

## RESEARCH ARTICLE

# Autonomic cardiac regulation facilitates acute heat tolerance in rainbow trout: *in situ* and *in vivo* support

Matthew J. H. Gilbert<sup>1,\*</sup>, Varsha Rani<sup>2</sup>, Sean M. McKenzie<sup>1</sup> and Anthony P. Farrell<sup>1,2</sup>

## ABSTRACT

Acute warming in fish increases heart rate ( $f_H$ ) and cardiac output to peak values, after which performance plateaus or declines and arrhythmia may occur. This cardiac response can place a convective limitation on systemic oxygen delivery at high temperatures. To test the hypothesis that autonomic cardiac regulation protects cardiac performance in rainbow trout during acute warming, we investigated adrenergic and cholinergic regulation during the onset and progression of cardiac limitations. We explored the direct effects of adrenergic stimulation by acutely warming an *in situ* working perfused heart until arrhythmia occurred, cooling the heart to restore rhythmicity and rewarming with increasing adrenergic stimulation. Adrenergic stimulation produced a clear, dose-dependent increase in the temperature and peak  $f_H$  achieved prior to the onset of arrhythmia. To examine how this adrenergic protection functions in conjunction with cholinergic vagal inhibition *in vivo*, rainbow trout fitted with ECG electrodes were acutely warmed in a respirometer until they lost equilibrium ( $CT_{max}$ ) with and without muscarinic (atropine) and  $\beta$ -adrenergic (sotalol) antagonists. Trout exhibited roughly equal and opposing cholinergic and adrenergic tone on  $f_H$  that persisted up to critical temperatures.  $\beta$ -Adrenergic blockade significantly lowered peak  $f_H$  by 14–17%, while muscarinic blockade significantly lowered the temperature for peak  $f_H$  by 2.0°C. Moreover, muscarinic and  $\beta$ -adrenergic blockers injected individually or together significantly reduced  $CT_{max}$  by up to 3°C, indicating for the first time that cardiac adrenergic stimulation and cholinergic inhibition can enhance acute heat tolerance in rainbow trout at the level of the heart and the whole animal.

**KEY WORDS:** Upper thermal tolerance, Autonomic regulation, Heart rate, *Oncorhynchus mykiss*, Cardiac arrhythmia

## INTRODUCTION

Teleosts are the most abundant class of fishes (>25,000 species) and utilize a variety of strategies in response to warming. Almost universally among these strategies is an increase in heart rate ( $f_H$ ), a response that has been found in Arctic, Antarctic, tropical and temperate species (Eliason and Anttila, 2017; Farrell and Smith, 2017). Furthermore, where measured, the increase in  $f_H$  mediates an increase in cardiac output ( $\dot{Q}$ ) (Brett, 1971; Ekström et al., 2016; Motyka et al., 2017; Penney et al., 2014; Steinhausen et al., 2008), which presumably services the increased tissue oxygen

consumption, as inferred from a proportional increase in whole-animal oxygen uptake (Ekström et al., 2014; Eliason and Anttila, 2017; Farrell, 2009; Farrell and Smith, 2017; Sandblom et al., 2016a). However, as fish approach their upper thermal limit,  $f_H$  reaches a peak and thereafter may decline and become arrhythmic (Anttila et al., 2014; Badr et al., 2016; Casselman et al., 2012; Ekström et al., 2014; Eliason et al., 2011). While the peak  $f_H$  and the temperatures for peak  $f_H$  and arrhythmia vary appreciably among species and even strains, with acclimation temperature and with ontogeny (Chen et al., 2015; Drost et al., 2016), in all cases the inability to increase  $f_H$  may constrain convective oxygen delivery at temperature extremes (Eliason and Anttila, 2017; Eliason et al., 2011, 2013; Fry, 1947; Steinhausen et al., 2008). Thus, given the central role of  $f_H$  in meeting increased oxygen demand, there is considerable interest in why  $f_H$  reaches a peak at warm temperatures and why it can become arrhythmic with further warming (Eliason and Anttila, 2017; Vornanen, 2016, 2017).

Extreme warming frequently causes a delay or loss of the QRS complex despite a normal, rhythmic P-wave in the electrocardiogram (ECG) (Badr et al., 2016; Casselman et al., 2012; Vornanen, 2016). As such, Vornanen (2016) proposed a temperature-dependent depression of electrical excitability hypothesis (TDEE), which suggests that the inward  $Na^+$  current ( $I_{Na}$ ) responsible for ventricular depolarization is depressed relative to the resting outward  $K^+$  current ( $I_{K1}$ ) at warm temperatures such that the threshold potential needed to initiate an action potential cannot be reached (Vornanen, 2016; Vornanen et al., 2014). Other putative mechanisms also exist to explain cardiac failure with warming. For example, increased proton leakage in cardiac mitochondria at warm extremes decreases mitochondrial efficiency, which may compromise ATP production and cellular pH (Chung et al., 2017; Iftikar et al., 2014; Leo et al., 2017; O'Brien et al., 2018). Potentially compounding compromised ATP production is a reduced myocardial oxygen supply from luminal venous blood, which is essential in all teleosts; at extremely warm temperatures, venous oxygen partial pressure often declines and blood residence time in the heart decreases because of the elevated  $f_H$  (Eliason and Anttila, 2017; Eliason et al., 2013; Farrell and Clutterham, 2003; Steinhausen et al., 2008). In addition, the acidemia and hyperkalemia accompanying high temperature also likely impair cardiac contractility (Farrell et al., 1983; Farrell, 2009; Gesser and Jørgensen, 1982; Hanson et al., 2006).

Despite the uncertainty regarding the exact mechanism(s) for the decline in cardiac performance in teleosts at high temperature, autonomic regulation of  $f_H$  may protect cardiac function. Autonomic regulation via the sympathetic and parasympathetic control systems involves  $\beta$ -adrenergic stimulation and muscarinic-cholinergic inhibition of the teleost heart (Sandblom and Axelsson, 2011). Together, they regulate the spontaneous depolarization frequency of pacemaker tissue, modifying the excitability and conductivity of atrial, ventricular and nodal tissues. Importantly, acutely warming fish greatly increases circulating catecholamines (adrenaline and

<sup>1</sup>Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4. <sup>2</sup>Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, BC, Canada V6T 1Z4.

\*Author for correspondence (gilbert1@zoology.ubc.ca)

 M.J.H.G., 0000-0001-8000-7568

noradrenaline) (Currie et al., 2013, 2008), which are known to protect the performance of *in situ* perfused hearts, cardiac strips and isolated cardiomyocytes under a range of physiological challenges including hypoxia, acidosis, hyperkalemia and temperature (Aho and Vornanen, 2001; Farrell et al., 1996, 1986; Graham and Farrell, 1989; Hanson et al., 2006; Shiels et al., 2003). An elevated cardiac  $\beta$ -adrenergic receptor density is also associated with improved thermal tolerance in wild sockeye salmon (Eliason et al., 2011). Furthermore, adrenergic agonists have long been recognized in human medicine as an effective first line clinical treatment when atrioventricular (AV) conduction or ventricular excitability is limited (i.e. AV block-type arrhythmia) (Bellet, 1964; Marshall, 1935). Cholinergic regulation of the heart is restricted to direct innervation of the nodal and atrial tissue and, importantly, does not act on the ventricle (Sandblom and Axelsson, 2011; Vornanen, 2017). A cholinergic mediated depression of  $f_H$  at high temperatures could increase the time for electrochemical recovery, cardiac filling and oxygen diffusion between heartbeats, as well as decrease cardiac energy and oxygen demands (Ekström et al., 2014; Eliason and Anttila, 2017; Farrell, 2007).

Despite these multiple lines of evidence to suggest an autonomic protection of cardiac performance during extreme warming, only Ekström et al. (2014) have directly tested this hypothesis by blocking  $\beta$ -adrenergic and muscarinic receptors while measuring cardiac output in rainbow trout (*Oncorhynchus mykiss*) as they were acutely warmed until they lost equilibrium ( $CT_{max}$ ). However, autonomic blockade had no significant effect on peak  $f_H$ , peak  $\dot{Q}$  or  $CT_{max}$  (Ekström et al., 2014). Instead, the reduced  $\beta$ -adrenergic stimulation of  $f_H$  observed at high temperatures indicated a possible decline in  $\beta$ -adrenergic sensitivity, as suggested previously (Ask et al., 1981; Eliason and Anttila, 2017; Sandblom and Axelsson, 2011; Shiels et al., 2003). Nonetheless, the sensitivity of the heart to  $\beta$ -adrenergic stimulation has yet to be directly quantified at temperatures that induce cardiac arrhythmia and loss of equilibrium. Also, the effect of dual cholinergic and adrenergic blockade on thermal performance has not been studied. Therefore, our objective was to test the hypothesis that sympathetic and parasympathetic mechanisms protect the rainbow trout heart and enhance warm tolerance. To test this hypothesis, sympathetic cardio-protection was directly studied by acutely warming an *in situ* working, perfused heart to an arrhythmic temperature while adjusting the adrenaline concentration in the perfusate. We predicted that  $\beta$ -adrenergic stimulation can stabilize or improve  $f_H$  near the upper thermal tolerance limit of rainbow trout. Also, we investigated whether parasympathetic and sympathetic regulation *in vivo* protects  $f_H$  in rainbow trout (as measured with ECG electrodes in a respirometer) during acute warming with and without injections of  $\beta$ -adrenergic and muscarinic antagonists (independently and together). We predicted that sympathetic and parasympathetic control of  $f_H$  exists near the upper critical temperature ( $CT_{max}$ ), which was measured as a humane endpoint for comparison of upper thermal tolerance with previous research.

## MATERIALS AND METHODS

### Animals

Animal housing and experimental protocols were approved by the University of British Columbia animal care committee (A16-0049 and A15-0035). Rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), obtained from Sun Valley Trout Farm (Mission, BC, Canada) were used to measure *in vivo*  $f_H$  and oxygen uptake ( $n=49$ ; both sexes; body mass:  $357\pm 6$  g, mean $\pm$ s.e.m.). Rainbow trout obtained from Miracle Springs Hatchery (Mission, BC, Canada)

were used to measure cardiac performance with an *in situ* working, perfused heart preparation ( $n=8$ ; all female; body mass:  $209\pm 8$  g). All fish were housed for at least 3 weeks prior to experimentation at the University of British Columbia Biological Sciences Aquatics Facility (Vancouver, BC) in 200–400 l holding tanks containing dechlorinated municipal tap water with partial recirculation and circular waterflow. The fish were acclimated to  $12^\circ\text{C}$  under a 12 h:12 h light:dark photoperiod.

### Assessment of the effects of acute warming and adrenergic stimulation on $f_H$ using an *in situ* working perfused heart preparation

The *in situ* working perfused heart preparation isolates the heart from any neural or humoral inputs while retaining an intact pericardium and allowing the heart to beat at its intrinsic, pacemaker rhythm, unless modulatory agents such as adrenaline are added to the perfusate (Farrell et al., 1988). As such, the preparation is ideal for examining the effects of acute warming on intrinsic  $f_H$  and the interactive effects of adrenergic regulation. The preparation in rainbow trout has previously been described in detail (Farrell et al., 1988; Hanson et al., 2006). Briefly, individual fish were anesthetized in freshwater at  $12^\circ\text{C}$  containing  $100\text{ mg l}^{-1}$  tricaine methanesulfonate (TMS) before transfer to an acrylic surgery cradle where their gills were irrigated with  $75\text{ mg l}^{-1}$  TMS (TMS solutions were buffered with  $110\text{ mg l}^{-1}$   $\text{NaHCO}_3$ ). Inflow and outflow cannula were inserted into the sinus venous via a hepatic vein and the ventral aorta, respectively, and all major remaining veins returning to the heart were ligated. The preparation was transferred to a jacketed bath containing 0.9% NaCl at  $12^\circ\text{C}$ , where the heart was perfused at a constant filling pressure with a physiological perfusate ( $\text{NaCl}$ ,  $140\text{ mmol l}^{-1}$ ;  $\text{KCl}$ ,  $2.8\text{ mmol l}^{-1}$ ;  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ ,  $1.8\text{ mmol l}^{-1}$ ;  $\text{MgSO}_4$ ,  $1.2\text{ mmol l}^{-1}$ ;  $\text{NaH}_2\text{PO}_4$ ,  $1.2\text{ mmol l}^{-1}$ ; TES acid,  $3.9\text{ mmol l}^{-1}$ ; TES sodium salt,  $6.1\text{ mmol l}^{-1}$ ; D-glucose,  $10\text{ mmol l}^{-1}$ ; pH 7.85 at  $10^\circ\text{C}$ ). The perfusate was contained in jacketed reservoirs for temperature regulation and bubbled with 100% oxygen. Precise temperature control was achieved by attaching two recirculating chillers (Isotemp 3016D, Fisher Scientific, Hampton, NH, USA) to the bath and perfusate reservoirs with valves that allowed the temperature control fluid to flow through either chiller. One chiller was used to control the rate of warming and the other was maintained at a cold temperature ( $>10^\circ\text{C}$  below target temperature) to allow rapid cooling when needed. Temperature was continuously recorded in the bath near to the heart (Microx 3, PreSens, Regensburg, Germany). Also, an ECG was acquired by placing one subdermal 21 gauge stainless steel electrode on the ventral surface over the heart, and another on the body. The inflow and outflow lines were fitted with pressure transducers (DPT 6100, Smiths Medical, Kirchseeon, Germany) to monitor filling pressure and afterload of the heart, respectively. A low afterload ( $2.94\text{ kPa}=30\text{ cmH}_2\text{O}$ ) was selected for the study to limit the potential for rundown with the knowledge that afterload has minimal effect on  $f_H$  in the current preparation (Farrell et al., 1986). A flow transducer (SWF-4, Zepeda Instruments, Seattle, WA, USA) was placed in the outflow line and used to monitor cardiac output ( $\dot{Q}$ ) at the start of the trial and ensure the preparations remained functional during warming.  $\dot{Q}$  was initially set on the upper end of the published range for routine values at similar temperatures *in vivo* ( $\sim 30\text{ ml min}^{-1}\text{ kg}^{-1}$ ; Farrell and Smith, 2017) by adjusting the filling pressure. Data for  $\dot{Q}$ , stroke volume and pressure measurements during the experiments are presented in Fig. S1 and Table S1, but were not corrected for the potential effects of temperature on measurement equipment. After the preparation had

stabilized with an adrenaline-free perfusate (~10 min), temperature controllers warmed the bath saline and perfusate reservoirs at a continuous rate of  $5^{\circ}\text{C h}^{-1}$  until a temperature was reached at which the heartbeat became arrhythmic ( $T_{\text{arr}}$ ).  $f_{\text{H}}$  did not exhibit marked beat-to-beat variation during warming and so the onset of arrhythmia was always unambiguous and characterized by a decrease in instantaneous  $f_{\text{H}}$  of approximately half or more when one or more beats were missed. At the onset of arrhythmia, the preparation was immediately and rapidly (~5–10 min) cooled by  $1\text{--}2^{\circ}\text{C}$  to restore a rhythmic heartbeat and stable  $\dot{Q}$  before adrenaline was added to the perfusate (final concentration:  $5\text{ nmol l}^{-1}$ ). The preparation was then rewarmed at the same rate until cardiac arrhythmia was again observed. This protocol of warming to produce arrhythmia, cooling to restore rhythmicity and rewarming to produce arrhythmia was repeated while progressively increasing the adrenaline concentration in the perfusate (10, 100 and  $1000\text{ nmol l}^{-1}$ ). The expectation was that  $1000\text{ nmol l}^{-1}$  would produce maximum adrenergic stimulation (Ask et al., 1981; Farrell et al., 1986; Graham and Farrell, 1989). The ECG signal was amplified and filtered ( $100\times$ , low pass: 3 Hz, high pass: 100 Hz) with a Grass P55 pre-amplifier (Natus, CA, USA). All electrical signals were digitally displayed and recorded using a PowerLab data acquisition unit and Labchart 8.0 software (ADInstruments, Colorado Springs, CO, USA). The ECG was then digitally filtered (low pass: 4–10 Hz, high pass: 40–45 Hz) to optimize automated heartbeat detection. Accurate beat identification was ensured through visual inspection. A moving 30 s average of  $f_{\text{H}}$  was calculated during each experiment and peak  $f_{\text{H}}$  at each adrenaline concentration was taken as the maximum 30 s average at that concentration.

#### Assessment of *in vivo* $f_{\text{H}}$ and oxygen uptake

Sub-dermal ECG electrodes represent a fairly minor surgical intervention to monitor  $f_{\text{H}}$  *in vivo* (Campbell et al., 2004) and follow  $f_{\text{H}}$  during acute warming. To this end, individual fish were anesthetized and transferred to a surgery cradle, as indicated above, to fit two 21 gauge sub-dermal needle electrodes placed diagonally on the ventral surface across the heart. An intraperitoneal (i.p.) cannula was inserted to permit injection of the antagonist drugs, as previously done by Ekström et al. (2014). The electrodes and the cannula were sutured in place at the site of insertion and at the rostral edge of the dorsal fin using 2-0 silk thread. Fish were placed into a 9.9 l respirometer (Loligo Systems, Viborg, Denmark) at  $12^{\circ}\text{C}$  to recover with recirculating flow and an automated 12 min intermittent flush cycle (420 s flush, 120 s wait and 180 s measure; Aquaresp 3.0 software, www.aquaresp.com; Svendsen et al., 2016). Fish were allowed to recover overnight for 15–18 h prior to the start of experimentation and random assignment to one of four treatment groups: saline control (0.9% NaCl), muscarinic blockade with atropine ( $1.2\text{ mg kg}^{-1}$ ), general  $\beta$ -adrenergic blockade with sotalol ( $2.7\text{ mg kg}^{-1}$ ) or dual blockade with both antagonists. All treatments were delivered as a  $1\text{ ml kg}^{-1}$  0.9% saline injection into the i.p. cannula, followed by a second  $1\text{ ml kg}^{-1}$  injection of saline to flush the cannula. Following the injection, fish were left for 24 min (two oxygen uptake measurement cycles) to allow the drugs to take effect and observe a change in  $f_{\text{H}}$ . This wait period was shorter than in a previous study (Ekström et al., 2014) and the full effect of the drugs may have occurred shortly thereafter. Fish were then warmed in  $1^{\circ}\text{C}$  increments every 12 min ( $5^{\circ}\text{C h}^{-1}$ ) until they lost equilibrium ( $\text{CT}_{\text{max}}$ ), at which point they were removed and killed with an overdose of buffered TMS ( $200\text{ mg l}^{-1}$ ). Warming was achieved by recirculating tank water through a stainless steel coil in a pre-heated bath. During the experiments, oxygen concentration and

temperature were continuously recorded every second using a 4-channel Firesting optical sensor (PyroScience GmbH, Aachen, Germany) while ECG signals were displayed and acquired as described above. *In vivo*  $f_{\text{H}}$  was calculated as the average over the final ~60 s segment at each temperature unless signal quality did not permit accurate beat detection, in which case a preceding ~60 s segment was used. If a fish did not complete a temperature step, final  $f_{\text{H}}$  was measured for ~60 s at that step. Peak  $f_{\text{H}}$  was then taken as the maximum average  $f_{\text{H}}$  from these ~60 s measurement periods. We also characterized the temperature at which an individual first approached its peak  $f_{\text{H}}$  by reporting the temperature at 95% of peak  $f_{\text{H}}$ . While this approach helped in dealing with situations where peak  $f_{\text{H}}$  exhibited a plateau, it underestimates the temperature when peak  $f_{\text{H}}$  was first reached by about  $1^{\circ}\text{C}$ . Because  $\text{CT}_{\text{max}}$  terminated the experiment, we report the percentage of fish that showed a plateau or collapse of peak  $f_{\text{H}}$  before  $\text{CT}_{\text{max}}$  was reached.

#### Data analysis

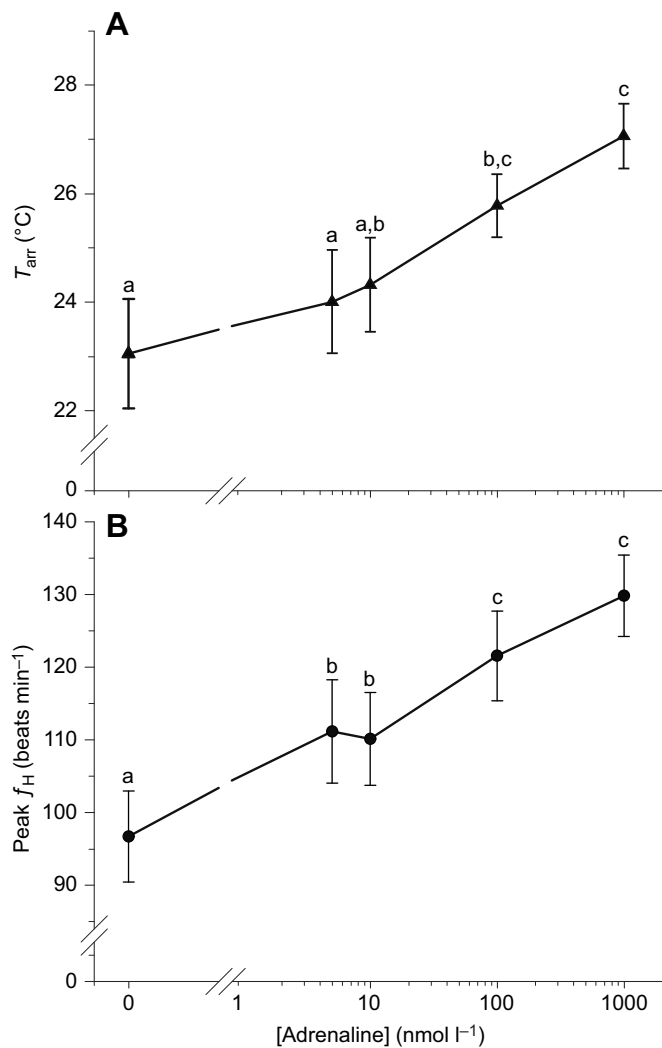
Repeated measures analysis of variance (ANOVA) was used to assess the effect of increasing adrenaline concentration on peak  $f_{\text{H}}$  and  $T_{\text{arr}}$  for the *in situ* perfused heart, with Holm's adjusted pairwise comparisons to evaluate differences among adrenaline concentrations. Linear mixed effects models were constructed with backwards elimination of non-significant interaction terms to assess differences in  $\dot{M}_{\text{O}_2}$  and  $f_{\text{H}}$  between treatment groups during warming from  $12$  to  $20^{\circ}\text{C}$ . In both cases, body mass was included in the initial model as a covariate and fish ID was included as a random factor to account for repeated measures. The  $12\text{--}20^{\circ}\text{C}$  temperature range was chosen to avoid peak  $f_{\text{H}}$  being reached in some fish above  $20^{\circ}\text{C}$ . We tested the *a priori* predictions that  $f_{\text{H}}$  at a given temperature would be lower than control with sotalol treatment and higher with atropine treatment using one-tailed *t*-tests at each temperature whenever  $>3$  individuals remained in the dataset. *P*-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. An ANOVA followed by Holm's adjusted pairwise comparisons was also used to assess *in vivo* differences in peak  $\dot{M}_{\text{O}_2}$ , temperature at peak  $\dot{M}_{\text{O}_2}$ , peak  $f_{\text{H}}$ , and temperature at 95% peak  $f_{\text{H}}$  and  $\text{CT}_{\text{max}}$  among treatment groups. As above, we used directional *a priori* predictions for the response of peak  $f_{\text{H}}$  with sotalol and atropine treatments and applied a one-tailed test for comparison with control fish. Again, body mass was included as a covariate in all ANOVA, but it was removed if it was not statistically significant. Differences in temperature at 95% peak  $f_{\text{H}}$  and  $\text{CT}_{\text{max}}$  among treatment groups were also evaluated as cumulative frequency or survival plots using Cox proportional hazards survival analysis. Data analysis and presentation were completed in R studio (R Development Core Team, 2014; <http://www.R-project.org>) and Sigmaplot 11.0 (Systat Software, San Jose, CA, USA) and  $\alpha$  was set at 0.05 for all analyses.

## RESULTS

### *In situ* perfused heart

Adrenaline-free perfusion produced an intrinsic  $f_{\text{H}}$  at  $12^{\circ}\text{C}$  of  $69.0\pm 2.6\text{ beats min}^{-1}$ , which gradually increased during acute warming to  $91.6\pm 3.9\text{ beats min}^{-1}$  at  $20^{\circ}\text{C}$  ( $Q_{10}$ :  $1.4\pm 0.1$ ; Fig. S1). Peak intrinsic  $f_{\text{H}}$  ( $96.8\pm 6.2\text{ beats min}^{-1}$ ) was reached at  $22.0\pm 1.0^{\circ}\text{C}$  (Fig. 1), a value similar to the intrinsic  $f_{\text{H}}$  measured *in vivo* at  $22^{\circ}\text{C}$  after dual pharmacological blockade ( $101.0\pm 6.4\text{ beats min}^{-1}$ ; see below). Further warming resulted in an arrhythmic intrinsic heartbeat at  $23.1\pm 1.0^{\circ}\text{C}$ .

Progressive increases in adrenaline concentration in the perfusate from 0 to  $1000\text{ nmol l}^{-1}$  significantly increased, in a dose-dependent manner, both peak  $f_{\text{H}}$  (by 34%:  $129.3\pm 6.4\text{ beats min}^{-1}$ ) and  $T_{\text{arr}}$  (by



**Fig. 1. Effect of increasing adrenergic stimulation and acute warming on temperature at arrhythmia and peak heart rate in an *in situ* perfused heart preparation.** (A) Temperature at arrhythmia ( $T_{arr}$ ). (B) Peak heart rate ( $f_H$ ). Dissimilar letters indicate significant differences between adrenaline concentrations ( $\alpha=0.05$ ).

17%:  $27.1 \pm 0.6^\circ\text{C}$ ) (peak  $f_H$ :  $F_{4,38}=19.2$ ,  $P<0.001$ ;  $T_{arr}$ :  $F_{4,38}=12.287$ ,  $P<0.001$ ; Fig. 1). Thus, the perfused heart performed significantly better during acute warming with  $\beta$ -adrenergic stimulation.

On average, peak  $f_H$  occurred  $0.4 \pm 0.1^\circ\text{C}$  below  $T_{arr}$  ( $F_{1,77}=3.76$ ,  $P=0.06$ ) and peak  $f_H$  was  $4.5 \pm 0.9$  beats  $\text{min}^{-1}$  higher than the average  $f_H$  at arrhythmia ( $F_{1,77}=8.55$ ,  $P=0.005$ ).

**Table 1. Morphometrics, peak  $f_H$  and  $\dot{M}_{O_2}$ , and corresponding critical temperatures for 12°C-acclimated rainbow trout during acute warming with and without autonomic blockade**

	Saline	+Atropine	+Sotalol	+Atropine+sotalol
<i>n</i>	11	14	13	12
Mass (g)	$377 \pm 14$	$344 \pm 12$	$363 \pm 14$	$346 \pm 13$
Length (cm)	$31.4 \pm 0.8$	$30.9 \pm 0.4$	$31.3 \pm 1.4$	$30.9 \pm 1.3$
Peak $f_H$ (beats $\text{min}^{-1}$ )	$125.0 \pm 6.0^a$	$129.7 \pm 3.9^a$	$107.5 \pm 4.2^b$	$116.4 \pm 6.5^{a,b}$
Temperature at 95% peak $f_H$ ( $^\circ\text{C}$ )	$24.3 \pm 0.3^a$	$22.3 \pm 0.3^b$	$23.8 \pm 0.3^a$	$23.1 \pm 0.4^{a,b}$
Peak $\dot{M}_{O_2}$ ( $\text{mg O}_2 \text{ kg}^{-1} \text{ min}^{-1}$ )	$8.1 \pm 0.5^a$ (10)	$7.3 \pm 0.4^{a,b}$ (13)	$6.8 \pm 0.3^{a,b}$ (11)	$6.6 \pm 0.5^b$ (11)
Temperature at peak $\dot{M}_{O_2}$ ( $^\circ\text{C}$ )	$24.8 \pm 0.7^a$ (10)	$23.0 \pm 0.5^b$ (13)	$24.1 \pm 0.3^{a,b}$ (11)	$24.1 \pm 0.5^{a,b}$ (11)
CT <sub>max</sub> ( $^\circ\text{C}$ )	$26.6 \pm 0.4^a$	$24.2 \pm 0.3^b$	$25.1 \pm 0.4^b$	$24.8 \pm 0.4^b$

Data are presented as mean  $\pm$  s.e.m. Dissimilar letters indicate significant differences between treatments within a row. Sample size (*n*) is shown in parentheses if different from the total number of fish used per group.

### ***In vivo* cardiorespiratory responses to acute warming with and without autonomic antagonists**

As expected,  $\dot{M}_{O_2}$  increased with temperature between 12 and  $20^\circ\text{C}$  ( $Q_{10}=2.1$ ), a response that was not significantly different among treatment groups [treatment:  $F_{1,40}=1.27$ ,  $P=0.298$ , temperature:  $F_{1,349}=508.82$ ,  $P<0.001$ ,  $\log(\text{mass})$ :  $F_{1,40}=18.19$ ,  $P<0.001$ ; Figs 2 and 3]. Nevertheless, peak  $\dot{M}_{O_2}$  during warming for the saline control group was significantly greater than for the dual antagonist treatment group, but not for either the atropine- or sotalol-treated group (treatment:  $F_{3,40}=4.19$ ,  $P=0.01$ , mass:  $F_{1,40}=11.34$ ,  $P=0.002$ ; Table 1). The temperature at peak  $\dot{M}_{O_2}$  was also higher in the saline group than in the atropine group (treatment:  $F_{3,40}=3.00$ ,  $P=0.04$ , mass:  $F_{1,40}=13.73$ ,  $P<0.001$ ; Table 1).

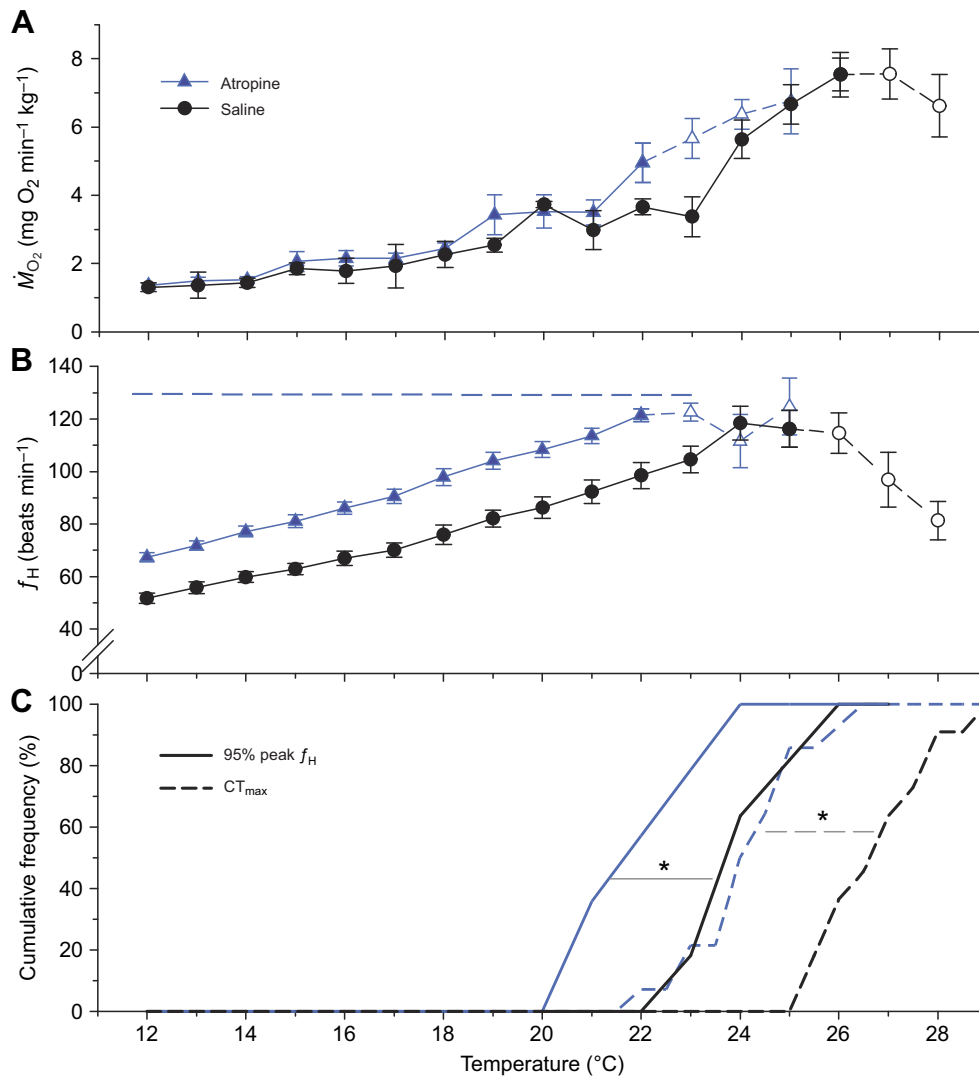
Warming between 12 and  $20^\circ\text{C}$  increased  $f_H$  by a similar amount for all four treatment groups (saline  $Q_{10}=1.9$ ; atropine  $Q_{10}=1.8$ ; sotalol  $Q_{10}=1.8$ ; both antagonists  $Q_{10}=2.0$ ) (treatment:  $F_{3,46}=16.62$ ,  $P<0.001$ , temperature:  $F_{1,399}=2303.62$ ,  $P<0.001$ ; Figs 2 and 3). As expected, atropine treatment significantly elevated  $f_H$  more than all the other treatments, while the sotalol-treated fish had a lower  $f_H$  than that of fish in all other treatments (Figs 2 and 3). In addition,  $f_H$  in fish treated with both antagonists was similar to  $f_H$  of the saline control fish (Fig. 3), indicating approximately equal and opposing adrenergic and cholinergic tone on the heart.

Peak  $f_H$  (and its plateau) occurred at a temperature lower than CT<sub>max</sub> in all control and atropinized fish, but in only 62% of sotalol-treated fish and in only 83% of fish that received both antagonists (Figs 2 and 3). While peak  $f_H$  was not significantly different in the atropine and saline treatment groups, the temperature at which a fish reached 95% peak  $f_H$  was significantly lower (–9%) in atropinized fish than in control (treatment:  $F_{3,46}=6.76$ ,  $P<0.001$ ; Fig. 2, Table 1). Peak  $f_H$  in sotalol-treated fish was significantly lower than following the atropine (–17%) and control (–14%) treatments (treatment:  $F_{1,40}=3.65$ ,  $P=0.02$ ; Table 1). Peak  $f_H$  in fish that received both antagonists was intermediate to that of the sotalol and atropine treatment groups (Table 1).

Relative to the saline control group, CT<sub>max</sub> was significantly reduced by a similar amount in all treatment groups (atropine –9.0%; sotalol –5.6%; both antagonists –6.8%) (treatment  $F_{3,46}=10.34$ ,  $P<0.001$ , mass:  $F_{1,46}=11.57$ ,  $P=0.001$ ; Table 1, Fig. 4) such that CT<sub>max</sub> was not significantly different among antagonist treatments.

### **DISCUSSION**

It has long been recognized for teleosts that both  $f_H$  and  $\dot{Q}$  increase with  $\dot{M}_{O_2}$  during acute warming, but they plateau or collapse at critically warm temperatures (Brett, 1971; Eliason and Anttila, 2017; Farrell, 2009; Fry, 1947). Past research suggests that autonomic regulation and specifically  $\beta$ -adrenergic stimulation of



**Fig. 2. *In vivo* oxygen uptake,  $f_H$ , and temperature at 95% peak  $f_H$  and loss of equilibrium in rainbow trout during acute warming with and without autonomic blockade.** Data for (A) oxygen uptake ( $\dot{M}_{O_2}$ ), (B)  $f_H$  and (C) 95% peak  $f_H$  and loss of equilibrium ( $CT_{max}$ ) with ( $n=11$ ) and without ( $n=14$ ) atropine (a muscarinic receptor antagonist) are presented (means $\pm$ s.e.m.). A dashed data line with open symbols indicates that individuals have been removed following loss of equilibrium, as shown in C. The horizontal dashed line indicates temperatures where  $f_H$  is significantly different between atropine and saline groups (Holm-adjusted pairwise comparisons;  $\alpha=0.05$ ). The asterisks and gray lines in C indicate a significant difference in distributions between treatments (Cox regression;  $P<0.001$ ).

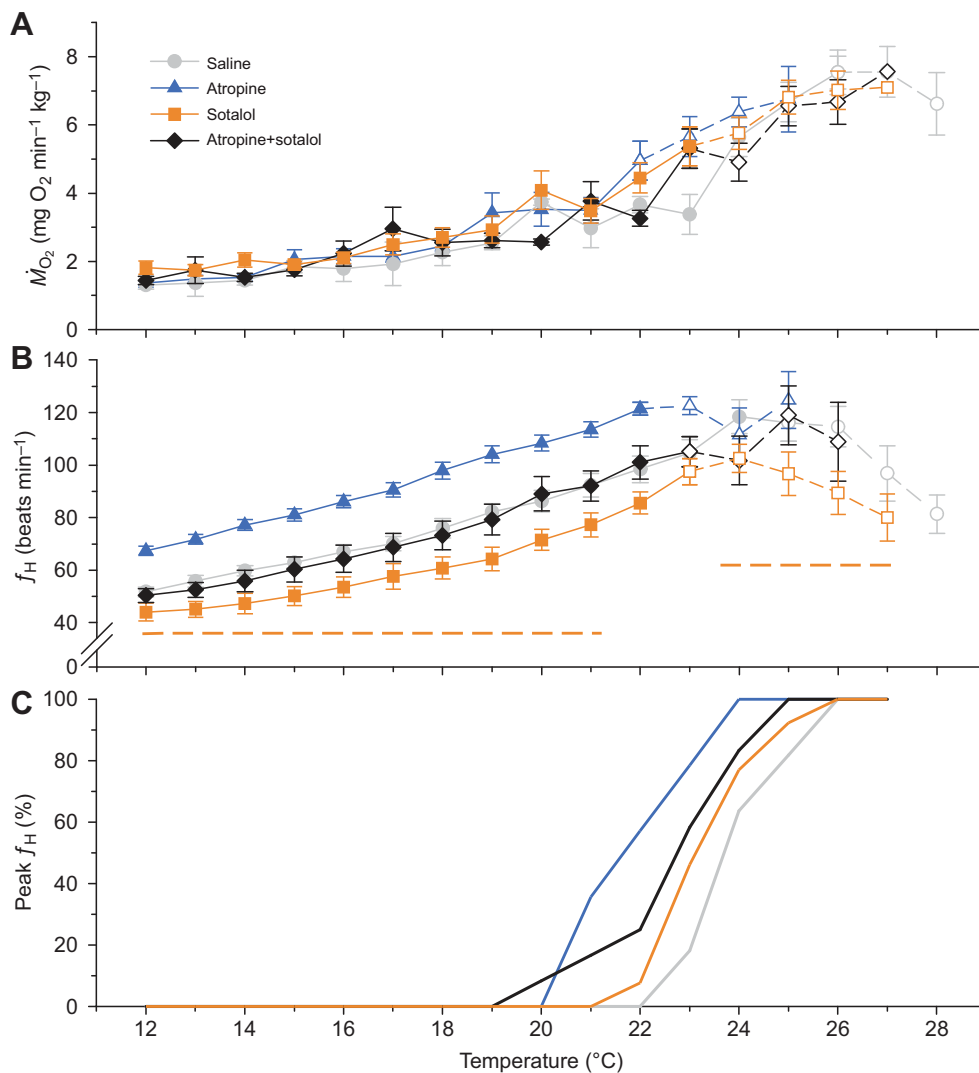
the heart may be cardio-protective during extreme warming (Aho and Vornanen, 2001; Eliason and Anttila, 2017; Eliason et al., 2011; Hanson and Farrell, 2007; Wood et al., 1979). However, Ekström et al. (2014) found no negative effects of autonomic blockade on cardiac or whole-organism thermal tolerance. In contrast, we found positive chronotropic and antiarrhythmic effects of  $\beta$ -adrenergic stimulation during acute warming of the *in situ* working heart preparation, and negative effects of *in vivo* autonomic blockade on both peak  $f_H$  and  $CT_{max}$ . As such, we argue below that autonomic regulation of  $f_H$  in rainbow trout can facilitate both cardiac and whole-organism thermal tolerance.

### $f_H$ , $\dot{M}_{O_2}$ and autonomic regulation over sub-critical temperatures

For commonly studied temperatures, our findings regarding  $f_H$ ,  $\dot{M}_{O_2}$  and autonomic tone align well with current literature. For example, the increase in routine  $f_H$  and  $\dot{M}_{O_2}$  that we observed with acute warming is well documented for salmonids, typically increasing with  $Q_{10}$  coefficients of between 1.5 and 2.5 for  $f_H$  and 2 and 4 for  $\dot{M}_{O_2}$ , as found here (Eliason and Anttila, 2017; Motyka et al., 2017; Penney et al., 2014; Rodnick et al., 2004; Steinhausen et al., 2008). While substantial variation in absolute  $\dot{M}_{O_2}$  and  $f_H$  values can exist among studies given variable genetic (e.g. between species and strains), environmental (e.g. thermal history) and methodological

considerations, notable similarities still exist between our results and previous ones. For instance, desert redband rainbow trout not only increased  $\dot{M}_{O_2}$  during acute warming from 14 to 26°C (1.96 to 6.86 mg O<sub>2</sub> kg<sup>-1</sup> min<sup>-1</sup>; Rodnick et al., 2004) with a quantitatively similar range to that seen here (1.63 to 7.24 mg O<sub>2</sub> kg<sup>-1</sup> min<sup>-1</sup>) but also exhibited a sharp transition at 24°C, as observed in the present study (Fig. 2A). Whether this sharp rise is related to an innate escape behavioral response to thermal stress, which has been documented previously (Peterson and Anderson, 1969), or a biochemical response is unknown. We also observed cholinergic and adrenergic cardiac tone and our estimates suggest they remained stable and approximately equal (~20–30% of intrinsic  $f_H$ ) up to near-critical temperatures, a suggestion supported by the fact that intrinsic and control  $f_H$  were nearly identical. Autonomic cardiac tone is normally calculated based on sequential injections of cholinergic and  $\beta$ -adrenergic antagonists (Altimiras et al., 1997), something that our study design precluded us from doing. Nevertheless, our estimates are within reported ranges for salmonids (i.e. 20–70% of intrinsic  $f_H$ ; Axelsson and Farrell, 1993; Ekström et al., 2016; Gamperl et al., 1995; Sandblom and Axelsson, 2011).

Beside the present study, only Ekström et al. (2014) have specifically investigated the role of autonomic regulation of  $f_H$  during acute warming to  $CT_{max}$ . At temperatures below  $CT_{max}$ , the quantitative similarities of the two studies are remarkable. For



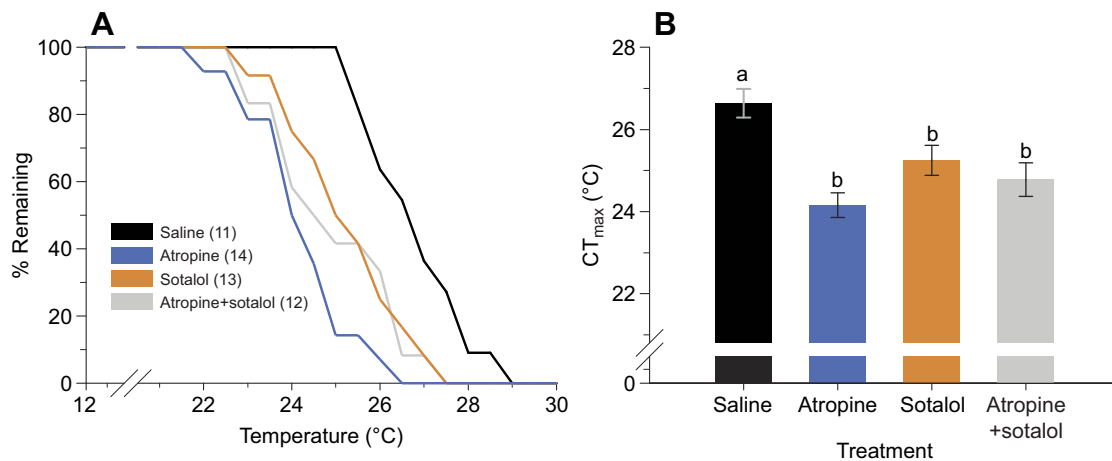
**Fig. 3. *In vivo*  $\dot{M}_{O_2}$ ,  $f_H$ , and temperature at 95% peak  $f_H$  and loss of equilibrium in rainbow trout during acute warming with  $\beta$ -adrenergic or dual blockade.** Data for (A)  $\dot{M}_{O_2}$ , (B)  $f_H$  and (C) 95% peak  $f_H$  in the presence of sotalol ( $\beta$ -adrenergic receptor antagonist,  $n=13$ ) or both atropine and sotalol (dual blockade,  $n=12$ ) are presented (means $\pm$ s.e.m.). Data for trout treated with saline or atropine (Fig. 2) are included for reference. A dashed data line with open symbols indicates that individuals have been removed following loss of equilibrium (Fig. 4A). The horizontal dashed line indicates temperatures where  $f_H$  is significantly different between atropine and saline groups (Holm-adjusted pairwise comparisons;  $\alpha=0.05$ ).

instance, atropine and sotalol treatment had a similar magnitude of effect on  $f_H$ , and  $f_H$  in all common treatments (saline, atropine and sotalol) increased with temperature in a nearly identical manner [e.g. atropine 12–20°C: 68 to 106 beats min<sup>-1</sup> for Ekström et al. (2014) versus 67 to 108 beats min<sup>-1</sup> in the present study]. A subtle difference between the two studies is that cholinergic tone appeared to increase at warm temperatures in Ekström et al. (2014) but was unchanged in the present study. Intrinsic  $f_H$  in the present study was similar to routine  $f_H$  and intermediate to that of atropine- and sotalol-treated fish in both studies, suggesting similar autonomic tone in the two studies even though Ekström et al. (2014) did not assess intrinsic  $f_H$ . Ekström et al. (2014) also did not measure routine  $\dot{M}_{O_2}$ , but instead measured ventilation rate and opercular pressure, both of which increased exponentially with temperature over sub-critical temperatures and were not affected by autonomic blockade, as was the case for  $\dot{M}_{O_2}$  in the present study. These similarities over moderate temperatures are highlighted to contrast the novelty of our findings at extreme warm temperatures regarding the cardio-protective role of autonomic regulation of  $f_H$ .

#### Cardio-protective effects of autonomic regulation at high temperatures

Previous *in situ* and *ex vivo* studies with rainbow trout have demonstrated the positive inotropic (Farrell et al., 1996; Shiels and

Farrell, 1997; Shiels et al., 2003) and chronotropic (Aho and Vornanen, 2001) effects of  $\beta$ -adrenergic stimulation (up to 22°C).  $\beta$ -Adrenergic stimulation is also known to be protective under adverse cellular conditions such as acidemia, hypoxemia and hyperkalemia (Hanson et al., 2006; Roberts and Syme, 2018), which are likely to occur *in vivo* at high temperatures. Furthermore, elevated  $\beta$ -adrenergic receptor density in sockeye salmon has been associated with improved aerobic performance at high temperatures (Eliason et al., 2011). However, the potential for direct  $\beta$ -adrenergic stimulation of the heart to protect against cardiac collapse with acute warming to critically warm temperatures has not previously been investigated. Here, we show that progressive  $\beta$ -adrenergic stimulation repeatedly rescued an *in situ* perfused working heart from warm-induced cardiac arrhythmia and increased peak  $f_H$  (+23%). Correspondingly,  $\beta$ -adrenergic blockade *in vivo* reduced the peak  $f_H$  attained during extreme acute warming (by 14–17%). Peak  $f_H$  of the perfused heart with maximal adrenergic stimulation was also similar to that observed *in vivo* in atropinized trout, suggesting that a similar stimulation may occur *in vivo* in the absence of cholinergic inhibition. Together, these results definitively show for the first time that  $\beta$ -adrenergic stimulation can facilitate achievement of peak  $f_H$  at critically warm temperatures, and is thus cardio-protective during extreme warming.



**Fig. 4. Thermal survival curves and  $CT_{max}$  for rainbow trout with and without autonomic blockade.** (A) Survival curves. (B)  $CT_{max}$ . Dissimilar letters indicate significant differences in  $CT_{max}$  between treatment groups (Holm-adjusted pairwise comparisons;  $\alpha=0.05$ ). Starting sample sizes for each treatment group are shown in parentheses.

Off-target effects of sotalol such as partial potassium channel blockade (Lynch et al., 2008) or actions on other aspects of circulatory physiology (e.g. blood pressure, vascular tone and red blood cell  $\beta$ -NHE activity) (Perry and Bernier, 1999; Sandblom and Axelsson, 2011) may have contributed, in part, to the reduction in peak  $f_H$  and  $CT_{max}$  observed with sotalol treatment. However, given the similarity in the change in peak  $f_H$  *in situ* and *in vivo* with  $\beta$ -adrenergic stimulation, and the fact that in mammals inhibition of  $K^+$  channels by sotalol does not typically occur until much higher doses than were used here (Lynch et al., 2008), we expect that the off-target effects of  $\beta$ -adrenergic blockade were marginal.

The relative  $\beta$ -adrenergic sensitivity of the rainbow trout heart tends to decrease with increasing temperature (Ask et al., 1981; Ekström et al., 2014; Eliason and Anttila, 2017; Keen et al., 1993; Sandblom and Axelsson, 2011; Shiels et al., 2003). For instance, Graham and Farrell (1989) found that for *in situ* perfused rainbow trout hearts, maximum adrenergic stimulation increased routine  $f_H$  and maximum  $\dot{Q}$  by 56% and 61% at 5°C, but by only 20% and 23% at 15°C. Both our study and that of Ekström et al. (2014) partially support this generalization, as we did not find a significant  $\beta$ -adrenergic tone at a critically warm temperatures, while  $\beta$ -adrenergic tone appeared to remain stable at sub-critical temperatures. Our study also suggests that while inotropic and chronotropic effects may be relatively diminished at high temperatures,  $\beta$ -adrenergic stimulation has a vital stabilizing role on heart function that was not captured in past assessments of  $\beta$ -adrenergic sensitivity. This stabilization does not directly increase  $f_H$  at critically warm temperatures but rather allows the heart to function at a higher temperature with a higher peak  $f_H$ . Thermal history must also be taken into consideration in discussions of  $\beta$ -adrenergic sensitivity (Eliason and Anttila, 2017). Many studies used to develop the generality that cardiac  $\beta$ -adrenergic sensitivity is lower at high temperatures have been conducted in warm-acclimated fish rather than acutely exposed fish. With acclimation to warm temperatures rainbow trout tend to down-regulate myocardial  $\beta$ -adrenoceptor expression (Gamperl et al., 1998; Keen et al., 1993). As such,  $\beta$ -adrenergic stimulation may not have the same cardio-protective effects at high temperatures after warm acclimation, and conversely may be relatively more important for acute cardiac heat tolerance following cold acclimation.

The collapse of  $f_H$  and loss of cardiac rhythmicity that occurs in fish at high temperatures is associated with a depression in ventricular excitability because the ECG often has delayed or

missing QRS complexes even though the P-wave tends to remain rhythmic (Badr et al., 2016; Casselman et al., 2012; Eliason and Anttila, 2017). Vornanen (2016) has attributed this depression to an imbalance in  $I_{Na}$  and  $I_{K1}$ . Although a direct mechanism by which  $\beta$ -adrenergic stimulation could circumvent or rectify this  $I_{Na}$  and  $I_{K1}$  imbalance has not been studied in fish, one possible linkage is through a depression of  $I_{K1}$ , which occurs in some mammals (Koumi et al., 1995). Indeed, the  $K^+$  channels responsible for generating  $I_{K1}$  in fish do have phosphorylation sites that could allow for  $\beta$ -adrenergically mediated modification (Hassinen et al., 2007; Vornanen, 2017).  $\beta$ -Adrenergic stimulation could also enhance rapid  $Ca^{2+}$  influx at relatively low membrane potentials through modification of T-type  $Ca^{2+}$  channels and the  $Na^+/Ca^{2+}$  exchanger (NCX), which could supplement  $I_{Na}$  and improve ventricular excitability at high temperatures. While this hypothesis will require further investigation, both T-type  $Ca^{2+}$  channels and the NCX are active in atrial and ventricular myocytes in some fish (Alday et al., 2014; Nemtsas et al., 2010; Vornanen, 2017). Furthermore,  $Ca^{2+}$  influx through NCX is enhanced with  $\beta$ -adrenergic stimulation in zebrafish (Xie et al., 2008), and in mammals,  $\beta$ -adrenergic stimulation enhances  $Ca^{2+}$  influx through both NCX and T-type  $Ca^{2+}$  channels, which contributes to cardiac excitation (Herrmann et al., 2013; Kaese et al., 2017; Li et al., 2012, 2018). Alternatively,  $Ca^{2+}$  kinetics may limit the ability of the ventricle to keep up with the pacemaker rate and an imbalance in  $I_{Na}$  and  $I_{K1}$  may not be to blame. If so, classical  $\beta$ -adrenergically mediated modification of  $Ca^{2+}$  cycling would account for the cardio-protective effects observed here.

As in Ekström et al. (2014), cholinergic tone in the present study persisted up to the point where  $f_H$  began to fail. This inhibitory action on pacemaker rate appeared necessary to sustain increasing  $f_H$  with temperature, as  $f_H$  began to peak at a lower temperature when cholinergic tone was blocked ( $-2^\circ\text{C}$ ). This limitation on  $f_H$  at high temperatures is unlikely to be a pacemaker limitation because Haverinen et al. (2017) showed that intrinsic pacemaker and sinoatrial rates continue to increase well above the temperature where  $f_H$  peaks, indicating the maximum pacemaker rate exceeds the maximum sustainable firing rate of atrial or, more likely, ventricular tissue (Haverinen et al., 2017). As such, we suggest that during extreme warming, cholinergic inhibition of pacemaker tissue acts as a brake to keep the pacemaker firing at a rate that is within the functional range of the ventricle. If the pacemaker rate did exceed

the physiological maxima of the ventricle, it would cause a progressive desynchrony in electrochemical events, which could account for the premature peak and decrease in  $f_H$  seen here with atropine treatment. Furthermore, the slowing of  $f_H$  through cholinergic inhibition would also allow for longer filling duration and blood residence time, which in turn could improve cardiac efficiency via the Frank–Starling mechanism and increase myocardial oxygenation (Eliason and Anttila, 2017; Farrell, 2007).

### Autonomic regulation facilitates $CT_{max}$

The decline in cardiac thermal tolerance that we observed in the absence of  $\beta$ -adrenergic stimulation and cholinergic inhibition was associated with a decrease in  $CT_{max}$ . Likewise, Ekström et al. (2017) found that coronary ligation reduced cardiac thermal tolerance and was also associated with a reduction in  $CT_{max}$ . It is important to note that in contrast to the present study, Ekström et al. (2014) did not find any reduction in  $CT_{max}$  with either atropine or sotolol treatment. However, given that they also did not identify any significant limitations in  $f_H$  with these treatments, this is not surprising. The mechanism by which a reduction in cardiac performance would reduce  $CT_{max}$  has not been explicitly tested. Yet, it is reasonable to postulate that one contributing factor would be impaired oxygen delivery to vital tissues. Given that maintaining equilibrium does not have a substantial aerobic cost, there is no *a priori* reason to expect that  $CT_{max}$  would necessarily be determined by oxygen availability. Instead, without additional stressors, loss of equilibrium at high temperatures may occur as a result of a direct temperature induced loss of nervous function (Friedlander et al., 1976) or some other indirect effect (e.g. acidosis). However, if this is the case, additional stressors (e.g. environmental hypoxia), compromised oxygen delivery (e.g. through coronary ligation) or elevated aerobic demand (e.g. elevated activity; Peterson and Anderson, 1969) could impose an oxygen limitation on  $CT_{max}$ , as was observed by Ern et al. (2017) when environmental  $P_{O_2}$  was reduced. As such, coronary ligation (Ekström et al., 2017) and autonomic blockade could sufficiently limit heart function and associated tissue oxygenation to reduce  $CT_{max}$ . Correspondingly, peak  $\dot{M}_{O_2}$  in all treatment groups in the present study tended to be lower than the control, although this difference was only significant when the two antagonists were applied together. That said, given that  $\dot{M}_{O_2}$  was not significantly greater in the control group at any given temperature, this reduction in peak  $\dot{M}_{O_2}$  may be a result of reduced  $CT_{max}$  rather than a direct cause. To test this, tissue oxygen supply and other aerobically sensitive measures of thermal tolerance (e.g. aerobic scope or swimming performance) should be tested with and without autonomic regulation in the future to determine the extent to which oxygen delivery is compromised at high temperatures in the absence of autonomic regulation.

### Conclusion

Our findings clearly demonstrate that, as previously hypothesized (Ekström et al., 2014; Eliason and Anttila, 2017; Farrell, 2007),  $\beta$ -adrenergic stimulation and cholinergic inhibition of the heart can facilitate acute cardiac and whole-organism heat tolerance. Autonomic regulatory physiology, however, varies extensively with thermal history as well as across genera, among closely related species and even between strains (Eliason et al., 2011; Gamperl et al., 1998; Sandblom and Axelsson, 2011; Sandblom et al., 2016b; Wood et al., 1979), which may explain why Ekström et al. (2014) did not find significant effects of autonomic blockade on cardiac or whole-organism heat tolerance whereas we did, despite using a similar methodology. Given these marked differences and the well-

documented diversity in autonomic regulatory physiology, further investigation is warranted to determine how cardiac autonomic regulation influences heat tolerance with environmental variation among teleosts.

### Acknowledgements

We thank the Drs Erika Eliason, Andreas Ekström and Erik Sandblom for their valuable feedback regarding this study at the 2017 Society for Experimental Biology meeting. We also thank the staff in the Zoology Workshop and Aquatics facility at the University of British Columbia for their assistance in the project.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: M.J.H.G., A.P.F.; Methodology: M.J.H.G., A.P.F.; Software: M.J.H.G.; Validation: M.J.H.G., V.R., S.M.M.; Formal analysis: M.J.H.G., V.R., S.M.M., A.P.F.; Investigation: M.J.H.G., V.R., S.M.M.; Resources: M.J.H.G.; Data curation: M.J.H.G.; Writing - original draft: M.J.H.G.; Writing - review & editing: M.J.H.G., A.P.F.; Visualization: M.J.H.G.; Supervision: M.J.H.G., A.P.F.; Project administration: M.J.H.G., A.P.F.; Funding acquisition: A.P.F.

### Funding

This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) through research grants to A.P.F., an Alexander Graham Bell (NSERC) Canadian Graduate Scholarship to M.J.H.G. and an NSERC Undergraduate Student Research Award to S.M.M.

### Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.194365.supplemental>

### References

- Aho, E. and Vornanen, M. (2001). Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol. B* **171**, 173–179. doi:10.1007/s003600000171
- Alday, A., Alonso, H., Gallego, M., Urrutia, J., Letamendia, A., Callol, C. and Casis, O. (2014). Ionic channels underlying the ventricular action potential in zebrafish embryo. *Pharmacol. Res.* **84**, 26–31. doi:10.1016/j.phrs.2014.03.011
- Altimiras, J., Aissaoui, A., Tort, L. and Axelsson, M. (1997). Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? *Comp. Biochem. Physiol. Part A* **118**, 131–139. doi:10.1016/S0300-9629(96)00402-1
- Anttila, K., Couturier, C. S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014). Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nat. Commun.* **5**, 4252. doi:10.1038/ncomms5252
- Ask, J., Stene-Larsen, G. and Helle, K. (1981). Temperature effects on the  $\beta$  2-adrenoceptors of the trout atrium. *J. Comp. Physiol.* **143**, 161–168.
- Axelsson, M. and Farrell, A. P. (1993). Coronary blood flow in vivo in the coho salmon (*Oncorhynchus kisutch*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **264**, R963–R971. doi:10.1152/ajpregu.1993.264.5.R963
- Badr, A., El-Sayed, M. F. and Vornanen, M. (2016). Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*. *J. Exp. Biol.* **219**, 1495–1504. doi:10.1242/jeb.138347
- Bellet, S. (1964). The drug treatment of complete A-V heart block. *Ann. N. Y. Acad. Sci.* **111**, 848–854. doi:10.1111/j.1749-6632.1964.tb53152.x
- Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.* **11**, 99–113. doi:10.1093/icb/11.1.99
- Campbell, H. A., Taylor, E. and Egginton, S. (2004). The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*. *J. Exp. Biol.* **207**, 1969–1976. doi:10.1242/jeb.00972
- Casselman, M. T., Anttila, K. and 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. doi:10.1111/j.1095-8649.2011.03182.x
- Chen, Z., Snow, M., Lawrence, C. S., Church, A. R., Narum, S. R., Devlin, R. H. and Farrell, A. P. (2015). Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). *J. Exp. Biol.* **218**, 803–812. doi:10.1242/jeb.113993
- Chung, D. J., Bryant, H. J. and Schulte, P. M. (2017). Thermal acclimation and subspecies-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (*Fundulus heteroclitus*). *J. Exp. Biol.* **220**, 1459–1471. doi:10.1242/jeb.151217



- Currie, S., Reddin, K., McGinn, P., McConnell, T. and Perry, S. F. (2008).  $\beta$ -adrenergic stimulation enhances the heat-shock response in fish. *Physiol. Biochem. Zool.* **81**, 414–425. doi:10.1086/589095
- Currie, S., Ahmady, E., Watters, M. A., Perry, S. F. and Gilmour, K. M. (2013). Fish in hot water: hypoxaemia does not trigger catecholamine mobilization during heat shock in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A* **165**, 281–287. doi:10.1016/j.cbpa.2013.03.014
- Drost, H. E., Lo, M., Carmack, E. C. and Farrell, A. P. (2016). Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *J. Exp. Biol.* **219**, 3114–3125. doi:10.1242/jeb.140194
- Ekström, A., Jutfelt, F. and Sandblom, E. (2014). Effects of autonomic blockade on acute thermal tolerance and cardioventilatory performance in rainbow trout, *Oncorhynchus mykiss*. *J. Therm. Biol.* **44**, 47–54. doi:10.1016/j.jtherbio.2014.06.002
- Ekström, A., Hellgren, K., Gräns, A., Pichaud, N. and Sandblom, E. (2016). Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout. *J. Exp. Biol.* **219**, 1106–1109. doi:10.1242/jeb.134312
- Ekström, A., Axelsson, M., Gräns, A., Brijs, J. and Sandblom, E. (2017). Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **312**, R549–R558. doi:10.1152/ajpregu.00536.2016
- Eliason, E. J. and Anttila, K. (2017). Temperature and the cardiovascular system. *Fish Physiol.* **36**, 235–297. doi:10.1016/bs.fp.2017.09.003
- Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109–112. doi:10.1126/science.1199158
- Eliason, E. J., Clark, T. D., Hinch, S. G. and Farrell, A. P. (2013). Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conserv. Physiol.* **1**, cot008. doi:10.1093/conphys/cot008
- Ern, R., Johansen, J. L., Rummer, J. L. and Esbaugh, A. J. (2017). Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. *Biol. Lett.* **13**, 20170135. doi:10.1098/rsbl.2017.0135
- Farrell, A. P. (2007). Tribute to P. L. Lutz: a message from the heart—why hypoxic bradycardia in fishes? *J. Exp. Biol.* **210**, 1715–1725. doi:10.1242/jeb.02781
- 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. doi:10.1242/jeb.023671
- Farrell, A. P. and Clutterham, S. M. (2003). On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *J. Exp. Biol.* **206**, 487–496. doi:10.1242/jeb.00100
- Farrell, A. P. and Smith, F. (2017). Cardiac form, function and physiology. *Fish Physiol.* **36**, 155–264. doi:10.1016/bs.fp.2017.07.001
- Farrell, A., MacLeod, K., Driedzic, W. and Wood, S. (1983). Cardiac performance in the in situ perfused fish heart during extracellular acidosis: interactive effects of adrenaline. *J. Exp. Biol.* **107**, 415–429.
- Farrell, A., MacLeod, K. and Chancey, B. (1986). Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. *J. Exp. Biol.* **125**, 319–345.
- Farrell, A. P., Johansen, J. A. and Graham, M. S. (1988). The role of the pericardium in cardiac performance of the trout (*Salmo gairdneri*). *Physiol. Zool.* **61**, 213–221. doi:10.1086/physzool.61.3.30161234
- Farrell, A., Gamperl, A., Hicks, J., Shiels, H. and Jain, K. (1996). Maximum cardiac performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their upper lethal limit. *J. Exp. Biol.* **199**, 663–672.
- Friedlander, M. J., Kotchabhakdi, N. and Prosser, C. L. (1976). Effects of cold and heat on behavior and cerebellar function in goldfish. *J. Comp. Physiol.* **112**, 19–45. doi:10.1007/BF00612674
- Fry, F. E. J. (1947). *Effects of the Environment on Animal Activity*. University of Toronto Press.
- Gamperl, A. K., Axelsson, M. and Farrell, A. P. (1995). Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **269**, R1258–R1266. doi:10.1152/ajpregu.1995.269.5.R1258
- Gamperl, A. K., Vijayan, M. M., Pereira, C. and Farrell, A. P. (1998).  $\beta$ -Receptors and stress protein 70 expression in hypoxic myocardium of rainbow trout and chinook salmon. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **274**, R428–R436. doi:10.1152/ajpregu.1998.274.2.R428
- Gesser, H. and Jørgensen, E. (1982). pH<sub>i</sub>, contractility and Ca-balance under hypercapnic acidosis in the myocardium of different vertebrate species. *J. Exp. Biol.* **96**, 405–412.
- Graham, M. S. and Farrell, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38–61. doi:10.1086/physzool.62.1.30159997
- Hanson, L. M. and Farrell, A. P. (2007). The hypoxic threshold for maximum cardiac performance in rainbow trout *Oncorhynchus mykiss* (Walbaum) during simulated exercise conditions at 18°C. *J. Fish Biol.* **71**, 926–932. doi:10.1111/j.1095-8649.2007.01533.x
- Hanson, L. M., Obradovich, S., Mouniargi, J. and 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. doi:10.1242/jeb.02237
- Hassinen, M., Pajananen, V., Haverinen, J., Eronen, H. and Vornanen, M. (2007). Cloning and expression of cardiac Kir2.1 and Kir 2.2 channels in thermally acclimated rainbow trout. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R2328–R2339. doi:10.1152/ajpregu.00354.2006
- Haverinen, J., Abramochkin, D. V., Kamkin, A. and Vornanen, M. (2017). Maximum heart rate in brown trout (*Salmo trutta fario*) is not limited by firing rate of pacemaker cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **312**, R165–R171. doi:10.1152/ajpregu.00403.2016
- Herrmann, S., Lipp, P., Wiesen, K., Stieber, J., Nguyen, H., Kaiser, E. and Ludwig, A. (2013). The cardiac sodium–calcium exchanger NCX1 is a key player in the initiation and maintenance of a stable heart rhythm. *Cardiovasc. Res.* **99**, 780–788. doi:10.1093/cvr/cvt154
- Iftikar, F. I., MacDonald, J. R., Baker, D. W., Renshaw, G. M. C. and Hickey, A. J. R. (2014). Could thermal sensitivity of mitochondria determine species distribution in a changing climate? *J. Exp. Biol.* **217**, 2348–2357. doi:10.1242/jeb.098798
- Kaese, S., Bögeholz, N., Pauls, P., Dechering, D., Olligs, J., Kölker, K., Badawi, S., Frommeyer, G., Pott, C. and Eckardt, L. (2017). Increased sodium/calcium exchanger activity enhances beta-adrenergic-mediated increase in heart rate: whole-heart study in a homozygous sodium/calcium exchanger overexpressor mouse model. *Heart Rhythm* **14**, 1247–1253. doi:10.1016/j.hrthm.2017.05.001
- Keen, J. E., Vianzon, D., Farrell, A. P. and Tibbitts, G. F. (1993). Thermal acclimation alters both adrenergic sensitivity and adrenoceptor density in cardiac tissue of rainbow trout. *J. Exp. Biol.* **181**, 27–48.
- Koumi, S., Wasserstrom, J. A. and Ten Eick, R. E. (1995). beta-adrenergic and cholinergic modulation of the inwardly rectifying K<sup>+</sup> current in guinea-pig ventricular myocytes. *J. Physiol.* **486**, 647–659. doi:10.1113/jphysiol.1995.sp020841
- Leo, E., Kunz, K. L., Schmidt, M., Storch, D., Pörtner, H.-O. and Mark, F. C. (2017). Mitochondrial acclimation potential to ocean acidification and warming of Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*). *Front. Zool.* **14**, 21. doi:10.1186/s12983-017-0205-1
- Li, Y., Wang, F., Zhang, X., Qi, Z., Tang, M., Szeto, C., Li, Y., Zhang, H. and Chen, X. (2012).  $\beta$ -Adrenergic stimulation increases Cav3.1 activity in cardiac myocytes through protein kinase A. *PLoS ONE* **7**, e39965. doi:10.1371/journal.pone.0039965
- Li, Y., Zhang, X., Zhang, C., Zhang, X., Li, Y., Qi, Z., Szeto, C., Tang, M., Peng, Y., Molkentin, J. D. et al. (2018). Increasing T-type calcium channel activity by  $\beta$ -adrenergic stimulation contributes to  $\beta$ -adrenergic regulation of heart rates. *J. Physiol.* **596**, 1137–1151. doi:10.1113/JP274756
- Lynch, J. J., III, Wilson, A. W., Hernandez, L. E., Nelson, R. A., Marsh, K. C., Cox, B. F., Mittelstadt, S. W. (2008). Dose-response effects of sotalol on cardiovascular function in conscious, freely moving cynomolgus monkeys. *Br. J. Pharmacol.* **154**, 1439–1445. doi:10.1038/bjp.2008.206
- Marshall, R. (1935). Treatment of heart-block. *Br. Med. J.* **2**, 75. doi:10.1136/bmj.2.3888.75
- Motyka, R., Norin, T., Petersen, L. H., Huggett, D. B. and Gamperl, A. K. (2017). Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*), but does not affect their upper thermal tolerance. *J. Therm. Biol.* **68**, 149–161. doi:10.1016/j.jtherbio.2016.03.007
- Nemtsas, P., Wettwer, E., Christ, T., Weidinger, G. and Ravens, U. (2010). Adult zebrafish heart as a model for human heart? An electrophysiological study. *J. mol. cell. cardiol.* **48**, 161–171. doi:10.1016/j.yjmcc.2009.08.034
- O'Brien, K. M., Rix, A. S., Egginton, S., Farrell, A. P., Crockett, E. L., Schlauch, K., Woolsey, R., Hoffman, M. and Merriman, S. (2018). Cardiac mitochondrial metabolism may contribute to differences in thermal tolerance of red- and white-blooded Antarctic notothenioid fishes. *J. Exp. Biol.* **221**, jeb177816. doi:10.1242/jeb.177816
- Penney, C. M., Nash, G. W. and Gamperl, A. K. (2014). Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. *Can. J. Fish. Aquat. Sci.* **71**, 1096–1105. doi:10.1139/cjfas-2013-0569
- Perry, S. F. and Bernier, N. J. (1999). The acute humoral adrenergic stress response in fish: facts and fiction. *Aquaculture* **177**, 285–295. doi:10.1016/S0044-8486(99)00092-7
- Peterson, R. H. and Anderson, J. M. (1969). Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, *Salmo salar*, acclimated to two temperatures. *J. Fish. Board Can.* **26**, 93–109. doi:10.1139/f69-008
- R Development Core Team. (2014). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2012. ISBN 3-900051-07-0.
- Roberts, J. C. and Syme, D. A. (2018). Effects of epinephrine exposure on contractile performance of compact and spongy myocardium from rainbow trout (*Oncorhynchus mykiss*) during hypoxia. *Fish Physiol. Biochem.* **44**, 49–62. doi:10.1007/s10695-017-0412-x

- Rodnick, K. J., Gamperl, A. K., Lizars, K. R., Bennett, M. T., Rausch, R. N. and Keeley, E. R. (2004). Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *J. Fish Biol.* **64**, 310-335. doi:10.1111/j.0022-1112.2004.00292.x
- Sandblom, E. and Axelsson, M. (2011). Autonomic control of circulation in fish: a comparative view. *Auton. Neurosci.* **165**, 127-139. doi:10.1016/j.autneu.2011.08.006
- Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T. and Jutfelt, F. (2016a). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nat. Commun.* **7**, 11447. doi:10.1038/ncomms11447
- Sandblom, E., Ekström, A., Brijs, J., Sundström, L. F., Jutfelt, F., Clark, T. D., Adill, A., Aho, T. and Gräns, A. (2016b). Cardiac reflexes in a warming world: thermal plasticity of barostatic control and autonomic tones in a temperate fish. *J. Exp. Biol.* **219**, 2880-2887. doi:10.1242/jeb.140319
- Shiels, H. and Farrell, A. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca<sup>2+</sup> to force development in isolated ventricular trabeculae from rainbow trout. *J. Exp. Biol.* **200**, 1607-1621.
- Shiels, H. A., Vornanen, M. and Farrell, A. P. (2003). Acute temperature change modulates the response of I<sub>Ca</sub> to adrenergic stimulation in fish cardiomyocytes. *Physiol. Biochem. Zool.* **76**, 816-824. doi:10.1086/378918
- Steinhausen, M. F., Sandblom, E., Eliason, E., Verhille, C. and 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. doi:10.1242/jeb.019281
- Svendsen, M. B. S., Bushnell, P. G. and Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *J. Fish Biol.* **88**, 26-50. doi:10.1111/jfb.12797
- Vornanen, M. (2016). The temperature dependence of electrical excitability in fish hearts. *J. Exp. Biol.* **219**, 1941-1952. doi:10.1242/jeb.128439
- Vornanen, M. (2017). Electrical excitability of the fish heart and its autonomic regulation. *Fish Physiol.* **36**, 99-153. doi:10.1016/bs.fp.2017.04.002
- Vornanen, M., Haverinen, J. and Egginton, S. (2014). Acute heat tolerance of cardiac excitation in the brown trout (*Salmo trutta fario*). *J. Exp. Biol.* **217**, 299-309. doi:10.1242/jeb.091272
- Wood, C. M., Pieprzak, P. and Trott, J. N. (1979). The influence of temperature and anaemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. *Can. J. Zool.* **57**, 2440-2447. doi:10.1139/z79-316
- Xie, Y., Ottolia, M., John, S. A., Chen, J.-N. and Philipson, K. D. (2008). Conformational changes of a Ca<sup>2+</sup>-binding domain of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger monitored by FRET in transgenic zebrafish heart. *Am. J. Physiol. Cell Physiol.* **295**, C388. doi:10.1152/ajpcell.00178.2008

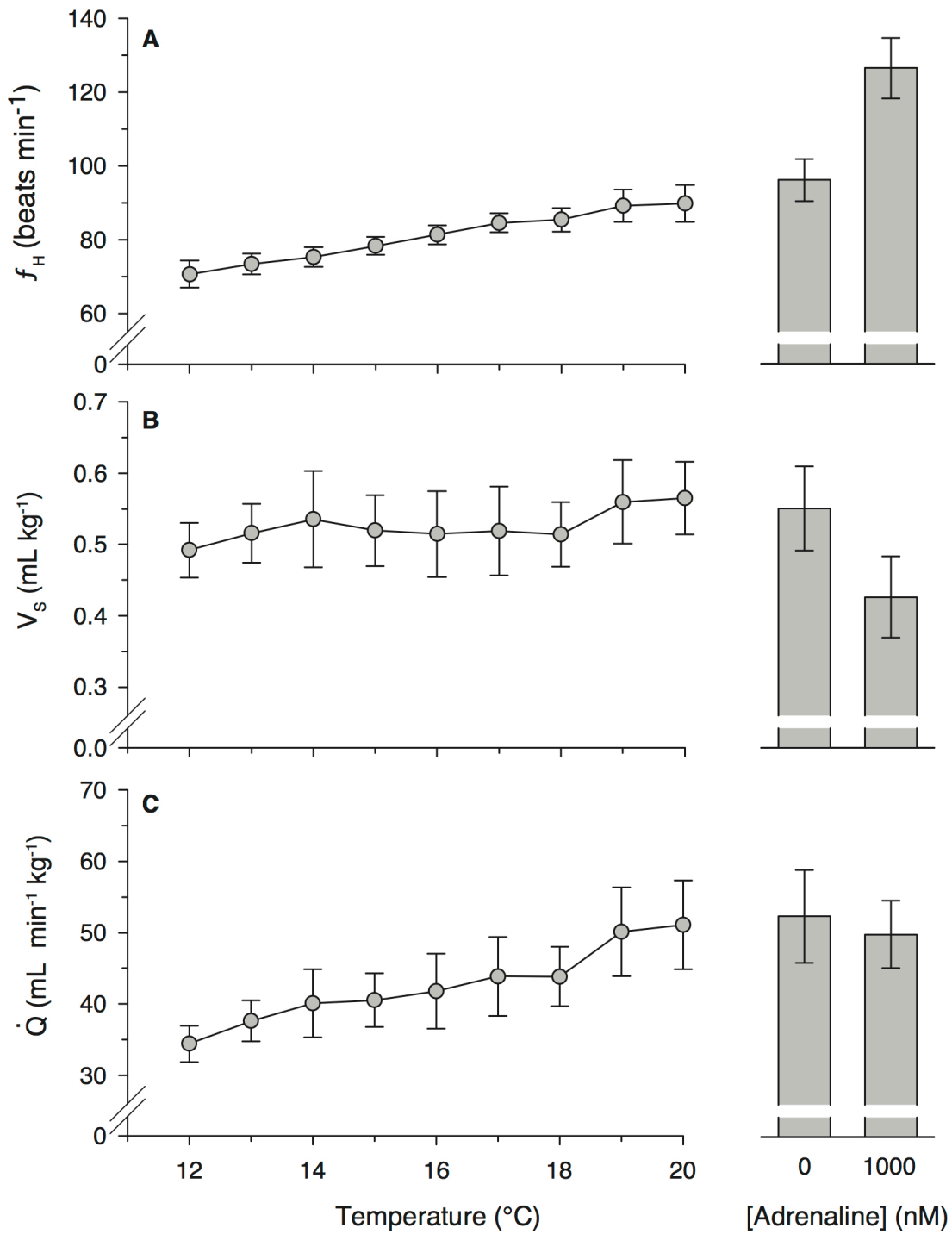


Figure S1. Heart rate ( $f_H$ ;A) stroke volume ( $V_s$ ;B) and cardiac output ( $\dot{Q}$ ;C) during acute warming to arrhythmia in an in situ perfused heart preparation ( $n=8$ ). Bar graphs show values at arrhythmia with and without maximal adrenergic stimulation. Flow measurements for  $V_s$  and  $\dot{Q}$  have not been temperature corrected.

## Table S1

[Click here to Download Table S1](#)