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Cardiac reflexes in a warming world: Thermal plasticity of barostatic control and autonomic tones in a temperate fish

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Abstract

Thermal plasticity of cardiorespiratory function allows ectotherms like fish to cope with seasonal temperature changes and is critical for resilience to climate change. Yet, the chronic thermal effects on cardiovascular homeostatic reflexes in fish are little understood although this may have important implications for physiological performance and overall resilience to climate warming. We compared cardiac autonomic control and baroreflex regulation of heart rate in perch (*Perca fluviatilis* L.) from a reference area in the Baltic Sea at 18-19°C with conspecifics from the 'Biotest enclosure', a chronically heated ecosystem receiving warmed effluent water (24-25°C) from a nuclear power plant. Resting heart rates of Biotest fish displayed clear thermal compensation and were 58.3±2.3 beats min^{-1} compared with 52.4±2.6 beats min^{-1} in reference fish at their respective environmental temperatures (Q_{10}: 1.2). The thermally-compensated heart rate of Biotest fish was a combined effect of elevated inhibitory cholinergic tone (105% in Biotest fish versus 70% in reference fish) and reduced intrinsic cardiac pacemaker rate. A barostatic response was evident in both groups, as pharmacologically-induced increases and decreases in blood pressure resulted in atropine-sensitive bradycardia and tachycardia, respectively. Yet, the tachycardia in Biotest fish was significantly greater, presumably due to the larger scope for vagal release. Acclimation of Biotest fish to 18°C for 3 weeks abolished differences in intrinsic heart rate and autonomic tones, suggesting considerable short-term thermal plasticity of cardiovascular control in this species. The heightened hypotensive tachycardia in Biotest perch may represent an important mechanism of ectothermic vertebrates that safeguards tissue perfusion pressure when tissue oxygen demand is elevated by environmental warming.

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Introduction

Fish represent the most thermally diverse ectothermic vertebrate group on Earth. Indeed, the group encompasses species that have evolved to function in extreme thermal environments ranging from sub-zero polar waters to warm hydrothermal springs and desert lakes, and some species experience seasonal temperature variations exceeding 30°C (Gamperl, 2011). Thermal acclimation (i.e. reversible phenotypic plasticity) of metabolism and cardiovascular function is crucial for fish and other ectothermic animals to cope with seasonal temperature changes and may be critical for resilience to climate warming (Sandblom et al., 2014; Schulte et al., 2011; Seebacher et al., 2015). However, while cardiac function is known to be important for thermal tolerance and overall performance in fish over relatively short timescales (Clark et al., 2008; Ekström et al., 2014; Farrell et al., 2009; Somero, 2011), the consequences of chronic temperature changes on circulatory reflexes and cardiac neuroendocrine control have not been thoroughly examined.

The barostatic reflex (i.e. the baroreflex) is a fundamental homeostatic cardiovascular mechanism in vertebrates, with the overall function to buffer changes in arterial blood pressure (Bagshaw, 1985; Jones and Milsom, 1982; Van Vliet and West, 1994). In fish, the gill vasculature is generally thought to be the main barosensitive area (Ristori, 1970; Ristori and Dessaux, 1970; Sundin and Nilsson, 2002), and at least in teleosts the efferent pathway of the reflex involves both a cardiac and a vascular limb (Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2011). The cardiac limb responds to baroreceptor stimulation (i.e. arterial hypertension) by reducing heart rate (f_H) and cardiac output through cholinergic vagal inhibition of the heart. Conversely, baroreceptor unloading (i.e. arterial hypotension) results in reduced vagal tone causing tachycardia and increased cardiac output. The vascular limb of the reflex primarily affects arterial resistance and venous capacitance

through changes in α-adrenergic vasomotor tone (Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2011).

During acute temperature changes that fish experience within their normal thermal range, f_H and cardiac output typically increase 2- to 3-fold along with metabolic rate for every 10°C increase in temperature (i.e. a Q_{10} of 2-3) (Clark et al., 2008; Dillon et al., 2010; Farrell et al., 2009). However, thermal acclimation can reset and lower f_H under chronically heated conditions (Ekström et al., 2016; Gamperl, 2011; Gamperl and Farrell, 2004). This is primarily achieved by two principal mechanisms. Firstly, the intrinsic heart rate (f_{Hintr}), which is the spontaneous depolarisation rate of the cardiac pacemaker irrespective of extrinsic hormonal or neural input, is reduced through remodelling of cellular attributes like pacemaker ion channel density and function (Aho and Vornanen, 2001; Bowler and Tirri, 1990; Harper et al., 1995; Haverinen and Vornanen, 2007). Secondly, the inhibitory cholinergic tone on the heart increases with temperature in some species, which depresses resting heart rate further (Ekström et al., 2016; Sandblom et al., 2016; Seibert, 1979; Sureau et al., 1989; Taylor et al., 1977). This latter adjustment could have important consequences for the chronotropic barostatic response as it may increase the scope for tachycardia through release of vagal tone during metabolically demanding situations and arterial hypotension. However, it is also possible that a higher routine cholinergic tone with warm acclimation may blunt the ability to depress heart rate through increased vagal tone during hypertensive events, but to our knowledge the effects of chronic temperature changes on barostatic reflexes have not been systematically examined in any ectothermic vertebrate. This lack of information is unfortunate as the thermal effects on cardiovascular reflexes may have important implications when examining the effects of ongoing climate warming on ectothermic animals like fish, as well as to decipher how homeostatic physiological mechanisms are affected by seasonal temperature changes.

In the present study, we took advantage of a more than 30-year-old, large-scale thermal experiment called the Biotest enclosure. This 90-hectare enclosure is located at the Baltic Sea coast adjacent to the nuclear power plant in Forsmark, Sweden. Heated cooling water effluents from two of the nuclear reactors have been pumped into the Biotest enclosure at a rate of 90 m^3 s^{-1} since the early 1980s, maintaining the water temperature approximately 5-10°C warmer than the surrounding archipelago (see table 1 and Hillebrand et al., 2010; Sandström et al., 1997; Sandström et al., 1995). European perch (*Perca fluviatilis* L.) inhabit this enclosure and the surrounding archipelago, both of which experience seasonal temperature fluctuations. Thus, this system presents a unique opportunity to study physiological responses of fish under chronically warm field conditions relevant to climate change. Previous studies have shown that the Biotest fish have smaller heart ventricles and reduced temperature-specific metabolic rates and heart rates relative to conspecifics from the surrounding areas (Sandblom et al., 2016). While the latter is due to both an increased cholinergic tone on the heart and reduced intrinsic heart rate, it is presently unclear how cardiorespiratory reflexes are affected and whether genetic differences (i.e. adaptation) and/or phenotypic plasticity underlies these modifications.

We hypothesised that the contrasting thermal environments experienced by reference and Biotest perch that has altered cardiac neurohumoral control, would affect cardiac reflex responses to changes in blood pressure. To test this, we characterised the cardiac limb of the baroreflex in fish from both sites using a closed-loop approach and pharmacological agonists to manipulate arterial blood pressure (see Altimiras et al., 1998). We also determined cardiac autonomic tones and intrinsic heart rates in reference and Biotest fish at their current environmental temperatures, as well as after 3-4 weeks of acclimation to reference thermal conditions to examine the thermal plasticity of cardiac control mechanisms (Altimiras et al., 1997; Sandblom et al., 2009).

Materials and methods

Study area and experimental animals

The Biotest enclosure has been described in greater detail elsewhere (Hillebrand et al., 2010; Sandblom et al., 2016; Sandström et al., 1995). Environmental thermal conditions are summarised in table 1. Adult perch (*Perca fluviatilis* L.) of mixed sexes were caught using hook and line throughout August 2013. Details on experimental fish body mass (M_b) and temperature conditions in specific experimental series are presented in table 2. Biotest fish were obtained from the inlet into the Biotest enclosure (i.e. downstream of the nuclear power plant), and reference fish living under a natural thermal regime were caught in the cooling water intake channel (i.e. upstream of the nuclear power plant). Fish were transported by car in holding tanks with aerated Baltic seawater to a laboratory facility located at the Biotest enclosure, where they were anaesthetised in Baltic seawater with tricaine methanesulfonate (MS-222 100 mg L^{-1}; Western Chemical Inc., Ferndale, WA, USA) and injected with a PIT tag in the abdominal cavity for individual identification. Fish were then kept at their respective environmental temperatures (see table 2) in 1200 L outdoor tanks with flow-through aerated seawater until experimentation. Following capture and PIT tagging, fish were allowed at least three days of recovery prior to experimentation and were not fed whilst in captivity.

To determine thermal acclimation capacity of cardiac control systems, a subset of fish from both locations were treated identically, but acclimated for 3-4 weeks to reference thermal conditions (see Experimental series 2 in table 2). In this experimental series, fish were fed daily with freshly killed bleak (*Alburnus alburnus*) up until 24 h prior to instrumentation. All tanks were partly covered and the photoperiod followed ambient conditions. Ethical permit #65-2012 covered all experimental procedures.

Surgery and experimental protocols

Fish were anaesthetised in aerated Baltic seawater containing MS-222 (100 mg L^{-1}) and placed on a surgery table covered with damp foam where the gills were continuously irrigated with aerated re-circulating seawater (~8°C) with MS-222 (50 mg L^{-1}). Following surgery, fish were allowed 24 h of recovery. Two separate experimental groups were instrumented as follows and subjected to the following experimental protocols:

Experimental series 1: Barostatic reflexes and cardiac autonomic control

Barostatic reflexes and cardiac autonomic control were studied in reference and Biotest fish at their respective acclimation temperatures (see table 2). Fish were cannulated with a PE-31 catheter (OD: 0.80 mm; Natsume, Tokyo, Japan) filled with heparinized (20 IU ml^{-1}) 0.9% saline that was occlusively inserted into the third afferent branchial artery until the tip was located close to the bifurcation of the ventral aorta (Axelsson and Fritsche, 1994). Correct placement was verified by positive pressure and unhindered withdrawal of blood. The catheter was attached with two silk sutures around the branchial arch and one in the skin.

During the experiments, fish were held individually in black-box type chambers (length: 440 mm; width: 100 mm; depth: 135 mm) to minimise visual stimuli. The chambers were supplied with aerated flow-through Baltic seawater at their respective acclimation temperatures (see table 2). Ventral aortic blood pressure (P_{VA}) and heart rate (f_H) were first recorded continuously for several hours to ensure stable resting values. Fish were then subjected to a pharmacological protocol to determine baroreflex responses and autonomic control of the heart as follows. Sodium nitroprusside (SNP, Sigma, St Louis, MO, USA) at concentrations of 5, 10, 25, 50 and 100 μg kg M_b^{-1} was injected via the arterial catheter to lower blood pressure, and phenylephrine (PE, Sigma, St Louis, MO, USA) at concentrations of 5, 10, 20, 30 and 60 μg kg M_b^{-1} was injected to increase blood pressure (Altimiras et al.,

1998). The order of the drug concentrations was randomized and hemodynamic variables always returned to pre-injection values before a new injection was administered. The drug concentrations were chosen based on the responses seen in preliminary experiments, whereby the highest concentrations for each drug had elicited maximal heart rate responses. All doses were injected as 0.5 ml kg^{-1} boluses followed by 0.3 ml saline to flush the catheter. Maximum f_H and P_{VA} responses following drug injection were recorded. Atropine sulphate (1.2 mg kg M_b^{-1}; Sigma, St Louis, MO, USA) was then injected to block muscarinic receptors and cardiovascular variables were allowed to stabilize for at least 30 min. The highest doses of SNP and PE as described above were then injected into the atropinized fish. Subsequently, propranolol (3 mg kg M_b^{-1}; Sigma, St Louis, MO, USA) was injected to block β-adrenergic receptors and cardiovascular variables were again allowed to stabilize for at least 30 min before recordings were taken. The heart rate after complete autonomic blockade reflects the intrinsic heart rate (Altimiras et al., 1997; Sandblom et al., 2009). Additionally, in some Biotest fish the f_{Hintr} was determined after the temperature had been reduced in the holding chamber over ~1 h to reference temperature to allow comparison of f_{Hintr} at a common temperature (see table 2).

Experimental series 2: Cardiac control after thermal acclimation to reference temperature

The autonomic control of heart rate was determined in fish from both sites that had been acclimated to reference thermal conditions for 3-4 weeks (see table 2). In this experimental group dual teflon-coated ECG electrodes (Cooner Wire, Chatsworth, CA, USA) were placed subcutaneously ventral to the heart and the position of the electrodes were adjusted on the surgery table to optimize the strength and quality of the ECG signal (Sandblom et al., 2010). Correct electrode placement was verified on the surgery table by briefly interrupting the water flow over the gills, which rapidly triggered a hypoxic bradycardia. A saline-filled PE-50

catheter (OD: 0.96 mm; Intramedic Clay Adams Brand, Becton Dickinson and Company, NJ, USA) was implanted into the abdominal cavity for drug administration using an injection needle to pierce the abdominal wall, taking care not to damage internal organs (Sandblom et al., 2010). The electrodes and the catheter were attached to the skin with two silk sutures.

During experiments, fish were held individually as described above at reference thermal conditions (see table 2). ECG signals were first recorded for several hours to ensure stable baseline recordings. Atropine sulphate (1.2 mg kg M_b^{-1}; Sigma, St Louis, MO, USA) was then injected via the abdominal catheter to block muscarinic receptors. The heart rate was allowed to stabilize at the new higher value for approximately 1 h. Subsequently, propranolol (3 mg kg M_b^{-1}; Sigma, St Louis, MO, USA) was injected to block β-adrenergic receptors and cardiovascular variables were again allowed to stabilize for at least 1 h. Finally, the temperature was increased to Biotest thermal conditions to allow determination of f_{Hintr} at this higher temperature (see table 2). Following the experiments, fish were euthanised with a sharp cranial blow.

Data acquisition and analysis

Ventral aortic blood pressure was recorded by connecting the catheters to DPT-6100 pressure transducers (pvb Medizintechnik, Kirchseeon, Germany) calibrated against a static water column with the water surface in the experimental tank serving as a zero pressure reference. The signals from the pressure transducers were amplified using a 4ChAmp pre-amplifier (Somedic, Hörby, Sweden). Raw ECG signals were recorded by connecting the ECG electrodes to bio-amplifiers (model ML136, ADInstruments, Castle Hill, Australia). Recordings were performed at 200 Hz with the electric ground placed in the surrounding water with the amplifiers set in EEG mode. The signals from the amplifiers were directed to a PowerLab system (ADInstruments, Castle Hill, Australia) connected to a PC running

LabChart Pro 7 acquisition software. Heart rate was calculated from the pulsatile blood pressure records using the blood pressure module in LabChart Pro, or from the ECG recordings using the ECG module in LabChart Pro. Environmental temperature values for 2013 were obtained from the nuclear power plant in Forsmark and are based on daily mean temperature records at approximately 2 m depth in the cooling water intake channel (Reference) and at the centre of the Biotest enclosure (Biotest) (see table 1). Holding tank temperatures were recorded throughout the experimental period using Testo 175-T3 temperature loggers (Testo AG, Lenzkirch, Germany) and expressed as daily means (see table 2).

To estimate sensitivity to the vasoactive drugs in untreated fish (i.e. before atropine or propranolol treatment), slopes were compared after fitting linear regressions to heart rate (beats kg M_b min^{-1} μg^{-1}) and blood pressure (cm H_2O kg M_b μg^{-1}) data points for individual fish obtained in response to all doses of SNP and PE. Similarly, after atropine, linear regressions were fitted to the data points after atropine alone and the high concentrations of SNP and PE. Cardiac autonomic tones (%) were determined using the heart beat interval [interval = $(60/f_H)$] as described by Altimiras et al. (1997). Cholinergic and adrenergic tones were calculated using equations 1 and 2, respectively:

(1) $$cholinergic\ tone = \frac{Interval_{cont} - Interval_{atr}}{interval_{intr}}$$

(2) $$adrenergic\ tone = \frac{Interval_{intr} - Interval_{atr}}{interval_{intr}}$$

Where $interval_{cont}$ is the heart beat interval in untreated fish, $interval_{atr}$ is the heart beat interval after atropine treatment and $interval_{intr}$ is the intrinsic heart beat interval after additional propranolol treatment (i.e. full autonomic blockade).

Statistics

Statistical analyses were performed in SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). For data only including one factor, Student t-tests were used for comparisons between experimental groups. For data including two factors (i.e. intrinsic heart rate in different experimental groups at two water temperatures), a linear mixed model with individuals as subject variables and water temperature as the repeated variable was used. Water temperature and experimental groups were included as fixed effects and we used 'unstructured' as covariance structure as this gave the best fit to the data (i.e. lowest Akaike's Information Criterion). If significant main effects were observed, these were further explored using pair-wise comparisons of the variable at both water temperatures. When multiple testing was performed p-values were adjusted using the Bonferroni method. Statistical significance was accepted at $p \leq 0.05$. Values are reported as means ± SEM unless otherwise stated.

Results

Routine cardiovascular status

Resting heart rates were 52.4±2.6 and 58.3±2.3 beats min^{-1} in reference and Biotest fish at their respective environmental temperatures (n=11 and 14 for reference and Biotest fish, respectively). These values were not significantly different (df=23, p=0.11) and represented a Q_{10} value of 1.2, suggesting considerable thermal compensation of resting heart rate (Fig. 1a). Similarly, routine blood pressures did not differ (df=23, p=0.35) and were 49.7±1.6 and 51.9±1.6 cm H_2O in reference and Biotest fish, respectively (Fig. 1b).

Effects of environmental temperature on barostatic reflexes

SNP and PE injections triggered a clear dose-dependent chronotropic barostatic response in both Biotest and reference fish at their respective environmental temperatures (n=10 and 13 for reference and Biotest fish, respectively; Fig. 1a). However, the tachycardic response to SNP was significantly more pronounced in Biotest compared to reference fish (slopes: 0.37±0.07 and 0.18±0.04 beats kg M_b min^{-1} μg^{-1}, respectively; df=21, p=0.016; Fig. 1a), although SNP only reduced ventral aortic blood pressure slightly in both groups (slopes: -0.07±0.01 and -0.10±0.01 cm H_2O kg M_b μg^{-1} for reference and Biotest fish, respectively; df=21, p=0.38; Fig. 1b). The bradycardia response following injection of PE did not differ significantly between groups (slopes: -0.28±0.08 and -0.19±0.03 beats kg M_b min^{-1} μg^{-1} for reference and Biotest fish, respectively; df=21, p=0.29; Fig. 1a), and caused a similar hypertensive response in both groups (slopes: 0.26±0.01 and 0.29±0.03 cm H_2O kg M_b μg^{-1} for reference and Biotest fish respectively, df=21, p=0.29; Fig. 1b).

Effects of environmental temperature on intrinsic heart rate and cardiac autonomic tones

Atropine treatment resulted in elevated resting f_H in both acclimation groups (n=8 and 13 for reference and Biotest fish, respectively), and following the injection f_H was significantly greater in Biotest compared to reference fish (129.9±3.0 versus 91.5±1.5 beats min^{-1}; df=19, p<0.0001; Fig.1c). While atropine did not affect resting blood pressure (df=19, p=0.52; Fig.1d), atropinisation abolished all heart rate responses to the highest doses of SNP and PE in both experimental groups (df=19, p>0.05; Fig. 1c).

Additional propranolol treatment revealed that Biotest fish (n=9) had a significantly higher intrinsic heart rate than reference fish (n=6) at their respective environmental temperatures (108.1±3.2 versus 81.7±2.5 beats min^{-1}, df=19, p<0.0001; Fig. 2). However, when Biotest fish were acutely lowered to reference temperature (i.e. 18-19°C) after complete autonomic blockade, the intrinsic heart rate was significantly lower compared to the intrinsic heart rate in reference fish at that temperature (65.1±2.1 versus 81.7±2.5 beats min^{-1}, df=19, p<0001; Fig. 2). Similarly, when reference fish where acutely increased to Biotest temperature (24-25°C) after complete autonomic blockade, the same relationship remained with a significantly lower intrinsic heart rate in Biotest fish (108.1±3.2 versus 125.4±7.0 beats min^{-1}, df=19, p<0001; Fig. 2).

When calculating the relative autonomic tones on the heart of Biotest (n=14) and reference (n=11) fish at the respective environmental temperatures, both cholinergic and adrenergic tones of Biotest fish were significantly higher compared to reference fish (104.8±6.5 versus 69.7±9.3%, df=23, p=0.004 and 16.0±1.4 versus 8.2±1.2%; df=23, p<0.0001 for cholinergic and adrenergic tones, respectively; Fig. 3). Thus, the compensatory reduction in resting heart rate of Biotest fish at the higher environmental temperature (see Fig. 1a) was a combined effect of reduced intrinsic heart rate and an elevated inhibitory cholinergic tone.

Cardiovascular plasticity following short-term thermal acclimation

There were no significant differences in resting heart rates, intrinsic heart rates or cardiac autonomic tones between the reference fish in experimental series 2 (n=6) that were exposed to 3-4 weeks of acclimation to reference thermal conditions in the lab (i.e. resting heart rate: 53.0±4.9 beats min^{-1}, intrinsic heart rate: 125.4±7.0 beats min^{-1}, cholinergic tone: 73±11%, adrenergic tone: 12±1%) and the reference fish tested immediately in experimental series 1 (see Figs. 1 and 2). Thus, for illustrative purposes only Biotest fish acclimated to reference temperature for 3-4 weeks (see table 2) were compared to the reference fish from the first series to examine the short-term thermal plasticity of heart rate and autonomic control in Figs. 2 and 3.

Indeed, the Biotest fish acclimated to reference conditions (n=7) displayed complete thermal remodelling of cardiovascular function because when tested at reference temperature there was no difference in resting heart rate compared with reference fish (n=11) (52.4±2.6 versus 54.6±5.1 beats min^{-1} for reference and Biotest fish, respectively; df=16, p=0.68). Similarly, the intrinsic heart rate did not differ between Biotest fish acclimated to reference temperature and reference fish regardless of test temperature (80.8±2.4 versus 81.7±2.5 beats min^{-1} at reference temperature; df=19, p=1.0 and 119.6±5.0 versus 125.4±7.0 beats min^{-1} at Biotest temperature; df=19, p=1.0; Fig. 2). The complete thermal remodelling of the Biotest fish acclimated to reference temperature was also evident in the cholinergic and adrenergic tones (n=7), which were 63.6±9.8 and 9.3±1.0%, respectively and did not differ from the tones (n=11) of the reference fish (df=17, p=0.67 and p=0.53 for cholinergic and adrenergic tones, respectively; Fig. 3).

Discussion

Temperature effects on barostatic reflexes in fish and other ectotherms

The efferent cardiac branch of the barostatic reflex appears to be an evolutionarily ancient circulatory trait ubiquitous to all jawed vertebrates including fish (Bagshaw, 1985; Sandblom and Axelsson, 2011). While no previous study has explicitly examined the effects of temperature on baroreflex responses in fish, there is some information for other ectothermic vertebrates. Hagensen et al. (2010) used a similar pharmacological protocol as in the present study in broad nosed caiman (*Caiman latirostris*) and noted that the tachycardia following SNP was greater when acutely warmed from 15 to 30°C. Similar patterns were observed in tilt experiments on restrained tiger snakes (*Notechis scutatus*) at different acutely altered temperatures (Lillywhite and Seymour, 1978), and after short-term (<2.5 weeks) acclimation to 13 and 3°C in red-eared sliders (*Trachemys scripta*) (Crossley et al., 2015). Nonetheless, to our knowledge, this study shows for the first time in fish that the cardiac limb of the baroreflex is strongly affected by chronic (i.e. >2-3 weeks) changes in environmental temperature.

When examined at their respective environmental temperatures, routine heart rate and blood pressure were similar in reference and Biotest fish (Fig 1). Consistent with previous studies on fish, the compensatory heart rate reduction in Biotest perch was explained both by a higher inhibitory cholinergic tone (Ekström et al., 2016; Seibert, 1979; Sureau et al., 1989; Taylor et al., 1977), as well as a reduced intrinsic pacemaker rate (Aho and Vornanen, 2001; Bowler and Tirri, 1990; Ekström et al., 2016; Harper et al., 1995; Haverinen and Vornanen, 2007) (see Figs. 2 and 3). Indeed, the pronounced thermal compensation of heart rate between acclimation groups observed here (Q_{10}=1.2) agrees with recent previous studies

on Biotest perch (Sandblom et al., 2016). Even so, the tachycardia following SNP was more pronounced in Biotest fish despite the blood pressure response being similar between groups (see Fig. 1). Since atropine abolished virtually all chronotropic responses, the most parsimonious explanation for these findings is that the release of vagal tone was more pronounced in the Biotest fish, which was most likely facilitated by the higher routine inhibitory cholinergic tone on the heart in this group (Fig. 3a). However, this did not translate to a reduced hypertensive bradycardia with PE (Fig. 1), suggesting that the potential for cholinergic vagal inhibition of heart rate was unaltered in Biotest fish.

Functional significance

The barostatic responses to decreases and increases in arterial pressure have fundamentally different physiological functions. The hypertensive bradycardia mainly prevents excessive pressure overload of the vasculature, which may prevent vessel damage and formation of tissue oedema in the particularly vulnerable and delicate respiratory vascular beds of the gills in fish and pulmonary vessels in reptiles and amphibians with an undivided circulation (West and Van Vliet, 1983; West and Van Vliet, 1994). In contrast, the hypotensive tachycardia probably has more of an autoregulatory function ensuring that perfusion pressure, and therefore tissue blood flow, is maintained to safeguard the tissues from ischemia (Van Vliet and West, 1994). Indeed, Hedrick et al. (2015) argued that the main role of the baroreflex in three anuran amphibians is to restore arterial blood pressure following hypotensive events, because resting mean arterial pressures were only slightly lower that the saturation points for the respective baroreflex curves, making the heart rate in these animals much more responsive to hypotensive than hypertensive events.

At present, we can only speculate on the functional significance of the observed changes in barostatic control with environmental temperature change. Although perch have a

well-developed capacity for metabolic thermal compensation, such acclimation capacity does not allow complete thermal compensation of tissue oxygen demand over the full range of temperatures experienced in nature. Indeed, in a study performed in parallel to the present study, standard metabolic rate was significantly higher in Biotest perch (Q_{10} 1.5) when reference and Biotest fish were compared at their respective environmental temperatures (Sandblom et al., 2016). Thus, the more profound tachycardic response to hypotension in the chronically warm Biotest fish may reflect a mechanism allowing a faster recovery of arterial perfusion pressure to secure tissue blood flow when tissue oxygen demand is elevated in warm environments. Similarly, assuming that the hypertensive bradycardia mainly serves to safeguard the respiratory tissues from hydrostatic pressure overload, it seems reasonable that this capacity should be relatively unaffected by environmental temperature; not least since the present study found that resting ventral aortic blood pressure was unaffected and responded similarly to the vasoactive drugs across environmental temperatures. Even so, since a closed-loop technique was employed in the present study, where vascular resistance and arterial blood pressure were pharmacologically manipulated, only the cardiac limb of the reflex was studied (for descriptions of closed- versus open-loop experimental approaches see Bagshaw, 1985; Van Vliet and West, 1994). These technical limitations prevented us from examining the vascular limb of the baroreflex and determining how the recovery of arterial blood pressure homeostasis is affected by temperature. Future studies adopting non-pharmacological methods to study barostatic reflexes in fish may be instrumental to further explore these topics (Sandblom and Axelsson, 2005).

When comparing the effect of the highest drug concentrations when the barostatic heart rate responses appeared saturated, it is clear that the heart rate response is not symmetrical as the tachycardia following SNP was markedly greater than the bradycardia after PE in both experimental groups (Fig. 1a). Nonetheless, the consistently greater heart rate

response to hypotension found here further strengthens the view that the role of the baroreflex in fish may not only be to prevent pressure overload, but also to prevent hypotension to maintain adequate tissue perfusion pressures. Similar arguments have been presented recently for anuran amphibians (Hedrick et al., 2015).

The finding that atropine abolished both hyper- and hypotensive chronotropic responses (Fig. 1C) is consistent with previous studies in fish (Sandblom and Axelsson, 2005), as well as some reptiles and amphibians (Millard and Moalli, 1980). However, it differs from other studies on various ectothermic vertebrates where adrenergic control of the heart rate response may dominate or act in concert with modulation of vagal tone (Bianchi-da-Silva et al., 2000; Crossley et al., 2003; Lillywhite and Seymour, 1978). Thus, although most teleost species possess a dual cardiac innervation from both cholinergic and adrenergic nerves (Nilsson, 1983; Sandblom and Axelsson, 2011), it appears that the β-adrenergic regulatory potential of the heart is not exploited in barostatic reflexes in teleosts such as perch and trout, at least not during thermal conditions tested so far.

Thermal plasticity of resting cardiac autonomic tones

While numerous biotic and environmental factors can affect resting cholinergic tone, the values in the range of 64-105% recorded here at different environmental temperatures (see Fig. 3) are clearly at the high end compared with most other fish species and experimental conditions examined so far (see figure 6 in Sandblom and Axelsson, 2011). While these findings provide evidence that our experimental animals had sufficiently recovered from the surgical protocol and that the influence of the surgical instrumentation was minimal, they are probably also partly explained by the relatively high environmental temperatures at the time of the experiments. Alternatively, the possibility that routine cholinergic tone is unusually high in perch compared with other fish species cannot be ruled out.

It is presently unclear whether the differences in cardiorespiratory function between perch from the Biotest enclosure and the reference fish from the surrounding archipelago are entirely explained by phenotypic plasticity at the individual level (i.e. acclimation), or whether there are also genetic or epigenetic mechanisms involved. Even so, 3-4 weeks of acclimation of Biotest fish to reference thermal conditions was sufficient to abolish any differences in resting heart rate as well as intrinsic heart rate and cardiac autonomic control (Figs. 2 and 3). This suggests that the thermal compensation of the intrinsic pacemaker rate and of resting heart rate through thermal plasticity of cardiac autonomic tones are relatively quick processes, but whether these changes to cardiac function occur in concert or in series is presently unknown. It is also unknown whether the increased vagal drive at warm temperatures is the result of increased CNS activity, or if changes at cholinergic synaptic or receptor levels may explain the patterns observed in the intact animals studied here.

Localization of baroreceptors

Although heart rate was clearly affected even by the lowest doses of SNP, this drug had a surprisingly small effect on ventral aortic blood pressure. While we cannot be certain how vascular resistances or trans-branchial pressure gradients were affected in the present study, it seems likely that the vasodilating effects of SNP may have been considerably more pronounced downstream of the gills (i.e. in the systemic vasculature), suggesting that the dorsal aortic pressure may have been reduced considerably more than the ventral aortic pressure. This line of reasoning is supported by studies on trout where SNP has virtually no effect on branchial resistance, but a profound vasodilatory effect on systemic vascular resistance (Olson et al., 1997). Moreover, the routine branchial vascular resistance is substantial and blood pressure typically falls by approximately 30% across the gills (Axelsson

and Nilsson, 1986; Farrell and Smith, 1981). The precise location of baroreceptors within the gills is not clear (see Jones and Milsom, 1982; Sundin and Nilsson, 2002), but the present study strongly suggests that the efferent side of the gill vasculature is the more likely site given the relatively profound tachycardia even with SNP concentrations that hardly affected ventral aortic pressure. Again, this reasoning further supports the view that an important role of the barostatic response in fish is to prevent post-branchial hypotension to ensure perfusion of systemic tissues.

Baroreflex sensitivity is often described as baroreflex gain; i.e. the change in heart rate as a function of the change in blood pressure (e.g. Altimiras et al., 1998). However, given the uncertainties regarding what pressure the baroreceptors experience in fish (i.e. dorsal or ventral aortic), and our finding that heart rate was markedly affected by SNP with only very limited changes in ventral aortic blood pressure, we were not able to reliably perform an analysis of baroreflex gain. Future studies involving measurements of heart rate changes in response to dorsal aortic blood pressure manipulations in fish species where this vasculature is more surgically accessible (e.g. salmonids) may therefore be informative.

Conclusions

We have demonstrated in a eurythermal temperate fish species that the neurohumoral control of resting heart rate exhibits considerable thermal plasticity and that the tachycardia response to pharmacologically-induced hypotension is exacerbated with chronic environmental warming. The latter response is likely explained by a greater scope for vagal release at the higher temperature, which presents a novel physiological mechanism in an ectothermic animal serving to safeguard arterial perfusion pressure as tissue oxygen demand increases with temperature. Whether this is a mechanism ubiquitous to all ectothermic vertebrates will not be known without further studies. What is known is that cardiovascular temperature

tolerance and cardiac acclimation capacity are important physiological traits determining resilience to climate warming in ectotherms. While perch exhibit a thermally flexible physiological phenotype with a well-developed capacity to adjust routine cardiac autonomic control and barostatic reflex control in relation to changes in environmental temperature, it is possible that other more stenothermal fishes (e.g. from polar and tropical environments) may not possess this ability and thus may be more susceptible to climate warming.

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Tables

Table 1. Environmental water temperatures in the Forsmark area in 2013.				
	Environmental temperatures (°C)			
	Experimental period mean	*Yearly Mean*	*Yearly minimum*	*Yearly maximum*
Reference	17.5±0.4	8.0±6.5	-0.2	18.5
Biotest	23.6±1.8	16.0±5.8	8.1	26.3
Environmental temperatures for 2013 when experiments were performed are based on daily mean temperature records at 2 m depth in the cooling water intake channel (*Reference*) and at the centre of the Biotest enclosure (*Biotest*) obtained from the nuclear power plant in Forsmark. Means±SD are reported for experimental period (n=18) and year (n=365). Yearly minimum and maximum environmental temperatures represent the daily mean temperature during the coldest and warmest days, respectively. The experimental period was August 11 to 28.				

Table 2. Experimental fish characteristics and experimental temperatures			
	Body mass (g)	*Holding temperature (°C)*	*Test temperature (°C)*
Experimental series 1: Barostatic reflexes and cardiac autonomic control			
Reference	466±170	18.4±1.0	18.8±0.4
Biotest	367±102	24.0±1.2	25.3±0.3 (18.9±0.2)
Experimental series 2: Cardiac control after thermal acclimation to Reference temperature			
Reference	173±92	18.2±0.8	18.8±0.6 (24.0±0.7)
Biotest	161±90	18.2±0.8	18.6±0.4 (23.7±0.6)
Experimental temperatures and body masses (n=12 for *Reference* and n=17 for *Biotest* in series 1 and n=8 for *Reference* and n=9 for *Biotest* in series 2) expressed as means±SD. Values within parentheses are experimental test temperatures after acute temperature change. There were no significant differences in body masses within experimental series. For details on temperature recordings and experimental protocols, see materials and methods.			

Figures

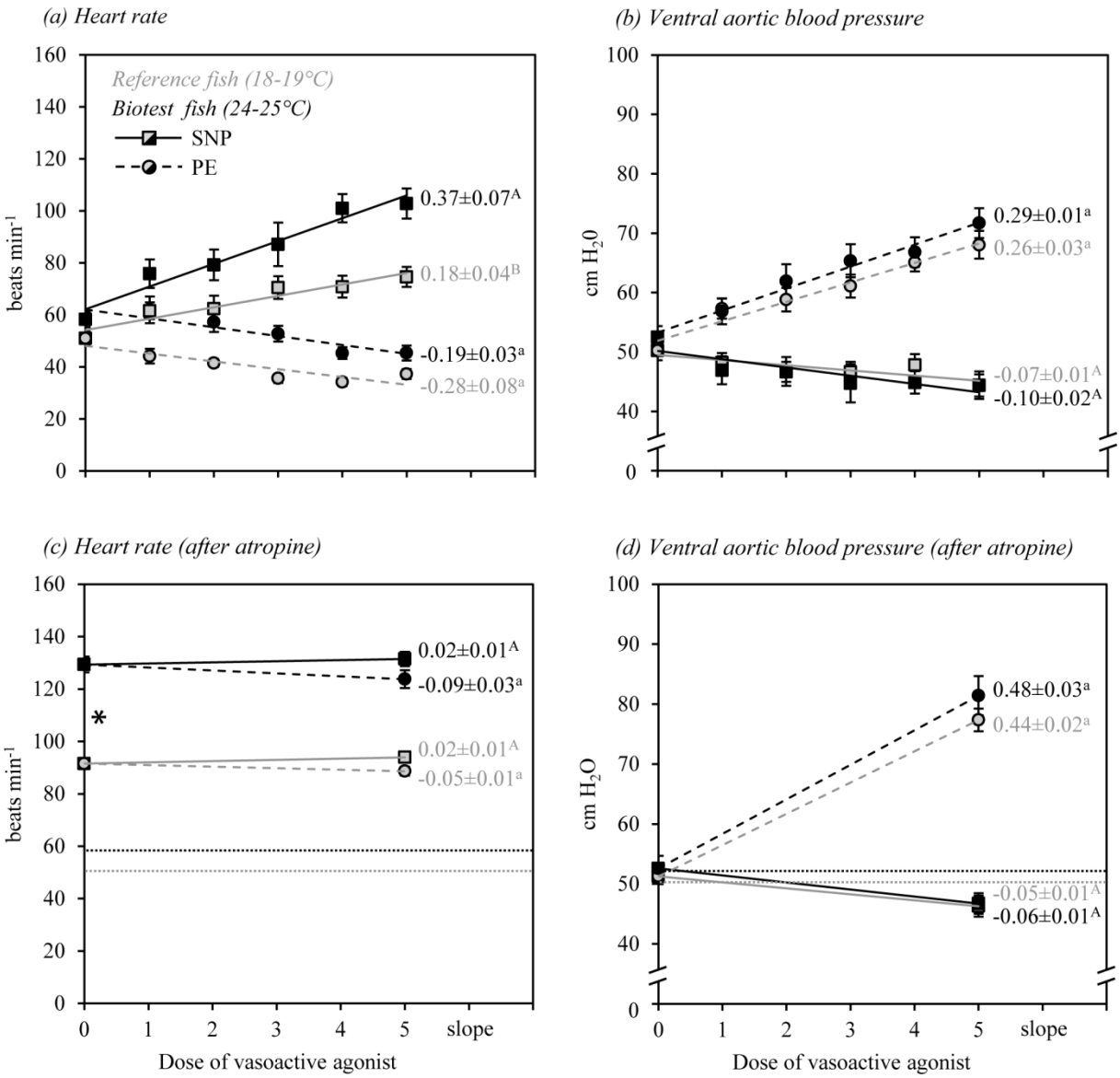

Figure 1. Heart rate (*a* and *c*) and ventral aortic blood pressure (*b* and *d*) in perch (*Perca fluviatilis* L.). Grey represent reference fish at reference thermal conditions (18-19°C) and black are Biotest fish at Biotest thermal conditions (24-25°C). Values in *a* and *b* represent untreated fish (dosage 0) and after sequential randomised injections of increasing

concentrations (dosages 1-5) of sodium nitroprusside (SNP; 5, 10, 25, 50 and 100 μg kg M_b^{-1}; squares and solid lines) and phenylephrine (PE; 5, 10, 20, 30 and 60 μg kg M_b^{-1}; circles and dashed lines). Values in *c* and *d* are after atropine alone (1.2 mg kg M_b^{-1}, dosage 0) and after the highest dosages of SNP (100 μg kg M_b^{-1}; squares and solid lines) and PE (60 μg kg M_b^{-1}; circles and dashed lines). Dotted horizontal lines in panels *c* and *d* represent values for untreated fish (i.e. 0 dosage values in panels *a* and *b*). Individual data points are presented as means±SEM (n=11-14). Mean slopes for each regression (i.e. responsiveness to the drugs) are presented for each agonist. Different superscript letters denote significant ($p \leq 0.05$) differences in agonist responsiveness (slopes) between reference and Biotest fish (capital letters for SNP and lower case letters for PE). An asterisk denotes significant ($p \leq 0.05$) difference in resting cardiovascular variables (i.e. 0 dosage values) between acclimation groups.

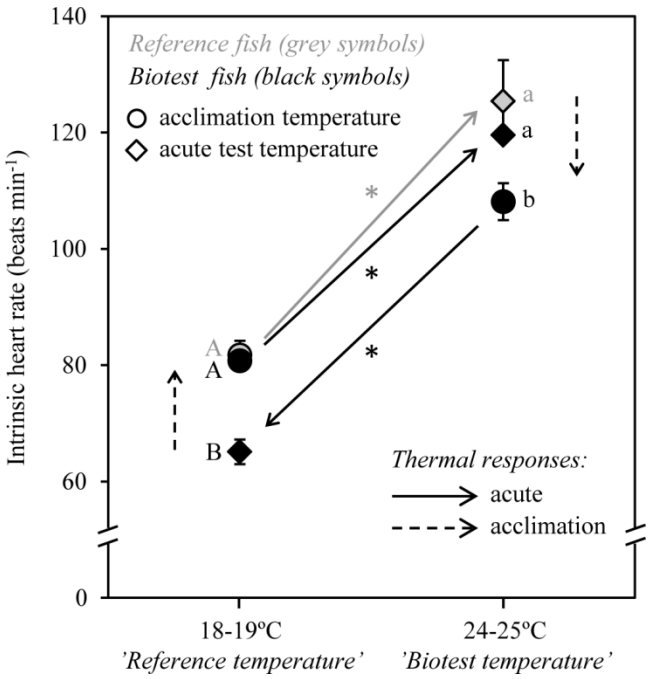

Figure 2. Intrinsic heart rate in perch (*Perca fluviatilis* L.) after double blockade of muscarinic receptors with atropine (1.2 mg kg M_b^{-1}) and β-receptors with propranolol (3 mg kg M_b^{-1}). Grey symbols are reference fish and black symbols are Biotest fish. Circles represent the initial environmental or acclimation temperature (i.e. 18-19 and 24-25°C for reference and Biotest thermal conditions, respectively). Diamonds represent values after an acute change in test temperature. Also included in the figure are Biotest fish acclimated for 3-4 weeks to reference temperature (18-19°C, black circle). Solid arrows indicate the direction of acute temperature change and dashed arrows represent the direction of thermal acclimation response. All values are means±SEM (n=6-9). Different letters denote significant ($p \leq 0.05$) differences between groups at a given temperature (capital letters for 18-19°C and lower case letters for 24-25°C). The asterisk denotes a significant ($p \leq 0.05$) main effect of the acute temperature change.

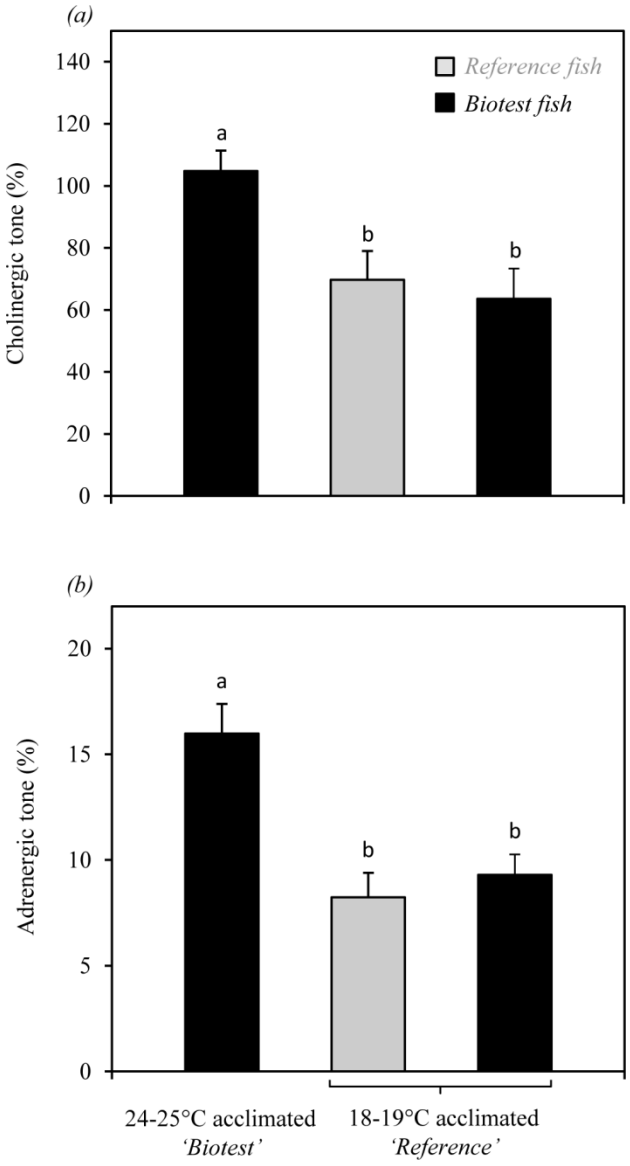

Figure 3. Cardiac cholinergic (*a*) and adrenergic (*b*) tones in perch (*Perca fluviatilis* L.). Grey bars are reference fish and black bars are Biotest fish. Temperatures represent test and acclimation temperatures. Variables are means +SEM (n=6-14). Different letters denote significant ($p \leq 0.05$) differences between treatment groups.

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