Thermal acclimation and seasonal acclimatization: a comparative study of cardiac response to prolonged temperature change in shorthorn sculpin

DOI: 10.1242/jeb.202242

Document Version
Final published version

Link to publication record in Manchester Research Explorer

Citation for published version (APA):

Published in:
The Journal of Experimental Biology

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester’s Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.
Thermal acclimation and seasonal acclimatization: a comparative study of cardiac response to prolonged temperature change in shorthorn sculpin

Tatiana S. Filatova¹,²,*, Denis V. Abramochkin¹,²,³ and Holly A. Shiels⁴

ABSTRACT
Seasonal thermal remodelling (acclimatization) and laboratory thermal remodelling (acclimation) can induce different physiological changes in ectothermic animals. As global temperatures are changing at an increasing rate, there is urgency to understand the compensatory abilities of key organs such as the heart to adjust under natural conditions. Thus, the aim of the present study was to directly compare the acclimatization and acclamatory response within a single eurythermal fish species, the European shorthorn sculpin (Myoxocephalus scorpius). We used current- and voltage-clamp to measure ionic current densities in both isolated atrial and ventricular myocytes from three groups of fish: (1) summer-caught fish maintained at 12°C (‘summer-acclimated’); (2) summer-caught fish kept at 2°C (‘cold acclimated’); and (3) winter-caught fish acclimatized to 2°C (‘winter-acclimatized’). At a common test temperature of 7.5°C, action potential (AP) was shortened by both winter acclimatization and cold acclimation compared with summer acclimation; however, winter acclimatization caused a greater shortening than did cold acclimation. Shortening of AP was achieved mostly by a significant increase in repolarizing current density (I_K) following winter acclimatization, with winter acclimation having only minor effects. Compared with summer acclimation, the depolarizing L-type calcium current (I_{Ca}) was larger following winter acclimatization, but again, there was no effect of cold acclimation on I_{Ca}. Interestingly, the other depolarizing current, I_{Na}, was downregulated at low temperatures. Our analysis further shows that ionic current remodelling is primarily due to changes in channel density rather than currents kinetics. In summary, acclimatization profoundly modified the electrical activity of the sculpin heart while acclimation to the same temperature for >1.5 months produced very limited remodelling effects.

KEY WORDS: Heart, Action potential, Electrophysiology, Hypertrophy, Thermal remodelling, Myoxocephalus scorpius

INTRODUCTION
Fishes living in temperate latitudes experience both acute and chronic fluctuations in their thermal environment. Acute temperature changes (minutes to hours) have a large influence on fish cardiac function through $Q_{10}$ effects on reaction rates (Galli et al., 2009a; Shiels et al., 2000; Vornanen et al., 2014). Acute cooling causes bradycardia (Keen et al., 1993) and a reduction in cardiac force production (Shiels et al., 2002; Vornanen et al., 2005; West and Driedzic, 1999), and these tissue-level changes are due, in part, to the direct effects of temperature on the ion channels and ion pumps that underlie cellular excitation–contraction coupling (Shiels et al., 2003; Vornanen et al., 2014). Although the effects of acute temperature change can be detrimental to contractile function, chronic exposure results in compensatory changes that limit their consequences.

The adjustment of biochemical and physiological processes following prolonged temperature exposure is called seasonal (or thermal) acclimatization (Driedzic and Gessler, 1994). When the thermal environment of an animal is changed within a laboratory setting, this set of physiological adjustments is referred to as thermal acclimation. The biochemical and physiological processes underlying acclimation of the fish heart have been widely studied because of the heart’s vital role in transporting gases and metabolic constituents around the body, supporting active biological processes across temperatures. Such studies show thermally-induced changes in cardiac contractility, pacemaker function, action potential (AP) configuration and ionic current densities (Aho and Vornanen, 1999; Galli et al., 2009a,b; Haverinen and Vornanen, 2007, 2004). Most of these adaptations are thought to be mediated by changes in the expression levels of certain ion channels or in the function of contractile myofilaments (Haverinen and Vornanen, 2004; Klaiman et al., 2014). The direction of these adaptive changes depends on an animal’s life style: whether it is cold active (like salmonids) or cold dormant (like crucian carp) (Haverinen and Vornanen, 2004; Klaiman et al., 2011).

Nevertheless, recent work has shown that naturally occurring seasonal acclimatization might induce a more pronounced change in cardiac function than lab-based thermal acclimation (Abramochkin and Vornanen, 2015; Hassinen et al., 2014). As global temperatures are changing at an increasing rate, resulting in latitudinal range shifts, it is important to understand the compensatory abilities of the fish heart to adjust under natural conditions. In the present study, our aim was to directly compare the acclimatization and acclamatory response of both chambers of the heart within a single eurythermal fish species, the European shorthorn sculpin (Myoxocephalus scorpius) to test the hypothesis that the changes caused by seasonal acclimatization are more prominent than those caused by laboratory acclimation.

The shorthorn sculpin is a widespread and common eurythermal benthic fish inhabiting a large geographic range (80°N–40°N; fishbase.org) from the Eastern coast of North America to Greenland, Iceland, the Baltic Sea, the White Sea and the Arctic Ocean (Luksenburg and Pedersen, 2002; Luksenburg et al., 2004). They...
are sexually dimorphic (Cardinale, 2000) opportunistic feeders (Raciborski, 1984) with a lifespan of 14–15 years for both sexes (Ennis, 1970a). There are differences in life history parameters between different sculpin populations. For example, northern populations of shorthorn sculpin are characterized by slower growth rates and late age of sexual maturation in comparison to the populations in central Europe (Luksenburg and Pedersen, 2002). In the Baltic Sea and in Newfoundland, shorthorn sculpins spawn from early December to February, in the White Sea they spawn from November to January, and in northern Norway they spawn from late January to the beginning of March (Ennis, 1970b; Lamp, 1966; Luksenburg et al., 2004). At high latitudes, the temperature of water during their spawning varies from 0°C to 2.1°C (Luksenburg et al., 2004). Thus, the shorthorn sculpin can be considered a temperature generalist (Farrell et al., 2013), able to adjust its physiology to a wide range of temperatures, which has allowed it to exploit a wide range of latitudes. Therefore, we expect its physiological response to temperature acclimation and seasonal acclimatization to be greater than those of stenothermic fish species (Franklin et al., 2013). Here, we utilize this eurythermal fish species to examine the effect of both seasonal acclimatization and thermal acclimation on the properties of all the major ionic currents underlying excitation–contraction coupling in freshly isolated atrial and ventricular myocytes using electrophysiology.

**MATERIALS AND METHODS**

**Experimental animals and acclimation protocol**

The research was performed at the White Sea Biological Station of Lomonosov Moscow State University. Fish were caught by hook and line in the Kandalaksha Bay near the Biological Station (Karelia, Russia; 66°19′50″N, 33°40′06″E) either in summer (July–August 2016) or in winter (early March 2017). The water temperature in Kandalaksha Bay in summer varied between 11°C and 13°C; in March, the water temperature varied within a range of 1.5–2.2°C. The European shorthorn sculpin [Myxocephalus scorpius (Linnaeus 1758)] captured in summer were divided into two groups. One group (the summer-acclimated animals) were kept in a marine aquarium under conditions that match those from which they were caught. Their tank was aerated and had fresh input from the sea at 12°C. They were fed every 3 days with minced meat of navaga (Eleginus navaga) and held for at least 1 month prior to use. The remaining summer-caught fish were placed in a separate aquarium where the water temperature was brought down from 12°C to 3°C by 2°C a day. This group was termed the cold acclimation group as they were summer caught and then thermally acclimated to a cold (i.e. winter) temperature. These fish were kept at 3°C for 1.5 months prior to use. This time period is in excess of the 4 weeks usually provided for biochemical remodelling of heart, liver and muscle function in other fish species (Graham and Farrell, 1989; Kent et al., 1988; Sephton and Driedzic, 1991). Since shorthorn sculpins tend to forage less in autumn and winter (Cardinale, 2000), this group was offered food every 5 days. The cold-acclimated fish, caught and thermally acclimated to cold in summer, allow the effects of cold acclimation and seasonal acclimatization to be distinguished. Indeed, previous studies have demonstrated that this protocol of acclimation can induce cardiac physiological remodelling in spite of seasonality (Chung et al., 2017; Vornanen, 1998).

The final group of fish were the winter-acclimatized animals that were caught by hook and line in March and were kept in a submerged sea cage (0.5 m³) at a depth of 8 m for at least 48 h prior to the experiments, as described previously (Abramochkin and Vornanen, 2015, 2017; Abramochkin et al., 2019; Hassinen et al., 2014). The natural photoperiod at the White Sea Biological Station was 15 h:9 h light:dark in August and 9 h:15 h light:dark in March. The summer-acclimated fish were kept at 15 h:9 h light:dark photoperiod, whereas the photoperiod in the cold-acclimated group was adjusted by 0.5 h a day until it reached 9 h:15 h light:dark. The light in the aquaria rooms was kept dim because these animals live at depth in the wild (ranging from 5 to 25 m) where light penetration is low. Moreover, the cold-acclimated group animals were kept in an opaque tank, which reduces light penetration in order to imitate winter conditions.

**Cell isolation**

Cardiomyocytes were obtained using a protocol previously described in detail (Vornanen, 1997, 1998). In brief, fish weighing 113.3±31.1 g (mean±s.e.m.) in the summer-acclimated group (n=11), 152.1±29.2 g in the cold-acclimated group (n=12) and 120.2±15.7 g in the winter-acclimatized group (n=12) were stunned by a blow to the head, the spine was cut and the brain was destroyed by pithing. The heart weighing 0.23±0.064 g in the summer-acclimated group (n=11), 0.31±0.053 g in the cold-acclimated group (n=12) and 0.27±0.086 g in the winter-acclimatized group (n=12) was rapidly excised, placed in a Langendorff apparatus and retrogradely perfused with nominally Ca²⁺-free low-Na⁺ solution (in mmol 1⁻¹): 100 NaCl, 10 KCl, 1.2 KH₂PO₄·2H₂O, 4 MgSO₄·7H₂O, 50 taurine, 10 glucose and 10 HEPES at pH 6.9. The perfusion was performed at room temperature to provide sufficient activity of the proteolytic enzymes together with maximum survival of the myocytes. After 5–7 min, the perfusion was switched to the same solution containing proteolytic enzymes: 1 mg ml⁻¹ collagenase type IA, 0.26 mg ml⁻¹ trypsin type IX, 0.66 mg ml⁻¹ fatty acid-free bovine serum albumin. After 18–25 min of enzymatic treatment, the atria and ventricles were separated, placed in low-Na⁺ solution, minced and gently pipetted. Isolated atrial and ventricular myocytes were stored separately in low-Na⁺ solution at +4°C. The storage conditions provided survival of the cells up to at least 8 h. All experiments conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the EU Directive 2010/63/EU for animal experiments.

**Recording of ionic currents**

The arithmetic average of the summer and winter temperatures (7.5°C) was chosen as the test temperature for direct comparison of

---

**List of symbols and abbreviations**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>APD25, APD50</td>
<td>action potential duration at 25%, 50% and 90% level of repolarization, respectively</td>
</tr>
<tr>
<td>G</td>
<td>conductance</td>
</tr>
<tr>
<td>I</td>
<td>registered current at a given membrane potential</td>
</tr>
<tr>
<td>I_Ca</td>
<td>calcium L-type current</td>
</tr>
<tr>
<td>I_Ka</td>
<td>fast sodium current</td>
</tr>
<tr>
<td>I_K1</td>
<td>inward rectifier potassium current</td>
</tr>
<tr>
<td>I_K0</td>
<td>rapid delayed rectifier potassium current</td>
</tr>
<tr>
<td>RMPI</td>
<td>resting membrane potential</td>
</tr>
<tr>
<td>TTX</td>
<td>tetrodotoxin, selective fast sodium current blocker</td>
</tr>
<tr>
<td>V_rev</td>
<td>reversal potential of the current</td>
</tr>
<tr>
<td>V_0.5</td>
<td>half activation potential</td>
</tr>
<tr>
<td>τ_slow</td>
<td>slow time constant of inactivation</td>
</tr>
<tr>
<td>τ_fast</td>
<td>fast time constant of inactivation</td>
</tr>
</tbody>
</table>
current densities and action potential configuration between the groups. By comparing ionic conductance from each treatment group at the same test temperature, we were able to separate the effects of thermal remodelling from the direct (i.e. $Q_{10}$) effects of temperature.

Whole-cell voltage clamp recording of ionic currents from both atrial and ventricular myocytes was performed with an Axopatch 200A amplifier (Molecular Devices, CA, USA). Isolated myocytes from either the atrium or the ventricle were placed into a small recording chamber (RC-26; Warner Instrument Corp, Brunswick, CT, USA; volume 150 µl) mounted on inverted microscope Eclipse Ti-S (Nikon, Tokyo, Japan) and superfused with physiological solution. Potassium currents ($I_{\text{K1}}$ and $I_{\text{K}}$) were recorded in K$^+$-based bath solution (containing in mmol l$^{-1}$): 150 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 1.2 MgCl$_2$, 10 glucose, 10 HEPES, with pH adjusted to 7.6 at 20°C with NaOH. The pH of the solutions at recording temperature (7.5°C) was 7.66 for the bath solution and 7.23 for the pipette solution. Tetrodotoxin (0.5 µmol l$^{-1}$) and nifedipine (10 µmol l$^{-1}$) were included to prevent sodium and calcium currents, respectively. Calcium L-type current ($I_{\text{CaL}}$) and fast sodium current ($I_{\text{Na}}$) were recorded in Cs$^+$-based bath solution with 5.4 mmol l$^{-1}$ CsCl instead of KCl to block potassium currents. The temperature of the bath solution was regulated and continuously monitored using a Peltier device (CL-100, Warner Instruments, CT, USA). Patch pipettes were pulled from borosilicate glass (Sutter Instruments, CA, USA) with a PIP 6 puller (HEKA Elektronik, Germany) and filled with a solution imitating the intracellular medium. The pipette solution for registration of potassium currents included (in mmol l$^{-1}$): 140 KCl, 1 MgCl$_2$, 5 EGTA, 4 MgATP, 0.3 Na$_2$GTP and 10 HEPES, pH 7.2. The pipette solution for registration of calcium and sodium currents included 130 mmol l$^{-1}$ CsCl and 15 mmol l$^{-1}$ TEA instead of KCl to prevent potassium currents. The resistance of filled pipettes was 2.6±0.2 MΩ. Access resistance, pipette capacitance and whole cell capacitance were completely compensated. Voltage-clamp protocols are provided in the figures. The analysis of current densities was performed using Clampfit 10.4 software (Molecular Devices, CA, USA). All of the analysed ionic currents are presented after subtraction of the current recorded in the presence of selective blocker in external solution: $I_{\text{Kr}}$ was blocked with E-4031 (1 µmol l$^{-1}$), $I_{\text{K1}}$ was blocked with Ba$^{2+}$ (1 mmol l$^{-1}$), $I_{\text{CaL}}$ was blocked with nifedipine (10 µmol l$^{-1}$) and $I_{\text{Na}}$ was blocked with TTX (0.5 µmol l$^{-1}$). $I_{\text{CaL}}$ and fast sodium current $I_{\text{Na}}$ amplitudes (pA) were analysed as the difference between the peak current and current at the end of the pulse. $I_{\text{K1}}$ amplitude was evaluated as absolute current amplitude after subtraction of leak current. The tail current peak amplitude after subtraction of leak current was used to assess $I_{\text{Kr}}$. The charge (PC) carried by an ionic current was calculated as the time integral of the inactivating part of the current. Current amplitude and charge transfer were normalized to cell capacitance (pA·pF$^{-1}$ and pC·pF$^{-1}$, respectively). The time constants of inactivation ($\tau$) for $I_{\text{CaL}}, I_{\text{Na}}$, and $I_{\text{Kr}}$ were calculated for the inactivating part of the current from Chebyshev exponential Eqn 1 using either single or double exponential fits as indicated in the figure legends. The $I_{\text{Kr}}$ time constant of inactivation was measured from the tail current:

$$f(t) = \sum_{i=1}^{n} A_i e^{-t/\tau_i} + C.$$

(1)

Steady-state voltage dependences of activation for $I_{\text{Na}}, I_{\text{CaL}}$ and $I_{\text{Kr}}$ were obtained by plotting normalized conductance as a function of membrane voltage and fitting it to the Boltzmann function with a positive slope:

$$y = \frac{1}{1 + e^{(V - V_{50})/S}},$$

(2)

where $V$ is membrane potential, $V_{50}$ is half-activation potential and $S$ is the slope of the curve. Conductance was obtained from current–voltage relationships using:

$$G = \frac{I}{V - V_{rev}},$$

(3)

where $G$ is conductance, $I$ is registered current at a given membrane potential and $V_{rev}$ is the reversal potential of the current.

**Recording of action potentials**

Action potentials (APs) were recorded from isolated ventricular myocytes using the standard whole-cell patch clamp method in current clamp mode at +7.5°C. Atrial myocytes isolated from sculpin had extremely small $I_{\text{K1}}$ current (see Results) and thus could not maintain sufficient resting membrane potential in current-clamp experimental conditions. Therefore, we only present AP data from ventricular myocytes. For these measurements, the external K$^+$ Tyrode solution and internal K$^+$ based pipette solution did not include blockers. The recording of cell electrical activity started after AP duration stabilized; ~1 min after whole-cell configuration was achieved. The analysis of AP waveform was performed with Clampfit 10.4 software and included the estimation of resting membrane potential (RMP), maximum upstroke velocity and AP duration at 25%, 50% and 90% levels of repolarization (APD25, APD50 and APD90, respectively).

**Drugs**

Collagenase type IA, trypsin, nifedipine and barium chloride were purchased from Sigma (St Louis, MO, USA). Tetrodotoxin and E-4031 were purchased from Tocris (Bristol, UK).

**Statistics**

Electrophysiological data are presented as means±s.e.m from n cells. The number of animals is indicated in figure legends. According to the previous experience in patch-clamp experiments with fish cardiomyocytes (Shiels et al., 2011; Vornanen, 1998), the number of animals was set at 10. The sample size (n) was no less than 6. No animals were excluded from the analysis. Cells were excluded from the analysis in case of low resistance of the contact between patch pipette and cell membrane resulting in unstable membrane potential. Current densities and AP duration were compared at a given voltage or duration (respectively) between acclimation groups after checking the distribution normality and the equality of variances. The comparison was performed using either one-way ANOVA followed by Bonferroni post hoc test or the ANOVA on ranks for normalized data. Details are provided in the figure legends. The significance limit for all statistical tests was set at $P<0.05$.

**RESULTS**

**Heart and myocyte size**

Heart mass relative to body mass did not vary between groups (Table 1). However, cold-induced hypertrophy was evident at the cellular level for both atrial and ventricular myocytes. Compared with summer-acclimated ventricular myocytes, winter-acclimatized ventricular myocytes had a greater cellular capacitance (an index of...
cell size). In both atrial and ventricular myocytes, the winter-acclimatized myocytes were larger than the cold-acclimated myocytes (Table 1).

**Action potential configuration**

At the common test temperature of 7.5°C, winter-acclimatized fish had shorter ventricular APD50 and APD90 (by 37.5±2.6% and 37±3.6%, respectively; mean±s.e.m.) compared with summer-acclimated fish, indicating seasonal thermal remodelling (Fig. 1A, B). Thermal acclimation had little effect on APD, with no significant differences in APD50 and APD90 between ventricular myocytes from the cold-acclimated animals and the other two groups (Fig. 1A, B). No significant differences between groups were found in RMP levels (Fig. 1C) or maximum upstroke velocity of APs (Fig. 1D).

**Delayed rectifier current (I_Kr)**

The shortening of AP in winter-acclimatized fish is consistent with an increase in the density of repolarizing potassium currents. Thus, we next examined the delayed rectifier potassium current (I_Kr) in both atrial and ventricular myocytes from each temperature group. Fig 2A,B,D and Fig 3A,B,D show representative recordings of I_Kr currents, IV curves and charge transfer, from ventricular and atrial myocytes, respectively, from all three groups of fish. As expected from the AP findings, I_Kr current density and charge transfer were dramatically increased in sculpin myocardium following seasonal

<table>
<thead>
<tr>
<th></th>
<th>Summer-acclimated</th>
<th>Cold-acclimated</th>
<th>Winter-acclimatized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative heart mass (%)</td>
<td>0.245±0.0182</td>
<td>0.219±0.0275</td>
<td>0.239±0.0248</td>
</tr>
<tr>
<td>Atrial myocyte capacitance (pF)</td>
<td>26.82±1.415</td>
<td>25.05±0.764*</td>
<td>28.92±0.814*</td>
</tr>
<tr>
<td>Ventricular myocyte capacitance (pF)</td>
<td>28.2±1.184*</td>
<td>27.2±1.922*</td>
<td>34.36±1.194*</td>
</tr>
</tbody>
</table>

Values are given as means±s.e.m., relative heart mass values were calculated for 11–12 animals in each group; cell capacitance values were obtained from 36–64 cells from 11–12 animals. **Significant differences between:** *summer-acclimated and winter-acclimatized groups; #cold-acclimated and winter-acclimatized groups; P<0.05, ANOVA. There were no differences between summer-acclimated and cold-acclimated groups.

Fig. 1. Ventricular action potential configuration. (A) Representative recordings of ventricular action potentials (APs), (B) means±s.e.m. for the APD at 90%, 50% and 25% repolarization, (C) resting membrane potential and (D) maximum upstroke velocity in isolated ventricular myocytes from summer-acclimated fish (12°C, red, n=6 myocytes from 5 animals), cold-acclimated fish (2°C, green, n=7 myocytes from 5 animals) and winter-acclimatized fish (2°C, blue, n=8 myocytes from 6 animals). Statistically significant difference between summer-acclimated and winter-acclimatized groups (*P<0.05, **P<0.01). Note that similar data could not be generated from atrial myocytes owing to the small I_K1 current density, which meant that cells failed to maintain a stable resting potential in current clamp mode.
acclimatization to winter, but not following thermal acclimation to the cold in the laboratory. For example, the density of outward tail current activated at +60 mV was 78.6±22.6% (mean±s.e.m.) greater in atrial myocytes and 307.7±15.6% greater in ventricular myocytes from winter-acclimatized (12°C, red, n=11 myocytes from 6 animals), cold-acclimated (2°C, green, n=9 myocytes from 5 animals) and winter-acclimatized sculpins (2°C, blue, n=12 myocytes from 7 animals). Statistically significant differences between: summer-acclimated and winter-acclimatized groups (**P<0.0001); summer-acclimated and cold-acclimated groups (††P<0.0001); winter-acclimatized groups and cold-acclimated groups (****P<0.0001); summer-acclimated and cold-acclimated groups (**P<0.05); NS, not significant.

Fig. 2. Characteristics of $I_{K_r}$ in sculpin ventricle. (A) Representative delayed rectifier potassium current recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of the $I_{K_r}$ tail current in isolated ventricular myocytes from summer-acclimated (12°C, red, n=11 myocytes from 6 animals), cold-acclimated (2°C, green, n=9 myocytes from 5 animals) and winter-acclimatized sculpins (2°C, blue, n=12 myocytes from 7 animals). Statistically significant differences between: summer-acclimatized and winter-acclimatized groups (****P<0.0001); winter-acclimatized groups and cold-acclimatized groups (††††P<0.0001); summer-acclimatized and cold-acclimatized groups (☆☆P<0.05); NS, not significant.

Inward rectifier current ($I_{K_1}$)
The inward rectifying potassium current ($I_{K_1}$), which is responsible for maintaining the resting membrane potential and can contribute to final phase of repolarization was also influenced by thermal exposure in the sculpin heart. At 7.5°C, both outward and inward components of $I_{K_1}$ were significantly higher in atrial and ventricular cells from winter-acclimatized sculpins when compared with cells from the other two groups (Fig. 4A,B). The physiologically significant outward current measured at −60 mV was 2.5-fold higher in atrial myocytes and 1.8-fold higher in ventricular myocytes from winter-acclimatized compared with the summer-acclimated animals. These changes in current density, were not reflected by RMP in our AP recordings. Although we did not observe noticeable changes in RMP in current-clamp mode, this fact does not exclude the possibility of finding such changes using a more sensitive method such as sharp electrode recording. On the other hand, another unknown current may be involved in setting RMP in the sculpin heart that was not investigated in this study. Similarly to the findings for $I_{K_r}$, cold acclimation did not significantly modify the current density of $I_{K_1}$ when compared with the summer-acclimated cohort.

L-type calcium current ($I_{CaL}$)
Similarly to the pattern observed with the key outward currents in cardiac excitation contraction coupling, thermal remodelling of inward Ca$^{2+}$ currents followed seasonal acclimatization but not thermal acclimation. Compared with the summer-acclimated fish, seasonal acclimatization but not thermal acclimation, induced a striking increase in the L-type calcium current in both chambers of the sculpin heart (Figs 5 and 6). The maximum current measured at +20 mV was 263.5±32.8% (mean±s.e.m) greater in atrial
myocytes and 204.6±37.5% greater in ventricular myocytes from winter-acclimatized animals compared with myocytes from the summer-acclimated group. Similarly, the charge transferred by $I_{Ca}$ in the winter-acclimatized group exceeds that in the summer-acclimated group by 157.2±23.6% in atrial cells and by 152.2±22.9% in ventricular cells (Fig. 5A,B,D and Fig. 6A,B,D). Current density and charge transfer did not differ significantly between summer-acclimated and cold-acclimated groups (Fig. 5A,B,D and Fig. 6A,B,D).

The greater charge transfer following winter acclimatization (and the trend with cold acclimation) can be attributed, at least in part, to the slowing of inactivation kinetics which allowed more time for Ca$^{2+}$ influx (Fig. 5E and Fig. 6E). Steady-state activation was also altered by winter acclimatization where $I_{CaL}$ activation curves in both atrial and ventricular cardiomyocytes were shifted toward more positive potentials when compared with summer-acclimated fish (Fig. 5C and Fig. 6C), and by the significant difference in half-activation potential ($V_{0.5}$) provided

### Table 2. Steady-state activation parameters for $I_{Kr}$, $I_{Ca}$ and $I_{Na}$ in cardiomyocytes isolated from shorthorn sculpin

<table>
<thead>
<tr>
<th></th>
<th>Ventricle</th>
<th>Atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{0.5}$</td>
<td>Hill slope</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold-acclimated</td>
<td>5.733±2.999</td>
<td>11.938±2.891</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer-acclimated</td>
<td>−17.75±1.844</td>
<td>8.811±1.904</td>
</tr>
<tr>
<td>Cold-acclimated</td>
<td>−14.31±1.297</td>
<td>8.211±1.124</td>
</tr>
<tr>
<td>Winter-acclimated</td>
<td>−8.865±1.214</td>
<td>6.412±0.718</td>
</tr>
<tr>
<td>$I_{Na}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer-acclimated</td>
<td>−37.24±0.524</td>
<td>2.838±0.411</td>
</tr>
<tr>
<td>Cold-acclimated</td>
<td>−38.44±1.045</td>
<td>2.465±1.334</td>
</tr>
<tr>
<td>Winter-acclimated</td>
<td>−36.28±0.436</td>
<td>2.874±0.267</td>
</tr>
</tbody>
</table>

Values are given as means±s.e.m. of 6–15 myocytes from 5–8 animals. $V_{0.5}$, half activation potential. Significant differences between: *summer-acclimated and winter-acclimatized groups; ‡summer-acclimated and cold-acclimated groups; #cold-acclimated and winter-acclimatized groups. P<0.05, ANOVA.
in Table 2. The shift in activation might impact excitability in winter-acclimatized individuals.

**Fast sodium current ($I_{Na}$)**

Unlike the other currents described thus far, the fast sodium current ($I_{Na}$) was downregulated in both of the cold temperature groups compared with the summer-acclimated fish for both atrial and ventricular myocytes (see Fig. 7A,B and Fig. 8A,B, respectively). In summer-acclimated fish, the peak density of the fast sodium current (at $-20$ mV) exceeded that of winter-acclimatized fish by 31.1±4.1% in the atrium and by 51.1±3.3% in the ventricle. The charge carried by winter-acclimatized $I_{Na}$ was 39.2±4.2% greater and by cold-acclimated fish $I_{Na}$ was 25.7±1.9% greater than that in ventricular cells from the summer-acclimated group. In atrial myocytes the differences in charge transfer between the groups were not statistically significant. This can be explained, in part, by the decrease in fast time inactivation constant ($\tau_{fast}$) in the summer-acclimated group compared with the winter-acclimatized group, since charge transfer depends both on current amplitude and its time-dependent inactivation.

In ventricular cardiomyocytes, neither $\tau_{fast}$ nor $\tau_{slow}$ differed between the groups; no changes were observed in the steady-state activation characteristics of $I_{Na}$ (Fig. 7D,E,F).

In summer-acclimated atrial myocytes the $I_{Na}$ steady-state activation curve was shifted toward more positive potentials when compared with the other two groups. This would reduce excitability in the summer compared with the winter- and cold-acclimated fish (Fig. 8D, Table 2). Thus, acclimation to low temperatures downregulates the density of $I_{Na}$ channels in sculpin heart, but in atrial myocardium, these changes are masked by the opposite changes in $\tau_{fast}$. Again here, the changes caused by naturally occurring acclimatization were more profound than those caused by artificial acclimation.

**DISCUSSION**

Electrical activity of the heart is altered by ambient temperature change due to the direct effect of temperature on the ion channels and ion pumps that underlie cardiac excitation–contraction coupling. The thermal sensitivity of ion flux in the cardiomyocytes is therefore very important for setting the thermal tolerance limits for ectothermic vertebrates (Haverinen and Vornanen, 2009). To ensure coordinated electrical and contractile function of the whole heart during prolonged temperature change, ion channel remodelling is known to occur in fishes (Aho and Vornanen, 2001; Badr et al., 2017; Hassinen et al., 2008, 2014; Haverinen and Vornanen, 2009; Keen et al., 2017; Korajoki and Vornanen, 2012; Matikainen and
Vornanen, 1992; Pelouch and Vornanen, 1996; Tiitu and Vornanen, 2001; Vornanen, 1994; Vornanen et al., 1998). This remodelling resets the chronotropic (Aho and Vornanen, 2001; Haverinen and Vornanen, 2007) and inotropic (Aho and Vornanen, 1999; Tiitu and Vornanen, 2001) output of the fish heart, presumably to compensate the direct effects of temperature. Many of the studies (listed above) which have investigated thermal remodelling in fish hearts have used animals acclimated in the laboratory to a number of temperatures. Although these studies provide valuable insight into the capacity of the fish heart to remodel in response to prolonged temperature change, there is growing evidence that the cardiac phenotype produced from such studies differs compared with that achieved when the thermal change occurs naturally, such as with changing of the seasons (i.e. thermal/seasonal acclimatization) (Abramochkin and Vornanen, 2015, 2017). As global temperatures are changing at an increasing rate, there is an urgent need to understand the compensatory abilities of the fish heart to adjust under natural conditions and to understand the applicability of the remodelling responses gleaned from thermal acclimation studies to the wild. Therefore, in this study we directly compared the thermal remodelling response of all of the major cardiac ion channels that underlie excitation–contraction coupling in both chambers of the heart of eurythermal fish species, the European shorthorn sculpin (Myxocephalus scorpius) in the summer with that following both seasonal acclimatization to winter and cold acclimation in the laboratory. Our key finding is that winter acclimatization was a stronger inducer of thermal remodelling than 6 weeks of cold acclimation for all ion currents in both atrium and ventricle of the shorthorn sculpin. If this observation holds true for other fishes, then perhaps there is greater capacity for remodelling of cardiac function in than previously determined through acclimation studies. This may extend the thermal limits for heart excitability in fish to a wider range of temperatures than that predicted from experiments exploiting the protocols of thermal acclimation in laboratory conditions.

**Thermal remodelling of the action potential and repolarizing currents**

One of the most consistent cardiac responses in fishes is compensatory decrease of APD following prolonged exposure to cold (Badr et al., 2017; Galli et al., 2009b; Haverinen and Vornanen, 2009). This AP shortening is thought to offset the prolongation known to occur with acute cooling and thus prevent the heart from becoming refractory at higher beat rates. Duly, cold acclimation of summer-acclimatized fish and seasonally acclimatized winter fish displayed shorter AP compared with summer-acclimated fish. However, winter acclimatization induced a stronger reduction in APD compared with 6 weeks of cold acclimation (discussed below). The thermally-induced decrease of APD in sculpin myocardium is explained by an increase in the density of the key repolarizing current, $I_{K}\text{C}$. This appears to be a ubiquitous mechanism for AP shortening.

---

**Fig. 5. Characteristics of $I_{Ca}$ in sculpin ventricle.** (A) Representative recordings, (B) current–voltage relationships, (C) steady-state activation curves, (D) charge transfer and (E) constant of time-dependent inactivation of $I_{Ca}$ current in isolated ventricular myocytes from summer-acclimated (12°C, red, $n=9$ myocytes from 5 animals), cold-acclimated (2°C, green, $n=11$ myocytes from 5 animals) and winter-acclimatized sculpins (2°C, blue, $n=12$ myocytes from 6 animals). Statistically significant differences between: summer-acclimated and winter-acclimatized groups (**$P<0.01$, ****$P<0.0001$); winter-acclimatized groups and cold-acclimatized groups (**$P<0.05$, ****$P<0.0001$).
across a range of fish orders including Cypriniformes (crucian carp, Carassius carassius; roach, Rutilus rutilus) Salmoniformes (rainbow trout, Oncorhynchus mykiss; pike, Esox lucius), Cadiformes (burbot, Lota lota; Arctic navaga cod, Eleginus navaga) (Hassinen et al., 2014) Perciformes (perch, Perca fluviatilis) (Badr et al., 2017; Haverinen and Vornanen, 2009) and Scophthalmiformes (Pacific bluefin, Thunnus orientalis) (Galli et al., 2009a,b). In the sculpin heart, we found that seasonal acclimatization induced a far greater increase in \( I_{Kr} \) current density than cold acclimation, which would explain the greater reduction in APD. The cold-induced increase in current density is likely due to a change in the number of functional channels at the sarcolemmal membrane, as steady-state activation kinetics were not significantly altered by either acclimatization or acclimation. Interestingly, in the rainbow trout, cold acclimation-induced remodelling nearly doubled the current density of \( I_{Kr} \), which was underpinned by a cold-induced increased \( I_{Kr} \) gene expression and provided nearly complete thermal compensation for the depressive effect of acute cold on \( I_{Kr} \) conductance (Hassinen et al., 2008; Vornanen et al., 2002). However, these studies did not look at the effect of seasonal acclimatization on \( I_{Kr} \) in the trout heart, and thus it remains unclear if a greater response could be induced with winter acclimatization compared with cold acclimation. In the roach, seasonal cold acclimatization doubled the atrial and tripled the ventricular \( I_{Kr} \) current density (Badr et al., 2017) when compared with summer-acclimatized fishes, and these data were supported by changes in gene expression; however, this study did not look at the effects of acclimation on roach \( I_{Kr} \). Similarly, in polar Arctic navaga cod (Eleginus navaga), winter acclimatization increased \( I_{Kr} \) gene expression and shortened AP in comparison with summer-acclimatized fish (Abramochkin and Vornanen, 2015; Hassinen et al., 2014), but the acclimatory response was not tested.

**Thermal remodelling of depolarizing currents**

Similarly to the pattern observed with the key outward currents in fish cardiac excitation contraction coupling, inward \( Ca^{2+} \) currents \( (I_{Ca}) \) were modulated by seasonal acclimatization but not thermal acclimation. A similar increase in \( I_{Ca} \) following winter acclimatization was seen in the roach heart (Badr et al., 2017), but the influence of thermal acclimation alone was not tested. These are interesting findings because previous cold acclimation studies with the rainbow trout and crucian carp saw no remodelling of \( I_{Ca} \) density in response to cold acclimation, although a similar shift in steady-state activation was observed in trout ventricular myocytes to that observed here for sculpin (Vornanen et al., 1998). On the other hand, \( I_{Ca} \) in cardiac myocytes of crucian carp was prominently downregulated following winter acclimatization (Vornanen and Paajanen, 2004). Thus, from the limited data available, it appears that the fish \( I_{Ca} \) is fairly resistant to remodelling following cold acclimation but does remodel following seasonal acclimatization to the cold. The increase in the \( I_{Ca} \) following winter acclimatization, together with increased \( Ca^{2+} \) sensitivity of the
myofilaments reported by Klaiman et al. (2014) may be necessary to provide sufficient contractility of the heart in the winter. However, we did not measure contractile strength of the sculpin heart, so we can only speculate that the larger $I_{\text{Ca}}$ in winter-acclimatized fish would have improved force production compared with the cold-acclimated group. Indeed, the cellular hypertrophy we observed following winter acclimatization would support the idea of morphological remodelling to increase cardiac contractile force in the cold (Aho and Vornanen, 1998, 1999; Keen et al., 2016; Klaiman et al., 2011; Vornanen et al., 2005). SR Ca$^{2+}$ cycling may also contribute to cellular Ca$^{2+}$ flux in this species and although we did not measure SR function in this study, other studies with fish show cold acclimation increases SR complement and SR function (i.e. in perch, Perca fluviatilis (Bowler and Tirri, 1990); Pacific Bluefin tuna, Thunnus orientalis (Shiels et al., 2011)). For example, cold acclimation increases the ryanodine sensitivity of cardiac isometric force production in rainbow trout (Aho and Vornanen, 1999; Shiels and Farrell, 1997) and biochemical studies indicate an increase in SERCA pump in rainbow trout (Aho and Vornanen, 1998). If similar patterns exist for sculpin, then SR Ca$^{2+}$ cycling could temper the influence of $I_{\text{Ca}}$. Nevertheless, it is clear that factors other than temperature alone are required for Ca$^{2+}$ flux modulation in fish hearts during the winter.

**Acclimation versus acclimatization**

In addition to changes in temperature, changing of the seasons can involve large and complex changes in a number of biotic and abiotic factors including day length, oxygen availability, food availability and reproductive status. At the latitude of the White Sea Biological Station, beyond the Polar Circle, day length dramatically changes during the course of the year: in June, maximum day length reaches 24 h, while in December, day length is only $\sim$1 h. Moreover, light availability is additionally decreased as the White Sea is covered with ice during the winter. In the current study, we tried to control for day length and light penetration such that conditions were similar for both cold groups of fish. However, the ice layer on the surface of the sea in winter would have limited light available to the winter-acclimatized fish to a greater extent than the opaque tank of the fish held in the laboratory, which needs to be considered when interpreting any seasonal versus temperature-dependent remodelling response.

The diet of shorthorn sculpins was not shown to vary between seasons (Cardinale, 2000), although the intensiveness of foraging decreases in winter. The composition of a diet is known to have a great influence on the composition of cell membranes (Corcoran et al., 2007; Leaf, 2006; Soriguer et al., 2000) and, therefore, on the lipid environment of the ionic channels, which, in turn, affects the function of channels (Tillman and Cascio, 2003). The membranes of different cell types isolated from fish tend to contain more polyunsaturated fatty acids after seasonal acclimatization, as well as after cold acclimation (Buda et al., 2006; Sellner and Hazel, 1982). On the other hand, there are reports that the seasonal shift in membrane composition towards unsaturated fatty acids can be due to changes in the expression of desaturases and thus can be referred to as
diet independent (Arnold et al., 2011; Hsieh and Kuo, 2005). There are no data on the direct comparison of the influence of cold acclimation and seasonal acclimatization on membrane lipids. However, based on the studies of Vornanen and colleagues (Hassinen et al., 2007, 2014; Vornanen et al., 2005), who showed that changes in ion channel expression occur in teleost heart following the adaptation to low temperatures, we can presume that the possible shift in lipid environment is an additional adaptive mechanism, although its contribution has to be studied more thoroughly.

Circulating hormones are known to be influenced by the seasons and by changes in temperature, which can have profound effects on the heart. For example, thyroid hormone increases with cold acclimation and mediates increased SR function and heart rate in zebrafish (Little and Seebacher, 2014) but not rainbow trout (Titu and Vornanen, 2003). Glucocorticoids are also seasonal (Wingfield and Grimm, 1977) and affect fish heart form and function (Johansen et al., 2011, 2017). Plasma cortisol varies seasonally in fish, with high levels during the spawning season and also directly following spawning when metabolic and nutritional reserves are low (Wingfield and Grimm, 1977). The European sculpin is a winter spawner (November to January in the White Sea population, (Luksenburg et al., 2004), thus it is probable that elevated cortisol contributed to the hypertrophy and the extensive ion channel remodelling observed in the winter-acclimatized fish in this study. Indeed, Johansen et al. (2011, 2017) found profound and pathological hypertrophic and fibrotic remodelling in salmonid hearts in response to artificially elevated cortisol levels. Moreover, a large number of studies demonstrated myocyte hypertrophy in fishes following cold acclimation (Aho and Vornanen, 1998; Driedzic et al., 1996; Farrell et al., 1988; Keen et al., 2016, 2018; Kent et al., 1988; Klaiman et al., 2011; Vornanen et al., 2005) where the influence of hormones may be less. In these studies, the hypertrophy has been attributed to the increased haemodynamic load on the heart caused by increased blood viscosity in the cold.

Sex steroids (i.e. testosterone) are known to affect K+ currents and heart repolarization in mammals (Bidoggia et al., 2000; Liu et al., 2003) and cardiac inotropy in rainbow trout (Farrar et al., 2006); thus it may be that changes in sex steroids in relation to winter spawning contributed to changes in ion channel density observed here. Indeed, the work of Badr et al. (2017) shows that roach, which also spawn in winter, have a powerful acclimatization response for cardiac K+-channels, which shorten AP. Future studies should investigate the influence of hormonal status on the expression of cardiac ion channels in fish. Lastly, nutritional status and fuel store/ utilization can change seasonally in a number of fish species and can modulate the activity of sex steroid and glucocorticoids. For example, the liver glycogen content is higher in the winter than summer in fresh water (Hyvärinen et al., 1985; Valtonen, 1974) and marine fishes (Shiels et al., 2011). Fuel utilisation may also influence the effect of sex steroids on fish heart contractility (Farrar et al., 2006). Thus, future work should try to consider the multiple biotic and abiotic factors that change with season, in addition to temperature, to better understand how cardiac function remodels to maintain adequate function across the seasons.

The ice layer on the surface of the sea in winter may also have affected oxygen availability to the winter-acclimatized fish, since it
obstructs the activity of photosynthetic algae and prevents oxygen dissolving into seawater from the air (Cottrell and Kirchman, 2009; Pomeroy, 1997). We did not measure oxygen content in the sea pens that held the winter-acclimatized fish. However, reduced activity and lowered metabolic rate (Hanson et al., 2008; Stehlík, 2009), reduced brain function (Vornanen and Paajanen, 2006), modified gill structure (Sollid et al., 2003), and reduced cardiac function (Aho and Vornanen, 1999; Matikainen and Vornanen, 1992; Titiú and Vornanen, 2001) are all associated with hypoxia and anoxia in fishes. Whether the oxygen content in winter could drive seasonal changes that have downstream effects on the electrophysiological phenotype of fish heart would be worthy of investigation in the future.

Conclusion
Thermal acclimation studies often assume that remodelling of a certain trait is both adaptive and functionally important for organisms in their natural environment. Although this may be the case, it is becoming clear that the relationship between capacities for temperature acclimation and seasonal acclimatization may be different. Seasonal biology is complex and its influence on physiology extends beyond temperature change.

Acknowledgements
Authors thank the director of the White Sea Biological Station, Prof. Alexander B. Tzetlin for general support of this work. Authors also would like to thank Alexander L. Gvozdev for capturing the fish and Dr Yanwen Wang at the University of Manchester for useful discussions about seasonal biology and ion channel remodelling.

Competing interests
The authors declare no competing or financial interests.

Author contributions

Funding
The study was supported by the Russian Foundation for Basic Research (18-315-20049 to D.V.A.).

Data availability


Highlighted Article: The effects of seasonal acclimatization on cardiac responses to low temperatures are stronger than those of acclimation to similar temperatures, highlighting the importance of seasonal cues in cardiac remodelling in a eurythermal fish.

Funding details

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Funder name</th>
<th>Funder ID</th>
<th>Grant ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Russian Foundation for Basic Research</td>
<td><a href="http://dx.doi.org/10.13039/501100002261">http://dx.doi.org/10.13039/501100002261</a></td>
<td>18-315-20049</td>
</tr>
</tbody>
</table>