://doi.org/10.1016/j.jtherbio.2025.104157>

882 Smith, K. E., Burrows, M. T., Hobday, A. J., King, N. G., Moore, P. J., Sen Gupta, A., Thomsen, M. S.,
883 Wernberg, T., & Smale, D. A. (2023). Biological Impacts of Marine Heatwaves. *Annual*
884 *Review of Marine Science*, 15(1), 119–145. [https://doi.org/10.1146/annurev-marine-](https://doi.org/10.1146/annurev-marine-032122-121437)
885 [032122-121437](https://doi.org/10.1146/annurev-marine-032122-121437)

886 Somero, G. N. (1978). Temperature Adaptation of Enzymes: Biological Optimization Through
887 Structure-Function Compromises. *Annual Review of Ecology and Systematics*, 9(1), 1–29.
888 <https://doi.org/10.1146/annurev.es.09.110178.000245>

889 Stanfield, P. R. (1972). Electrical properties of white and red muscle fibres of the elasmobranch
890 fish scyliorhinus canicula by p. R. Stanfield. *The Journal of Physiology*.

891 Stevens, E. D., Bennion, G. R., Randall, D. J., & Shelton, G. (1972). Factors affecting arterial
892 pressures and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon*

893 elongatus. *Comparative Biochemistry and Physiology Part A: Physiology*, 43(3), 681–695.
 894 [https://doi.org/10.1016/0300-9629\(72\)90255-1](https://doi.org/10.1016/0300-9629(72)90255-1)

895 Sugioka, T., Tanimoto, M., & Higashijima, S. (2023). Biomechanics and neural circuits for
 896 vestibular-induced fine postural control in larval zebrafish. *Nature Communications*,
 897 14(1), 1217. <https://doi.org/10.1038/s41467-023-36682-y>

898 Verberk, W. C. E. P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., & Terblanche, J. S.
 899 (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current
 900 evidence. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*
 901 *Physiology*, 192, 64–78. <https://doi.org/10.1016/j.cbpa.2015.10.020>

902 Vornanen, M. (1997). Sarcolemmal Ca influx through L-type Ca channels in ventricular myocytes
 903 of a teleost fish. *American Journal of Physiology-Regulatory, Integrative and Comparative*
 904 *Physiology*, 272(5), R1432–R1440. <https://doi.org/10.1152/ajpregu.1997.272.5.R1432>

905 Vornanen, M. (2016). The temperature dependence of electrical excitability in fish hearts.
 906 *Journal of Experimental Biology*, 219(13), 1941–1952.
 907 <https://doi.org/10.1242/jeb.128439>

908 Vornanen, M. (2020). Feeling the heat: Source–sink mismatch as a mechanism underlying the
 909 failure of thermal tolerance. *Journal of Experimental Biology*, 223(16), jeb225680.
 910 <https://doi.org/10.1242/jeb.225680>

911 Vornanen, M., Haverinen, J., & Egginton, S. (2013). Acute heat tolerance of cardiac excitation in
 912 the brown trout (*Salmo trutta fario*). *Journal of Experimental Biology*, jeb.091272.
 913 <https://doi.org/10.1242/jeb.091272>

914 Vornanen, M., Ryökkönen, A., & Nurmi, A. (2002). Temperature-dependent expression of
 915 sarcolemmal K⁺ currents in rainbow trout atrial and ventricular myocytes. *American*
 916 *Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 282(4),
 917 R1191–R1199. <https://doi.org/10.1152/ajpregu.00349.2001>

918 Walker, M. G. (1971). Effect of Starvation and Exercise on the Skeletal Muscle Fibres of the Cod
 919 (Gadus Morhua L.) and the Coalfish (Gadus Virens L.) Respectively. *ICES Journal of*
 920 *Marine Science*, 33(3), 421–427. <https://doi.org/10.1093/icesjms/33.3.421>

921 Wang, T., Lefevre, S., Iversen, N. K., Findorf, I., Buchanan, R., & McKenzie, D. J. (2014). Anaemia
 922 only causes a small reduction in the upper critical temperature of sea bass: Is oxygen
 923 delivery the limiting factor for tolerance of acute warming in fishes? *Journal of*
 924 *Experimental Biology*, jeb.104166. <https://doi.org/10.1242/jeb.104166>

925